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A PSYCHOBIOLOGICAL APPROACH TO IMPROVE EXERCISE ADHERENCE

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CONTENTS

Frequently used abbreviations

HR	heart rate (beats per minute)
PPO	peak power output (watts)
VO_2	oxygen consumption ($L^* min^{-1}$)
VCO_2	carbon dioxide consumption ($L^* min^{-1}$)
VE	minute ventilation ($L^* min^{-1}$)
CO	cardiac output ($L^* min^{-1}$)
SBP	systolic blood pressure (mm Hg)
DBP	diastolic blood pressure (mm Hg)
RPE	rating of perceived exertion
FS	Feeling Scale

CHAPTER 1

GENERAL INTRODUCTION

1.1 *Exercise adherence: definition and impact*

Adherence is generally defined as the level of participation in a behavioral regimen once a person has agreed to undertake it [1]. Looking at adherence from a clinical point of view is important point for a fully understanding of its usefulness in a whatever program. In fact, as indicated by Osterberg and Blaschke [2], the term adherence is preferred to compliance based on its active participation pattern to the treatment. Physical activity and adherence to exercise programs have had a huge impact on the likelihood of illnesses reduction in the last decades [3]. In fact, as indicated in several researches, there is a dose – response relationship between physical activity level, frequency, intensity and duration and the relative risk ratio of certain illness conditions, like CV diseases, colon cancer, obesity, depression, etc. [4,5]. Since several researchers made effort in order to define theories and models for a better understanding of this phenomenon, they have always tried to define determinants addressed for different categories, i.e. psychological factors, social aspects, biological factors etc. [6]. Nowadays, it is important to use integrated ecological models that take into account the interaction between personal, social and environmental aspects [7]. As indicated by Bauman et al. [3], all these determinants are important and contributors for a possible success in a behaviour change. One of the major problems of physical activity is the total amount performed by the individuals [8]. In fact, there has been a rapid growth of death in the low and middle-income countries related to physical inactivity as non communicable disease. Moreover, although people decide to pass the physical inactivity barrier, there is a high likelihood of dropping out usually after 3 months from the beginning [6]. For these reasons appear to be very important try to find strategies and models in order to increase the level of physical activity.

1.2 *Current approaches to improve exercise adherence*

It is well known that physical activity improves physical fitness and several health outcomes if performed on a regular basis, at the right intensity, duration and frequency [9]. As reviewed by Dishman R.K et al.[6], physical activity behaviour is determined by several factors [10]. Previous studies on exercise adherence improvement have used psychological and behavioural interventions targeting determinants like environmental factors, intensity and volume of exercise, and imagery [11,12,13,14,15,16,17,18].

On the other hand, the effort for publishing researches on interventions targeting the relationship between exercise adherence and exercise intensity is still poor. As indicated by Bauman et al.[3], one challenge in the interpretation of behaviour changes is the lack of presence of longitudinal data collection. Indeed, a distinction has to be done between correlates and determinants of physical activity. The main difference between the two variables referred to the possibility of causal relationships between factors acting on the exercise domain and physical activity studying determinants. Analyzing the literature, it is possible to find several studies targeting exercise adherence by using different perspectives. Most of the studies aimed on the definition of the relationships between determinants and correlates to physical activity. As indicated by Bauman et al.[3], it has been possible to summarize determinants and correlates in five broad categories, demographic and biological, psychosocial, behavioural, and social and cultural variables [3]. Moreover, other studies have taken into account the possibility to increase the level of physical activity studying environmental correlates [3]. These parameters are divided in three main different correlates outcomes categories, like transport activity outcome, leisure activity outcome and total physical activity outcome [19,20]. On the basis of these studies, there is the concept based on the huge importance of behavioural and psychological factors and theories in determining a physical activity behaviour change. As indicated by Biddle S.J.H. and Mutrie N. [21], motivation, emotions, time and facilities availability are barriers for the increase of exercise adherence. Another important psychological factor in exercise adherence is the income level. Indeed, some studies [3,6,21] have indicated how physical activity can be modified by different domains. Some of those become a daily pattern for a life sustaining, i.e. transport, occupational and household physical activity in low and middle-income countries. On the other hand, in high-income countries, physical activity is mainly based on leisure time activities. A different approach related to physical activity and adherence is based on a popular theory indicating that high exercise intensity is negatively related to exercise adherence [22,23]. This relationship would be mediated by exercise intensity and affective responses (low and moderate intensity exercise = positive affect; high intensity exercise = negative affect).

Therefore, it has been suggested that people should self-select their own exercise intensity to elicit a positive affective response during exercise [22] eliciting a higher adherence rate. However, to date, there is no evidence highlighting that this behavioural intervention improves exercise adherence. The analysis of the relationship between adherence and exercise has also to be done verifying how different exercise parameters (i.e. intensity, frequency, volume) contribute in determining the behaviour change. One of the strategies implemented by several world organisations in order to try to increase exercise adherence it has been the reduction of the exercise intensity [23,24,25], shifting from exercise performed to develop cardiorespiratory fitness to moderate intensity activities. Although it is true that moderate intensity exercise confers significant health benefits, vigorous exercise improves health and physical fitness even further [24,25,26]. During the last decades, most guidelines on physical activity actually recommend moderate intensity exercise [24,27]. The rationale for the use of this approach is based on several studies showing that a higher rate of adherence was obtained performing moderate compared to high intensity exercise [28,29,30]. One of the reasons indicated by the investigators was that moderate intensity has a higher adoption and maintenance due to a reduced injury rates compared to higher intensities [27,28]. Furthermore, the higher rates of self-reported injuries could have represented an excuse for not completing an activity that reaches a high degree of subjective perception of discomfort. In addition to these investigations, the aspect of different exercise frequency was also studied [14,28]. Results showed a higher rating of adherence in the high frequency exercise and moderate intensity group. This is a demonstration that frequency per se is not a negative determinant of exercise adherence and has to be encouraged in the exercise prescription phase. Moreover, Anton S.D. [14] also indicated that previously active individuals taking part in exercise activities during adulthood, can affects the preferred level of intensity and frequency of the exercise. Other investigations over these aspects were performed analyzing differences in responses between different activity modalities like high intensity interval running compared to moderate continuous running on exercise enjoyment [33]. The results, based on the approach of a higher enjoyment rating achievement during the exercise, showed greater enjoyment perception performing high intensity exercise compared to the moderate intensity group. Another aspect that can describe this phenomenon is the exploration of interventions impacting the behaviour change by affective expectations. More precisely, nevertheless a relatively unexplored topic, there are evidences of an improved adherence rate comparing interactive exercise videogaming with traditional physical activity environments [15,34,35,36]. The framework and basis for the implementation of this approach was the extended theory of planned behaviour model [37,38]. This theory is based on its utility on the prediction of physical activity by the presence of discriminant affective parameters. Indeed, the TPB applied on the physical activity domain, suggests that a behaviour enhancement is predicted by intention (i.e motivation) and its perceived behavioural control (i.e. perceived ability to perform the behaviour) [36]. Intention is subsequently influenced by enjoyment/pleasure assessment of the behaviour, the instrumental attitudes, subjective norms and perceived behavioural control [36]. The results of these investigations produced a significant difference in adherence in favor of the videogaming condition compared to classical one [15,36]. Thus, another possible approach likely to be implemented in order to increase exercise adherence is based on nutritional supplements [39,40]. In fact, several studies have shown that a pre-exercise nutritional supplements administration (i.e. caffeine, ephedra, etc.) increased performances in athletes. As indicated in this paragraph, the majority of the strategies implemented for improving exercise adherence use behavioural and psychological interventions in order to improve attitudes and motivation to exercise activity. A part of the investigations have been also made analyzing the effects of exercise characteristics itself on adherence. As mentioned above, it seems that the way for inducing a higher adoption and maintenance of physical activity is desirable with a reduction of the its intensity, producing at the meantime a lower efficacy in terms of cardiovascular risk factors reduction [4]. For these reasons, interventions that can increases adherence without reducing exercise intensity would be highly desirable to maintain positive effects on health and physical fitness. To date, on the top of our knowledge, there are not present interventions using this kind of approach for improving exercise adherence.

1.3 Perception of effort: its evidences as physical activity determinant

Perception of effort is defined as how hard, heavy and strenuous a physical task is [41]. From a neurophysiological point of view, this feeling should be mainly dependent on feeling of effort in the active limbs and sensation of heavy breathing [42,43]. As shown by Taylor and Gandevia [44] perceived exertion increases during a constant load exercise over time and this phenomenon is a clear symptom of fatigue. Furthermore, psychological factors such as personality, mood, somatic perception, locus of control, and self-efficacy can affect RPE [42]. Based on these concepts, RPE is correlated to different afferent feedbacks that sending cues from peripheral and central factors can influence perceived exertion [43]. Looking at this process from a statistical point of view, it has been demonstrated that correlation does not mean causation. Indeed, analyzing the literature based on this topic, it is possible to focus the attention on some other researches (e.g. epidural anaesthesia) aimed to understand the central role of RPE. An example is represented by some interventions aimed to block afferent inputs (from muscles spindles and golgi tendon organs, or type III and IV afferent fibres) to the central nervous system. The results did not show any effect on RPE [45,46]. Other researchers during the last decades have tried to implement theories in order to support and corroborate the central role of RPE. One of the researchers that more than others has given his effort in trying to understand this phenomenon is Samuele Marcora. Indeed, in one of his works [47], he well explained the reasons why it is necessary to support a brain based model rather than an afferent feedback one. Actually, his model is based on the so called motivational intensity theory [48] and its name is "Psychobiological Model". Following this model, it is possible to understand that individuals mainly disengage from a task for the reason that the effort required for achieving a defined task is too demanding for a subject, then they are no longer willing to exert their maximum effort in order to succeed in a certain task [47,48].

RPE has also been studied as determinant of physical activity. Investigations on this topic aimed to find a relationship between both supervised and self-selected physical activity in different populations. As indicated by Dishman R.K. et al.[6], supervised physical activity is negatively associated to perceived exertion. More specifically, as they shown the “perceived discomfort during an exercise program, regardless of exertion” could have produced either a positive or negative result in terms of adherence and drop out. In a recent research conducted by Aadhal M. et al. [49] they found an inverse relationship between level of self rated fitness and RPE in more than 35 different activities. Moreover, another study [50] shown as the level of relative intensity of physical activity measured using rating of perceived exertion is a strong predictor of lower cardiovascular risk rate. This means that the higher the intensity of the exercise, the better will be the likelihood in terms of reduction of cardiovascular diseases [32]. Unfortunately, the relationship between exercise intensity and RPE in terms of exercise adherence still remains a problem.

1.4 Motivational Intensity Theory: a general overview

Exercise habits depend on several factors. As indicated by Prochaska et al. [51] in his "stages of change framework" or transtheoretical model, people move through different stages. Indeed, when they start thinking to be physically active, they are not actually active yet. Even when individuals start to perform exercise, this model indicates that the different stages are irregular. This happens when people that are physically active decide to disengage from the exercise domain [52]. For these reasons, researchers tried to give an explanation of what regulates exercise and physical activity from a psychological point of view. One of the fathers of psychology of physical activity, Dishman R.K., made efforts for the definition of determinants of physical activity [6,10,53,54]. Moreover, other researchers studied this phenomenon starting from psychological theories. One of the most used able to explain the relationship between exercise and adherence is the so called "Motivational Intensity Theory", proposed by Brehm and Self [55]. They have defined effort as the mobilization of resources in order to carry out instrumental behaviour. Moreover, they indicate that effort primarily refers to the intensity aspect of motivation [55]. The presence of effort is of importance for overcoming obstacles and deterrents, which constitute barriers between the individual and the goal to be achieved. According to the difficulty law of motivation [56,57], effort is proportionally mobilized to the difficulty of effort [58]. Another very important aspect of Brehm's theory is the presence of a construct that is not take into account in all the other effort theories. Indeed, two parameters design this approach in a very interesting manner. The first one is called potential motivation, defined as the amount of effort that people would be willing to exert in order to satisfy a motive. The second, called motivation intensity is defined as the maximum effort that individuals actually expend [59]. These two different concepts lead to another law, the so-called "law of parsimony". The law indicates that simple theories should be preferred over complex ones, unless the complex theories explain more completely the outcome of interest. Its logic indicates that theoretical distinctions are of value to the degree that they improve, or refine, the prediction of defined outcomes [59]. Analyzing the studies produced on Brehm's distinction, it is definitely possible to declare that his theory has a huge prediction on effort outcomes [59,60]. Furthermore, another important aspect has to be considered dealing with effort theories. In fact, motive strength should be a key in determining the subject's effort. Motive determines the potential motivation and where effort requirements are warranted and possible to be achieved, the person should expend effort as it needed. On the other hand, individuals tend to hold effort in reserve for other motives. More specifically, what has been explained in the previous rows indicates the existing relationship between effort, potential motivation and difficulty. Figure n.1 explains this relationship.

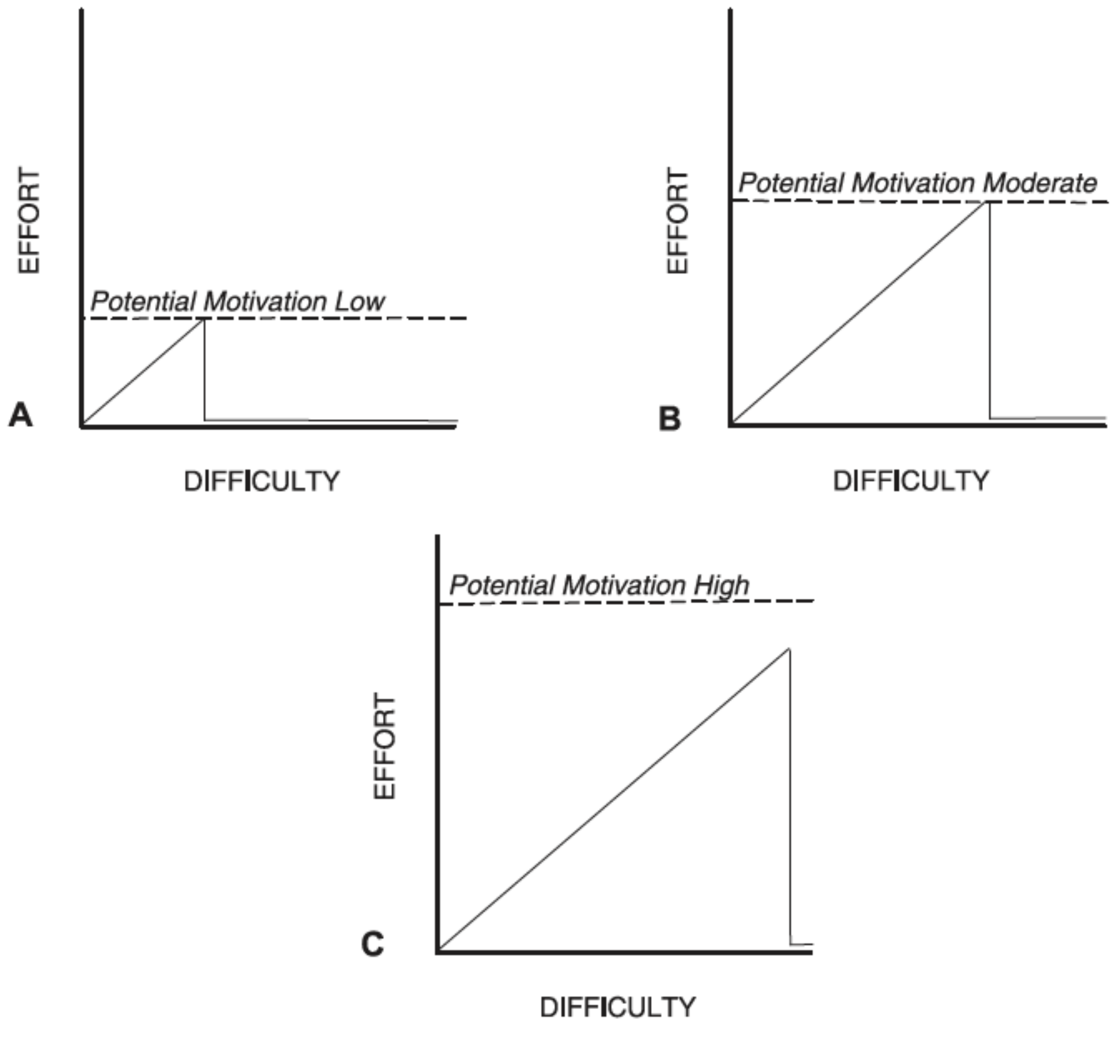


Figure1. Potential motivation and motivation intensity variations based on the task difficulty [59].

As indicated on the first panel (A), a subject with a low level of potential motivation is not able to overcome a higher task in terms of difficulty. This aspect indicates that the amount of effort that the subjects would be willing to exert is not enough for increasingly difficulty tasks. The second panel (B) indicates an example with a moderate level of potential motivation. In this case, on the basis of the task difficulty, the effort that individuals would be actually willing to exert allow to achieve easy and moderately difficult tasks, but no difficult tasks are warranted. For this aspect, is important to focus the attention on the third panel (C). Indeed, in this case, individuals are willing to exert a high level of effort in order to cope with high difficulty tasks [59].

1.5 The psychobiological model

The analysis of the motivational intensity theory of Brehm [55] has an impact on the physical activity domain. Before to explain the application of this model to exercise and physical activity, it is important to focus the attention on an important aspect related to the reason of the use of this model. On the scenario of psychological theories explaining the relationship between exercise and adherence there is another one that wanted to highlight the characteristics of this phenomenon. According to the Hedonic theory of motivation [22], Ekkekakis proposed a model based on the affective aspects of the exercise [22,61]. More precisely, the issue is the relationship between moderate and high intensity exercise with adherence. Based on this model, moderate intensity exercise should be perceived as enjoyable and positive by the people leading to adoption and maintenance of physical activity behaviour. On the other hand, an exercise intensity perceived as uncomfortable, based on this approach as over the ventilatory threshold, should results in a higher rate of individuals with tendency to give up from the activity [22,62,63]. Unfortunately, as indicated by several papers and public reports published by universities and health organizations, based on the level of moderate intensity activity, is possible to understand how the rate of individuals that meet the criteria for being physically active is low [64]. This firstly demonstrates that people do not engage in all categories of intensity of the exercise, and secondly highlights that the hedonic theory implemented by Ekkekakis has a lack of construct validity.

The application of the Brehm's theory on the exercise domain has been recently proposed by Marcora S.M. [47,65] in the so-called psychobiological model. According to this model, people either do not engage in any kind of physical activity or disengage from the task whether they are active, because the effort required by the activity itself is equal to the maximum effort they are willing to exert, or because the individuals believe to have exerted a true maximal level of effort and continuation of the activity or the planned program is no longer possible. The motivational intensity theory can be implemented using two different approaches. As indicated in the figure n.2, the first approach is the classical way, used in order to try to increase the level of potential motivation (i.e. from panel A to panel B) to obtain a task improvement. This strategy is the most popular, because of its use in all the treatments based on psychological interventions (i.e. 1 to 1 sessions, different enviroments).

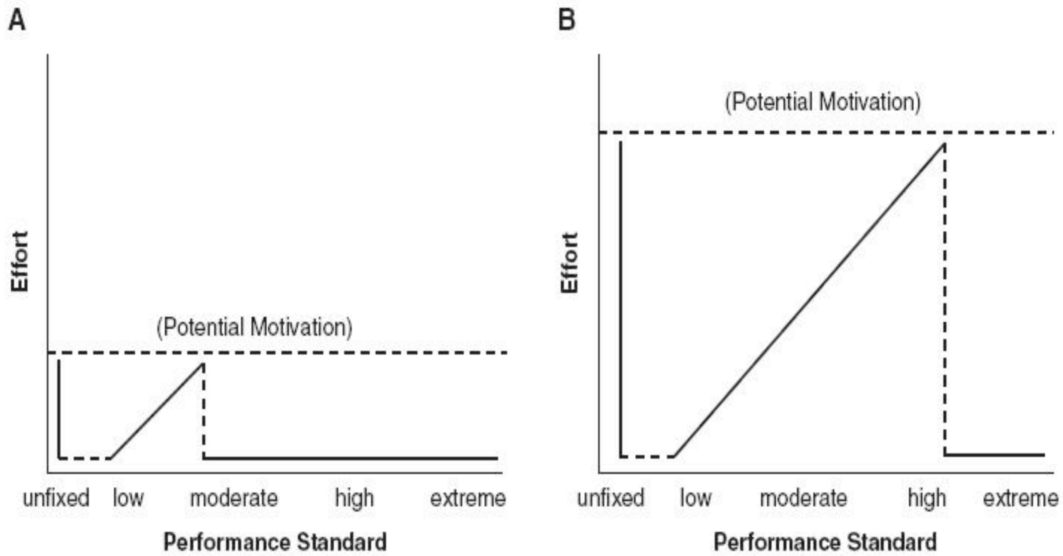


Figure 2 A general description of the classical use of Brehm motivational intensity theory. A) pre-test condition with a low to moderate potential motivation. B) post-test condition with increased level of potential motivation. [59]

The second approach, showed in the figure n.3, has a different starting point. Actually, from a general point of view the concept is based on the ability to reduce the demand of a task, in order to maintain a good level of potential motivation without reaching the point of task disengagement. Based on this strategy, people should maintain their task engagement for a longer period of time and whether they decide to disengage from the task, they should do it later on.

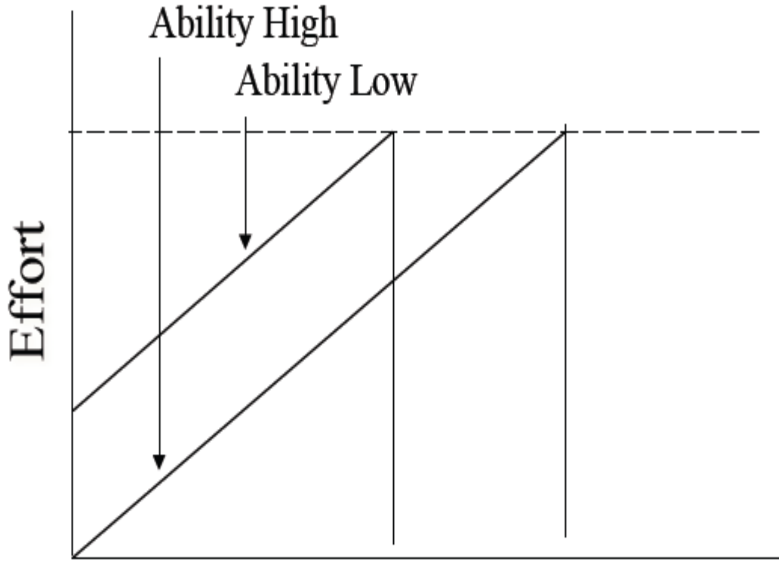


Figure 3 Alternative approach of the Brehm's motivational intensity theory. The ability to reduce the task difficulty assures a reduction in terms of effort resulting in a longer task engagement.

1.6 The use of caffeine on exercise adherence improvements

Caffeine is one of the most widely used drugs in the world, as demonstrated by several researches [40,66]. Almost the totality of caffeine daily intake comes from dietary sources, with a higher percentage coming from coffee and teas. As indicated, the use of caffeine on a daily use does not represent a risk for the general health even if in the literature it is possible to find contradictory results due to the possibility of caffeinism, dependence and withdrawal [67]. Caffeine has also been studied in the physical activity and exercise domain as well as in competitive sports [39,40,68]. In fact, athletes are well known to use caffeine in order to improve mental and physical performances due to its ergogenic effects [69]. The main action of caffeine on the body is its role as antagonist of adenosine in the brain [70,71]. The result, as indicated in a meta-analysis conducted by Doherty M. et al. [40] is a delayed onset of the fatigue process. Moreover, another aspect plays a role when caffeine is taken during the exercise. The contribution of RPE in the aerobic exercise domain as limiting factor has been well demonstrated in athletes during the last decades [42,47,72,73]. Indeed, as indicated in a systematic review published by Doherty et al. [40] there is an inverse relationship between the fitness level expressed as VO_2 max and percentage of reduction in RPE. The studies collected for this meta-analysis included individuals with a fitness level above 45 ml*O_2*min^{-1}, as indicated in figure n.4.

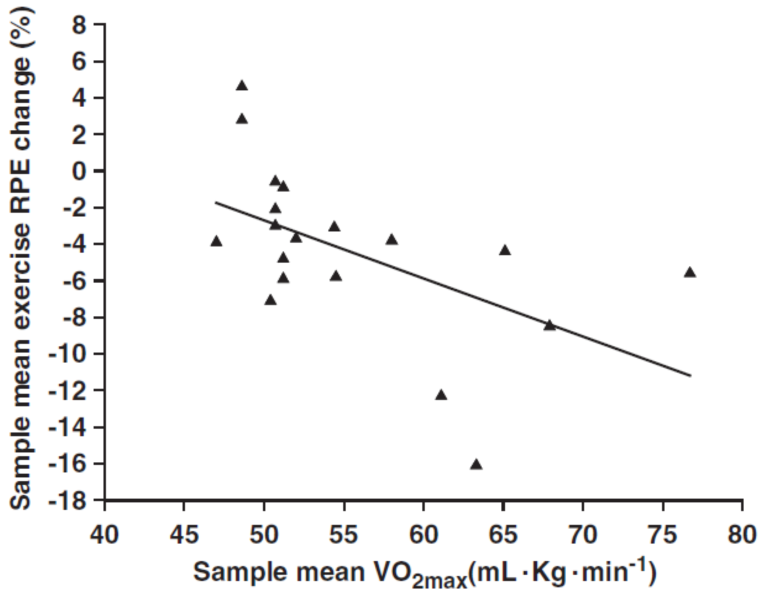


Figure 4 Scatterplot of sample mean VO2max and sample mean exercise ratings of perceived exertion (RPE) change following caffeine ingestion (n=20). RPE change (%) = -13.21+(VO2max [mL/kg/min] _0.32); $r^2$50.29 ($P<0.05$).These data suggest a good relationship between VO_{2max} and the likelihood to reduce RPE following caffeine ingestion in athletes than participants that have a low VO_{2max}. [40]

Furthermore, there are some evidences indicating that the effect of caffeine on perception of effort is positively related to VO_{2max} [74,75]. To date, only very few studies have been conducted either in sedentary individuals or in people with a poor level of fitness [76,77]. For these reasons it is of importance to see whether the use of a nutritional strategy could lead to an improvement of exercise adherence. As indicated in the paragraph above, it will be possible to implement the second way of using the Bremh's motivation intensity theory. Indeed, in the context of physical activity for health, the objective is to use caffeine in order to target perception of effort without reducing the intensity of the exercise. In this way it could be possible to maintain for a longer period individuals as physically active, leaving them to disengage later on.

1.7 Aims of the research program

The aims of this thesis was to implement a nutritional approach based on the psychobiological model using caffeine for addressing the relationships between caffeine on perception of effort, physiological variables and adherence in sedentary adults. (inserire proof of concept che sam ha dichiarato durante la telefonata). We have performed two studies:

Study n. 1: Effects of caffeine on perception of effort and physiological responses during sub-maximal aerobic exercise in sedentary adults.

Aim of the study: to examine the effects of different caffeine dosages and placebo on perception of effort, affect, blood lactate and cardiovascular parameters during a sub-maximal training simulation.

Study n. 2: Effect of caffeine on exercise adherence in sedentary adults.

Aim of the study: to examine how caffeine affects perception of effort and adherence parameters during a 12 weeks aerobic training program in sedentary adults.

CHAPTER 2

Effects of caffeine on perception of effort and physiological parameters during sub-maximal exercise in sedentary adults (Study I)

STUDY I

Title: **Effects of caffeine on perception of effort and physiological responses during sub-maximal aerobic exercise in sedentary adults.**

2.1 ABSTRACT

Rating of perceived exertion (RPE) is a negative determinant of physical activity [6]. This conscious sensation is related to the intensity of the performed task [41]. It is well known that high-intensity exercise is associated to a lower rate of adherence when compared to a moderate intensity regimen [30]. Caffeine is one of the most widely used drugs in the world [78]. Furthermore, it has been well established that it can enhances performance in athletes either by a higher power production [40,79]. The effect of caffeine on RPE in unfit people is poorly understood and controversial [76]. It has been suggested that RPE could be not affected by caffeine in this population during a sub-maximal aerobic exercise [76]. The aim of this study was to establish whether caffeine affects RPE in sedentary healthy subjects during a sub-maximal aerobic exercise. A secondary aim was to determine the effects of caffeine on cardiovascular and physiological variables. 16 healthy subjects were involved in the study. In this randomised cross-over study participants visited the laboratory four times. After a $VO_{2\ MAX}$ determination each subject was asked to perform a sub-maximal aerobic simulation taking in a randomised order 400, 200 mg, and placebo. There was a significant treatment x workload interaction of caffeine on RPE ($p<0.05, p=0.006$). Pairwises comparisons indicated that a dosage of 200 mg (M=15.66,SD=1.69) ($p<0.05, p=0.014$) and 400 mg (M=15.47,SD=1.26) ($p<0.05, p=0.000$) of caffeine were significantly different in reducing RPE compared to placebo (M=16.94,SD=1.94). No significant differences were found between the 200 mg and 400 mg of caffeine ($p=1.000$). No significant treatment x workload interaction was found on VO_2 ($p=0.480$). There was a significant main effect of workload on VO_2 on the three different conditions ($p<0.05, p=0.000$). No treatment x workload interaction on VCO_2 ($p=0.408$). Significant main effect of workload ($p<0.05, p=0.000$) was found. No treatment x workload interaction on VE ($p=0.388$). Significant main effect of workload ($p< 0.05$, $p=0.000$) was found. No

treatment x workload interaction on HR ($p=0.548$). Significant main effect of workload ($p<0.05, p=0.000$) was found. No treatment x workload interaction on La^- ($p=0.390$). A significant main effect of workload ($p<0.05, p=0.045$) was found. No treatment x workload interaction on RPM ($p=0.659$). A significant main effect of workload ($p<0.05, p=0.004$) was found. No significant treatment x workload interaction on SV ($p=0.284$). A significant main effect of workload ($p<0.05, p=0.049$) was found. No significant treatment x workload interaction on CO ($p=0.285$). A significant main effect of workload ($p<0.05, p=0.001$) was found. No significant treatment x workload interaction on SBP ($p=0.538$) A significant main effect of workload ($p<0.05, p=0.001$) was found. No significant treatment x workload interaction on DBP ($p=0.972$). There was not any significant main effect of workload ($p= .194$).

In conclusion, this study demonstrated that 200 mg of caffeine taken on a chewing-gum based pill 15 minutes before a sub-maximal aerobic exercise were able to reduce RPE in sedentary individuals.

2.2 INTRODUCTION

Observing literature it is possible to highlight that RPE is indicated as a negative key variable and an important limiting factor during both exercise and physical activity programs [10,22,41]. Another key variable of exercise adherence is the intensity of the exercise [6,10]. As indicated in a study conducted by Perri et al. [31], low – moderate intensity exercise are associated with a greater adherence than prescription for vigorous activities. For these reasons, it is necessary to define a strategy in order to improve attrition to exercise programs. One possibility is related to the use of caffeine. This drug is considered the most widely used, with both young and adults consuming it on a regular basis [80]. It is well known that caffeine enhances performances in athletes [39,40,79]. Another study, conducted by Davis et al. [81] demonstrated that a central nervous system infusion of caffeine in rats provided an increased run time to fatigue. In a recent Review by Glade [78], he demonstrated the beneficial effects of moderate (100 to 200 mg, every 3 to 4 h, 5 times/day) caffeine consumption in humans. As stated by Glade, an important issue of the use of caffeine are both the decrease of “sense of effort” associated with physical activity and the decrease of mental fatigue. Other studies, conducted in humans, have shown some alterations of participant perceptual responses during testing activities. Some of these responses are based on an increased work output at a given rating of perceived exertion, or a reduction in RPE at a constant exercise intensity [40]. As indicated by the authors, they found a relationship between $VO_{2\ max}$ and RPE. Indeed, after the ingestion of caffeine, subjects with a higher fitness level showed a better reduction in terms of RPE. Moreover, fitness level might play an important role in the reduction of perception of effort [75,79]. Therefore, there are not studies conducted with sedentary subjects [40]. The aim of the study was to investigate the effects of caffeine on perception of effort during sub-maximal exercise in unfit people. A secondary aim was to determine whether caffeine could affects cardiovascular parameters (i.e. CO, SBP, DBP) due to the controversial results published on peer review journals on this topic.

2.3 METHODS

Subjects and Ethical issues

Sixteen eligible subjects [8 men and 8 women; mean ± SD, age 28.0 ± 6 yr, height 172.2 ± 6.4 cm, weight 70.5 ± 14.3 kg, peak oxygen uptake (VO_{2peak}) 36.5 ± 4.8 ml · kg^{-1} · min^{-1}, peak power output (PPO) 200 ± 43 W] signed an informed consent form describing the study protocol. The eligibility criteria were being between 18 and 45 for men, and from 18 to 55 for women, being no involved in aerobic activities for more than 3 times per week in the past 3 months, being free of any know illness condition and with a VO_{2peak} below "GOOD" according the Heyward cardiorespiratory fitness classification [27]. All subjects were informed and given written instructions describing all procedures related to the study. Participants believed that the study was conducted on the effect of two different psycho stimulants on the physiological responses to sub-maximal aerobic exercise. At the end of the last visit, subjects were clarified on the real aims of the study, thanked for their participations and they were also asked to not talk to the other subjects the real aims of the research.

Study design and procedures

In this study we employed a double - blind, randomized cross-over design. Subjects visited the laboratory four times. During the first visit were asked to participants to complete the International Physical Activity Questionnaires (IPAQ) [82] to ensure the absence of any regular physical exercise in the last three months. Moreover, subjects were asked to complete a caffeine questionnaire in order to obtain informations about the frequency and quantity of any beverage and food containing caffeine in the last three months. Subjects were also asked to perform both body size composition and an incremental exercise test (3 min at 50 W + 25 W every 1 min with a pedal frequency from 60 to 100 revolutions/min) in order to determine $VO_{2\,peak}$ and Peak Power Output (PPO) calculated according to the equation of Kuipers et al.[83] on an electromagnetically-braked cycle ergometer (Lode, Groningen, The Netherlands). Tidal volume (V_T), breathing frequency (B_F), minute ventilation (VE), oxygen consumption

(VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were measured using a breath by breath gas Analyzer (Metalyzer, Cortex, Netherlands) connected to an oro–(mouth) mask. The highest VO_2 value measured during the test was considered maximal (VO_{2peak}) when any two of the following criteria were met: a respiratory exchange ratio ≥ 1.15; a maximal heart rate higher than the 90% of the subject's age-predicted maximal heart rate; a plateau in the VO_2 with an increase in work rate; leg or chest RPE higher than 18 on the 15 point Borg Scale. The cycle ergometer was regularly checked for accuracy of power output and cadence. Prior to the incremental test the ergometer was adjusted for each subject. Settings were recorded to allow the reproduction in the subsequent visits. Subjects were familiarized and received instructions for the rating of perceived exertion (RPE) using the 15 point scale developed by Borg [42] .

In the second, third and fourth visit, after randomization (www.randomization.com), subjects taken either a 200 mg caffeine based chewing gum, 400 mg caffeine based chewing gum or a placebo chewing gum. Prior to testing participant informed consent and informations were provided in order to refrain from caffeine, nicotine, alcohol and any medication in the 24 h prior each testing session and during all the day of the experimental conditions, as well as to avoid exercise for 24 h prior [84,85]. At each visit to the lab, subjects were asked to complete a pre-test checklist to ascertain that they had complied with the instructions given to them. Each testing visit consisted in a 4 intensities sub-maximal aerobic simulations. More precisely, each subject was asked to pedal 4 min at 20, 40, 60 and 80 % of their PPO obtained during the first visit after a 4 min step seated on the cycle ergometer to allow basal measurement of blood lactate, SBP, DBP, SV and CO. Each workload was interspersed with a rest of 1 min. Cardiovascular parameters were measured during the second and fourth minute of each intensity. La^- was also measured immediately after each stage. Perceptual responses to the exercise i.e. RPE and FS, were measured during the last 15 seconds of the second and fourth minute of each load. (see Physiological and perceptual responses to exercise for details). Each subject completed all the testing sessions over a period of two weeks with a time recovery in between of at least 48 h. Environmental conditions during the laboratory were kept constant between 18 and 22 °C for the temperature and 45 and 60 % for humidity.

Treatment

Experimental treatment

Subjects involved in the study were deceived and they were informed of a testing of the effects of two different psychostimulants on physiological responses in order to avoid any changes in perceptual responses due to the different composition of the treatment. Every visit, in a randomized order they were asked to chew 4 chewing gums either 2 *Stay Alert* and two placebo chewing gums, 4 *Stay Alert*, or 4 placebo chewing gums. At the end of the data collection subjects were informed of the real nature of the aims of the study and the composition of the treatments. The chewing gums were chewed for 5 minutes taking 2 chewing gums each time. After each 5 minutes periods were asked to the subjects to expectorate the gums. Fifteen minutes after assuming treatment, subjects performed a 4 minutes baseline step. Than, subjects performed 4 bouts at 20 – 40 – 60 - 80 % of PPO of 4 minutes each, defined as LOW - MODERATE - HIGH - VERY HIGH INTENSITY. The rest interspersed within each bout was 1 minute. At the end of the VERY HIGH intensity bout subjects performed a 3 minutes bout at 10% of PPO as cool down.

Stay Alert ® is a commercial available caffeine-based chewing gum. Its caffeine concentration is 100 mg for any piece. We used a maximal caffeine concentration of 6 $mg*kg^{-1}$. As shown in the study of S.A. Syed et al. [84] subjects rested for 15 minutes after the assumption of the gums and warm up. The reason is the required time that is needed to achieve the maximum caffeine concentration using chewing gum form [84]. Due to the presence of the buccal mucosa site, the gum formulation provides a significantly faster absorption rate [84]. Indeed, this site is known to be a rapid site for drugs absorption. The amount of caffeine that subjects were asked to take ranged from 3 to 6 $mg*kg^{-1}$. We decided to use this range of caffeine concentration based on studies that demonstrated the absence of any side effect in healthy subjects who assumed caffeine in a range from 3 to 10 $mg*kg^{-1}$ [78].

Control treatment. Control treatment consisted of performing the same protocol as indicated in the “experimental treatment” section. The main difference was related to the pills that subjects were asked to take. They chewed 4 normal chewing gums with a similar taste compared to caffeine-based chewing gums. The chewing gums were sugar free.

Physiological and perceptual responses

Heart rate (HR), stroke volume (SV) and cardiac output (CO) were measured during exercise using a bioimpedance device (Physioflow PF05L1; Manatec, Petit-Ebersviller, France). Minute ventilation (VE), oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were measured using a breath by breath gas Analyzer (Metalyzer, Cortex, Netherlands) connected to an oro–(mouth) mask. Two sets of two electrodes (Ambu Blue Sensor VL, Ambu A/S, Ballerup, Denmark), one transmitting and the other one receiving a low amperage alternating electrical current, were applied on the supraclavicular fossa at the left base of the neck and along the xiphoid. Another set of two electrodes was used to monitor a single ECG lead in the V1/V6 position. All electrodes placement areas were shaved if necessary, cleaned with an alcohol pad and dried with a paper towel. Wires connected to the electrodes were fixed on the body using tape to reduce movement artifacts. Stroke volume (ml), was estimated by this computerized device from changes in transthoracic impedance during cardiac ejection according to the method described in detail by Charloux et al. [86]. CO ($l \cdot min^{-1}$) was calculated as CO = (HR x SVi x BSA) / 1000, where BSA was body surface area (m^2) calculated according to the Haycock formula [BSA= 0.02465 x body mass (kg) 0.5378 x stature (cm)0.3964] and SVi (ml m-2) = SV / BSA. Heart rate (min-1) was based on the R-R interval determined from the first derivative of the ECG. All data were averaged over 1 min periods before statistical analysis. Accuracy of CO estimation has been validated against direct Fick methods [86]. Blood pressure was monitored both at rest and during the exercise during the 2nd and 4th minute using an automated blood pressure monitor (Tango stress blood pressure monitor; SunTech Medical, Morrisville, NC) interfaced with physioflow. The size of the cuff, which was placed on the left arm of the subject, was based on individual arm girth. Mean arterial pressure (MAP) (mmHg) was calculated as MAP = [(2 x diastolic pressure) + systolic pressure] / 3. Total peripheral resistance (TPR) (mmHg l^{-1}) was calculated as TPR = MAP / CO. Capillary blood was sampled from the earlobe immediately after each stage during the 1-min break and analyzed using Biosen C_line, Clinic.

During the last 15 seconds of the 2nd and 4th minute of exercise, subjects were asked to rate their perception of effort using the 15-point Borg Scale (1998). Moreover, immediately after their perception of effort declaration, there were also asked to the subjects to rate their amount of pleasure/displeasure using the so-called feeling scale [89].

Statistical analysis.

All data are presented as mean ± SD. The effects of condition (caffeine 200 mg, caffeine 400 mg and control) and the intensity (LOW - MODERATE – HIGH – VERY HIGH) on physiological, perceptual responses and psychological variables, were tested using a 3 x 4 factorial fully repeated measures ANOVA.

2.4 RESULTS

Effect of caffeine on physiological responses

As indicated in figure 5 the average HR compared in the 3 different conditions did not present any interaction (p = 0.548) at each of the four different workloads, while HR increased from the LOW to VERY HIGH intensity workload (main effect of workload (P< 0.05, p = 0.000)).

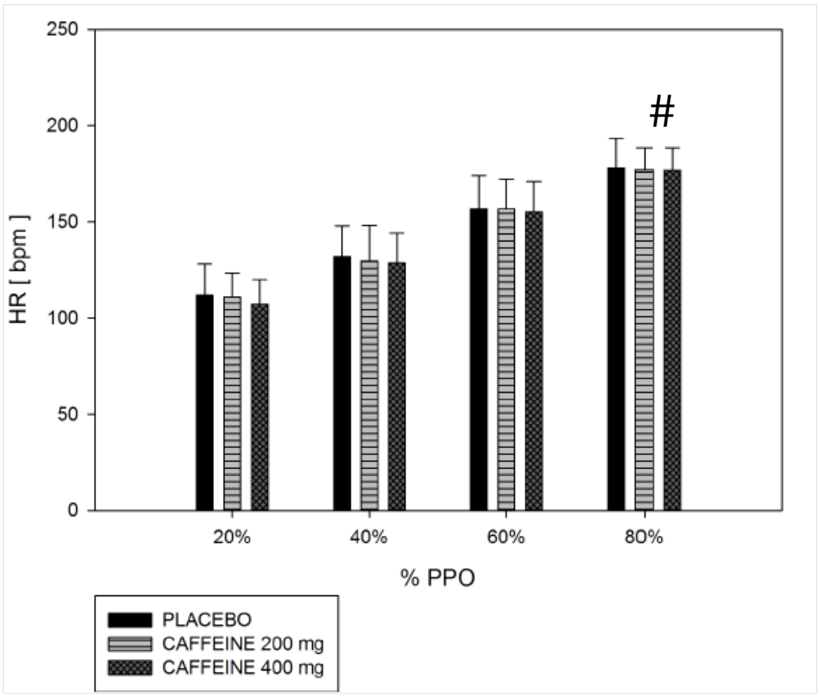


Figure 5 Effect of caffeine on heart rate during sub-maximal aerobic exercise. # Significant main effect of workload (P<0.05, p = 0.000). Data are presented as mean ± SD.

Average VO_2 during the sub-maximal aerobic exercise at each workload did not reveal any interaction with treatments (p = 0.480). VO_2 changed significantly over each workload (main effect of workload, p< 0.05, p = 0.000) (Fig. 6).

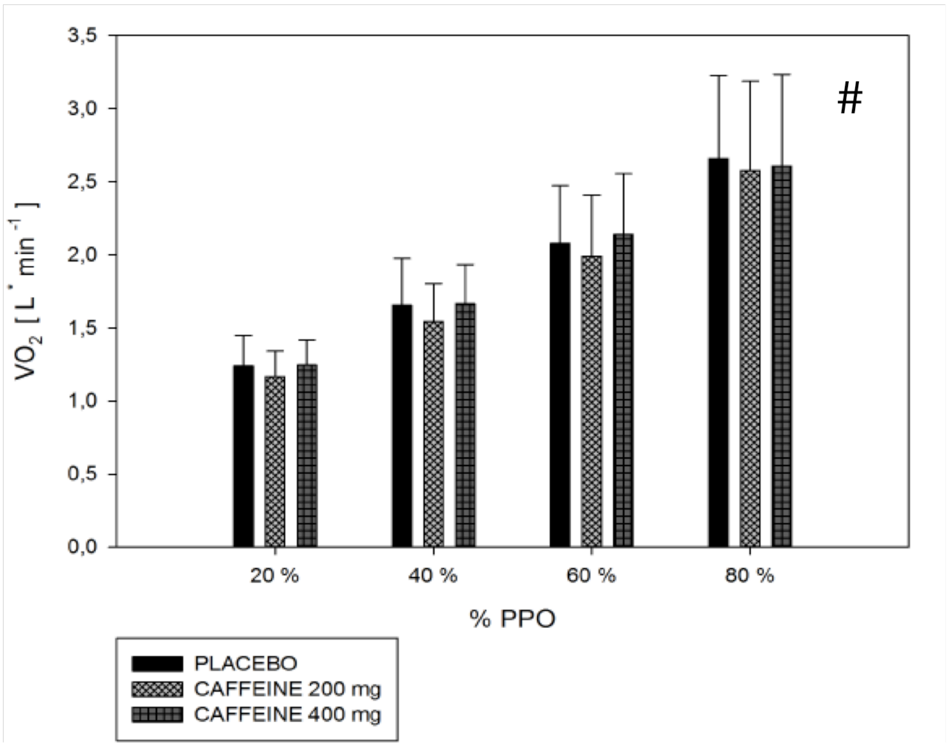


Figure 6 Effect of caffeine on Oxygen consumption during sub-maximal aerobic exercise. # Significant main effect of workload ($P<0.05$, $p = 0.000$) . Data are presented as mean ± SD.

VCO_2 and VE changed significantly over each workload (all main effects of workload $P<0.05$, $p = 0.000$) but there were no significant interaction between the two caffeine dosages and the workloads, respectively ($p = 0.408$) and ($p = 0.388$) (Fig. 7-8).

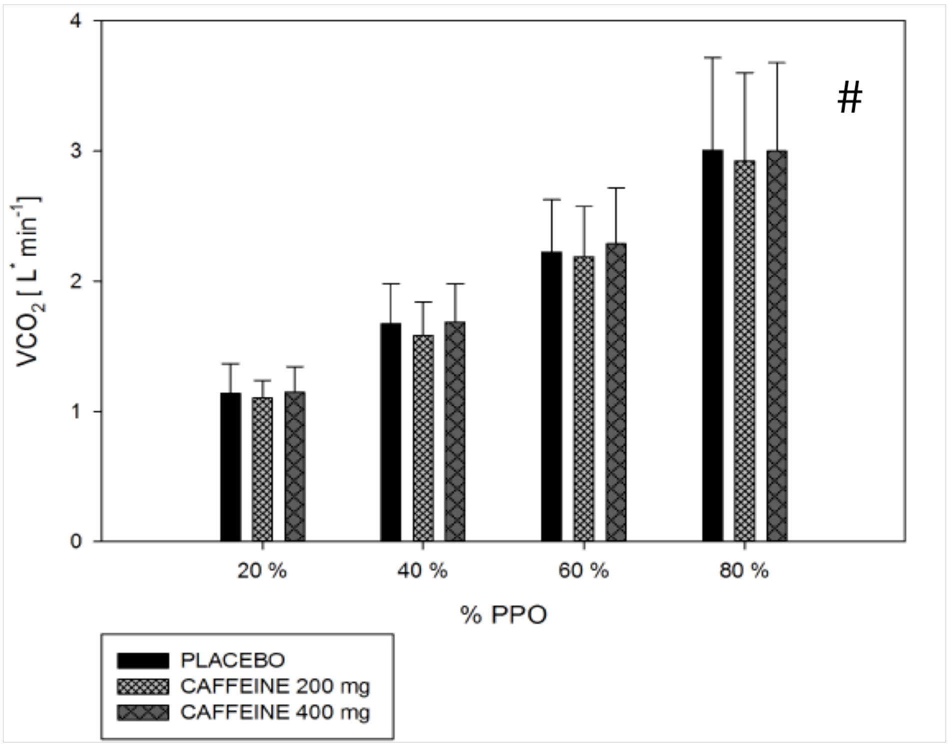


Figure 7 Effect of caffeine on Carbon Dioxide consumption during sub-maximal aerobic exercise. # Significant main effect of workload ($P<0.05$, $p = 0.000$). Data are presented as mean ± SD.

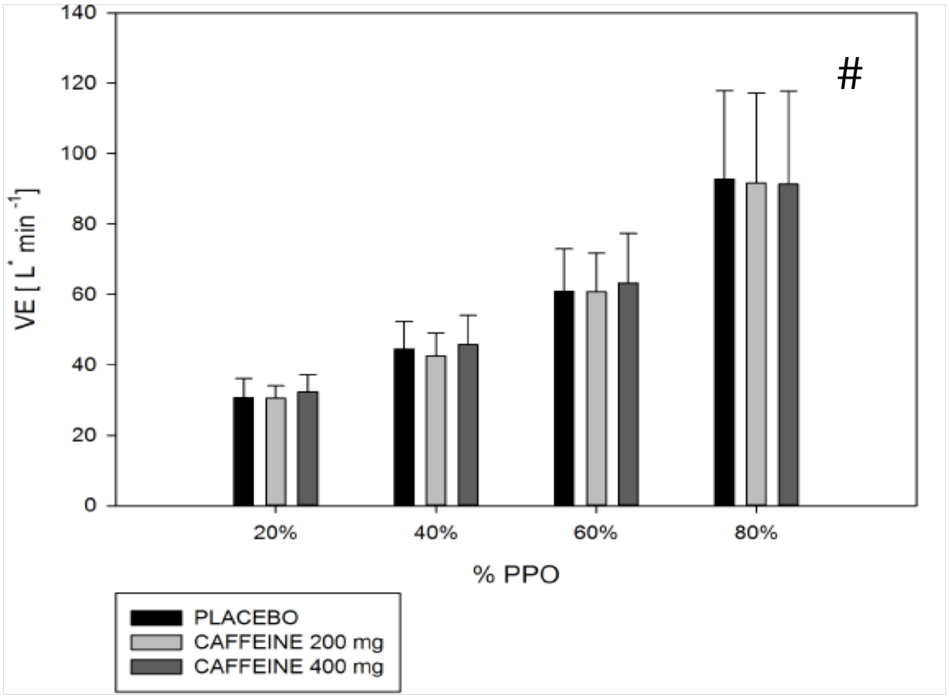


Figure 8 Effect of caffeine on minute ventilation during sub-maximal aerobic exercise. # Significant main effect of workload ($P<0.05$, $p = 0.000$). Data are presented as mean ± SD.

Blood lactate results in the three different conditions presented a main effect of workload ($p < 0.05$, $p = 0.045$) but there was not any interaction between treatments and workloads ($p = 0.390$) (Fig. 9).

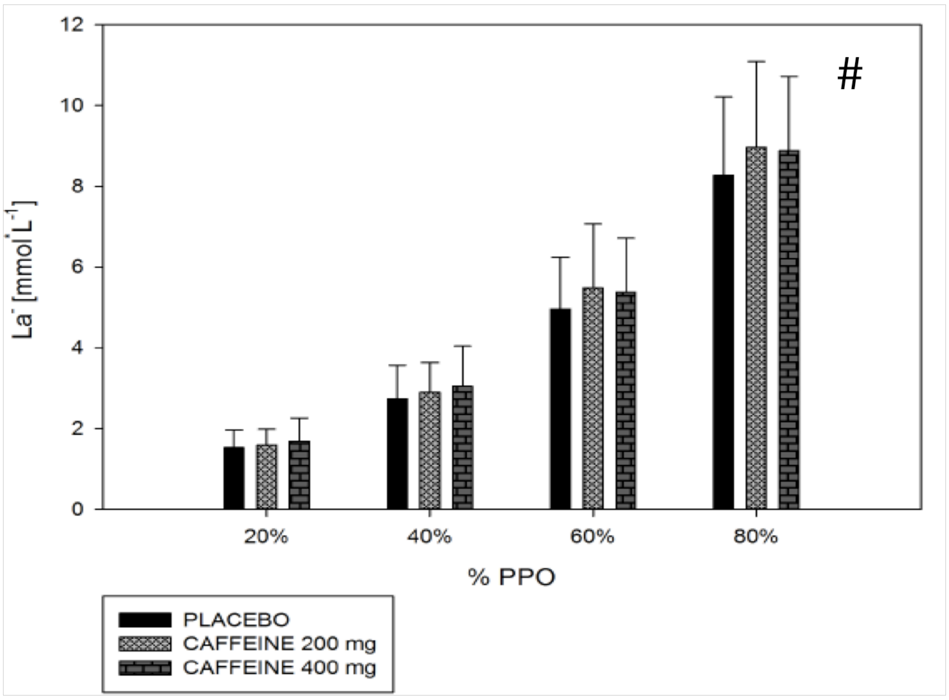


Figure 9 Effect of caffeine on Blood Lactate during sub-maximal aerobic exercise. # Significant main effect of workload ($P<0.05$, $p = 0.045$) . Data are presented as mean ± SD.

Cardiovascular parameters during exercise, i.e. SV, CO, SBP, DBP, did not reveal any interaction between treatment and workload, respectively ($p = 0.284$) for SV, ($p = 0.285$) for CO, ($p = 0.538$) for SBP, and ($p = 0.972$) for DBP. All those parameters, unless for DBP ($p = .194$), revealed a significant main effect of workload ($P< 0.05$) (Fig.10).

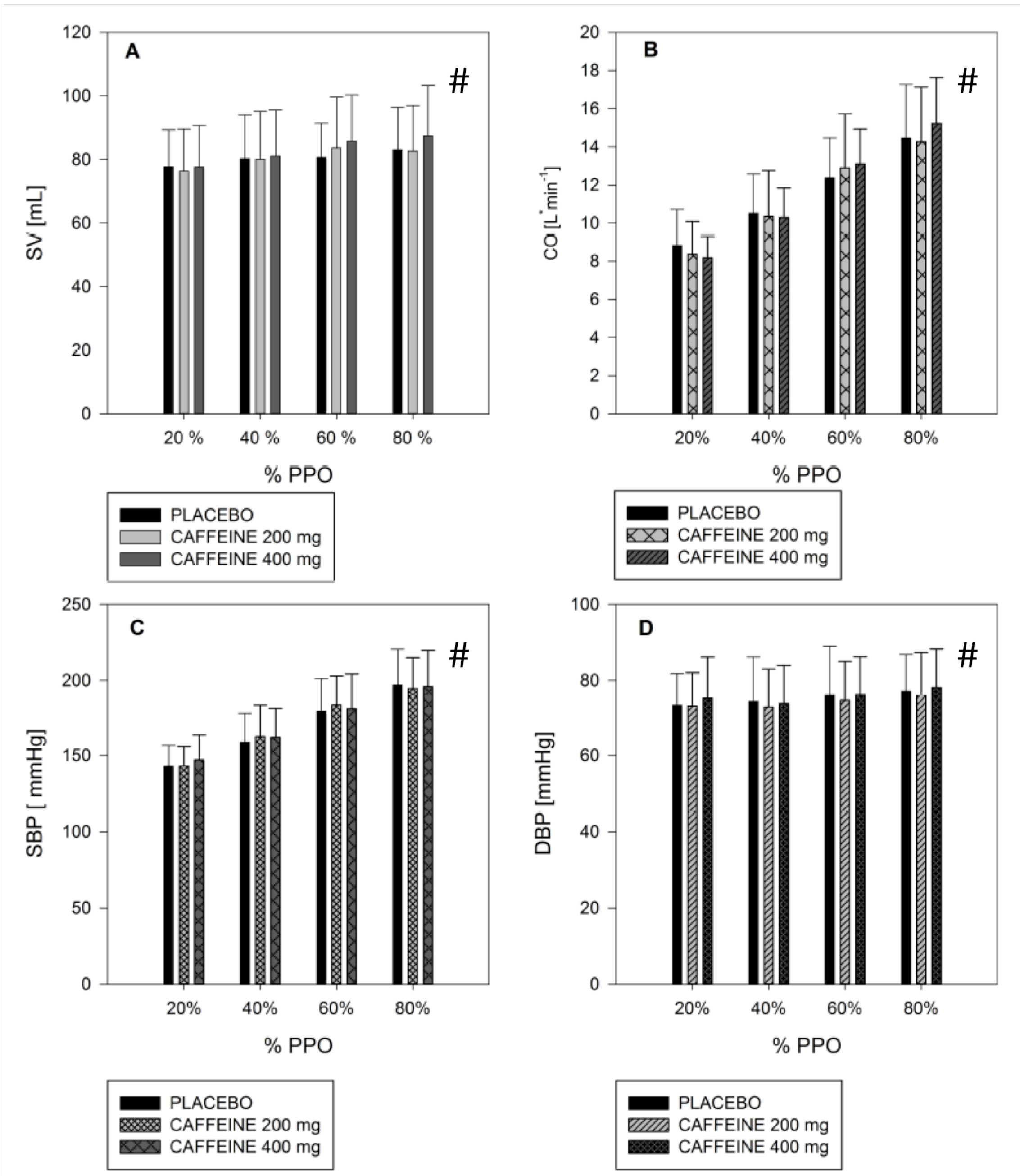


Figure 10 Effects of caffeine on cardiovascular parameters during sub-maximal aerobic exercise. A) Stroke volume. B) Cardiac Output. C) Systolic blood pressure. D) Diastolic blood pressure. # Significant main effect of workload ($P<0.05$, $p = 0.049$; $P<0.05$, $p = 0.001$; $P<0.05$, $p = 0.001$). Data are presented as mean ± SD.

Effects of caffeine on perceptual responses

A significant treatment x workload interaction ($p < 0.05$, $p = 0.006$) was found on RPE at 80 % of PPO. Pairwise comparisons indicated at the overall 0.05 level that caffeine 200 mg (M = 15.66,SD = 1.69) ($p < 0.05$, $p = 0.014$) and 400 mg (M = 15.47,SD = 1.26) ($p < 0.05$, $p = 0.000$) were significantly different in reducing RPE compared to placebo (M = 16.94,SD = 1.94). No significant differences were found between Caffeine 200 and 400 mg ($p = 1.000$) (Fig. 11).

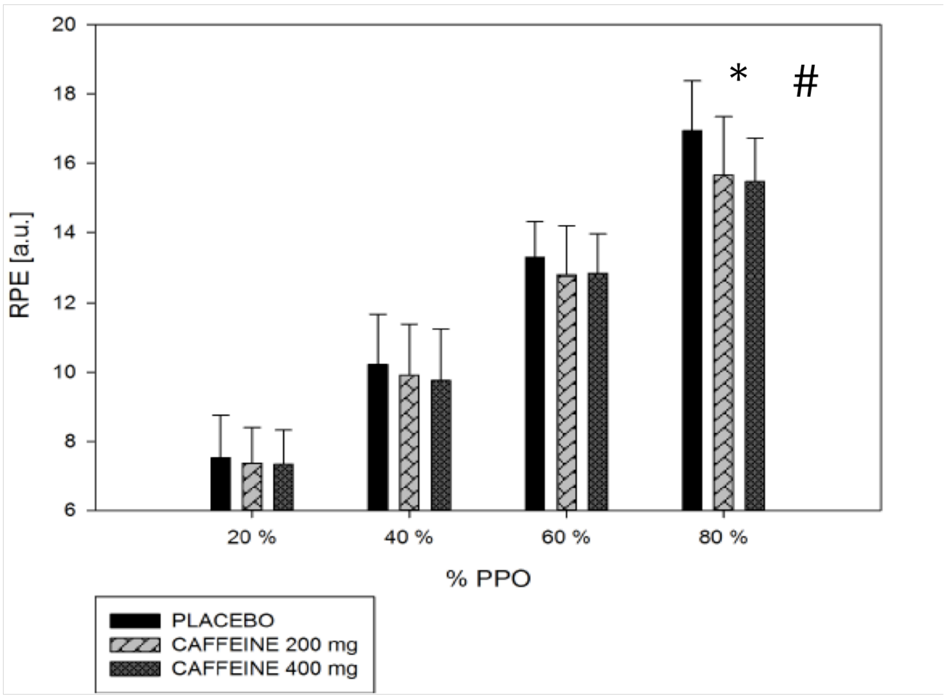


Figure 11 Effect of caffeine on RPE during sub-maximal aerobic exercise. ∗ Significant treatment x workload interaction ($p < 0.05$, $p = 0.006$). # Significant main effect of workload ($P<0.05$, $p = 0.049$; $P<0.05$, $p = 0.001$; $P<0.05$, $p = 0.001$). Data are presented as mean ± SD.

The experienced mood measured using F.S. during the sub-maximal aerobic exercise did not reveal any interaction between treatments and workload (p = .565). Feeling scale results presented both a main effect of workload (P < 0.05, p = 0.003) and treatment (P < 0.05, p = 0.043) (Fig. 12)

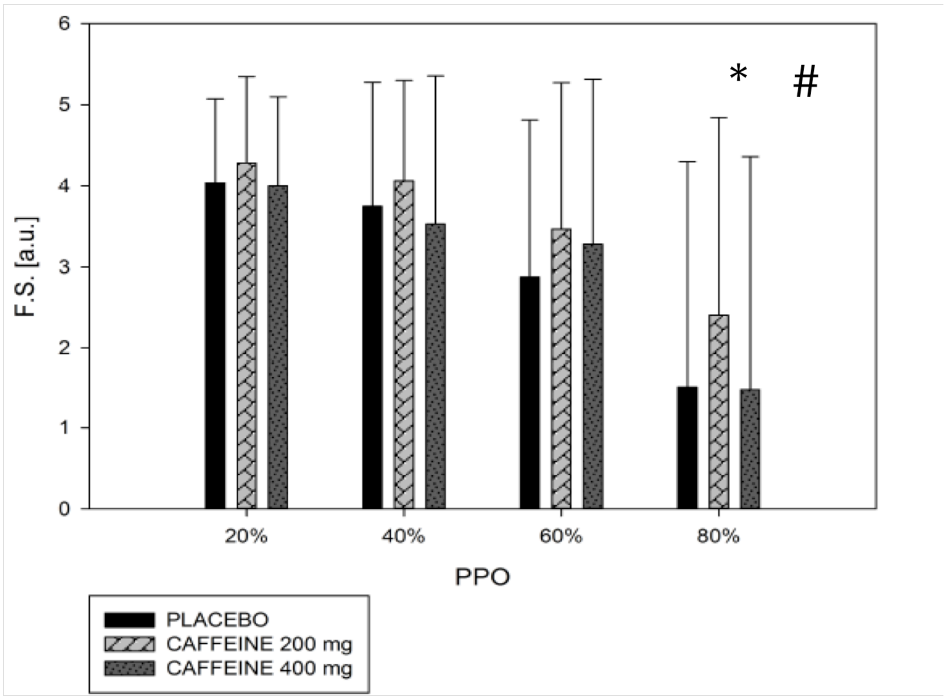


Figure 12 Effect of caffeine on FS during sub-maximal aerobic exercise. ∗ Significant main effect of workload (p< 0.05, p = 0.003). # Significant main effect of treatment (P<0.05, p = 0.043). Data are presented as mean ± SD.

2.5 DISCUSSION

Although the ergogenic effects of caffeine are well established in the athletes population as indicated by Doherty and Smith [40] informations on how caffeine behaves in sedentary individuals still remains a point of interest from a scientific point of view. On the first study we demonstrated how different caffeine dosages had a neutral effect on the cardiovascular parameters measured during the 4 steps sub-maximal aerobic exercise performed by the subjects. This finding was in contrast to some other published studies on this topic [92,93] in which they showed a significant raise of SBP, HR during exercise assuming different amounts of caffeine. Cardiovascular parameters could be interpreted as not influenced by either caffeine or coffee without taking into account the genetic aspects of each individual [66]. Furthermore, VO_2, VCO_2, VE did not reveal significant differences between treatment and placebo. Also this result was in contrast to other published data investigating the effect of caffeine on the metabolic parameters [76] in which they presented a significant increase of VO_2 during the task conducted assuming caffeine. Those results indicate that the glycogen sparing theory cannot be used to explain the peripheral effects of caffeine ingestion, as there are not data supporting any significant difference between caffeine and placebo during different exercise tasks [94]. To support this aspect, the most important finding of the first study was the ability to significantly reduce RPE at 80% of PPO during both 200 and 400 mg of caffeine compared to placebo. To date, on the top of our knowledge, there were not acute studies demonstrating a significant positive effect of caffeine on perception of effort in sedentary individuals. A possible explanation for this phenomenon is related to the central effect of caffeine as indicated by Fredholm et al. [66]. Indeed, caffeine assumption plays an important role in the block of the circulating brain adenosine. More specifically, caffeine acts on adenosine A1 and A2 receptors in order to produce a delay on the onset of the fatigue process by binding on adenosine receptors. This aspect has been recently demonstrated on rats by Davis J.M. et al. [81]. Indeed, they demonstrated that rats assuming a dose of caffeine spent more time on running compared to those that were given placebo. Furthermore, the results on rating of perceived exertion obtained on this study and all the previous informations are in line with the psychobiological model proposed by Marcora S.M. [41]. As stated in his paper, perception of effort seems to be generated from motor to sensory areas of the cerebral cortex. Therefore, we demonstrated the validity of the Brehm's motivational intensity theory based on the ability to reduce the effort necessary to perform a task (.i.e. sub-maximal aerobic exercise). In this study, the increase in central motor command necessary to perform the same task is represented by the increase of perception of effort at the same exercise intensity comparing placebo with the two dosages of caffeine. The results of the first study were of importance as set up and starting point for the caffeine dosages necessary to use in the second one, the clinical trial. Moreover, study I worked as proof of concept because we were able to demonstrate the possibility to reduce RPE using a nutritional strategy as taking caffeine before an exercise session giving also the likelihood to increase exercise adherence if assumed on a regular bases.

2.6 CONCLUSION

We can conclude that this study demonstrated for the first time that 200 mg of caffeine consumed 15 min before sub-maximal high-intensity exercise can reduce RPE in sedentary subjects. From a physiological point of view we demonstrated that the assumption of either 200 mg or 400 mg of caffeine does not represent a risk for a dangerous increases of all the cardio respiratory parameters that we measured. On the basis of this finding and the psychobiological model of exercise, we hypothesize that consumption of caffeine supplements before each training session could increases exercise adherence in sedentary subjects.

CHAPTER 3

EFFECT OF CAFFEINE ON PERCEPTION OF EFFORT AND EXERCISE ADHERENCE IN SEDENTARY ADULTS: A RANDOMIZED CONTROLLED TRIAL

STUDY II

Title: **Effect of caffeine on perception of effort and exercise adherence in sedentary adults: A randomized controlled trial**

3.1 ABSTRACT

It is well known that physical activity improves physical fitness and several health outcomes whether performed on a regular basis, at the right intensity, duration and frequency [10]. As reviewed by Dishman [6] physical activity behaviour is determined by several factors [10,22,88]. Previous studies on exercise adherence have been focused on psychological and behavioural interventions in order to improve this behaviour [11,12,13,14,15,16,17,18]. To date, there are just few studies that used nutritional strategies in order to improve exercise adherence [8,76]. The aim of the study was to implement in a randomized controlled trial a 3 times per week for 12 weeks aerobic training in order to test the hypothesis that an assumption of 200 mg of caffeine before each training session could increase the adherence to a vigorous aerobic training program designed according to the ACSM Guidelines for the improvement and maintenance of fitness [82]. After randomization was asked to each subject to perform a pre-test $VO_{2\ MAX}$ in order to define the PPO [W] and training intensities for the first 6 weeks of training. The same procedures were performed for the mid-test and post-test. Was also asked to each subject to perform a baseline mid and post-test assessment of IPAQ, EMI-2 and BRUMS questionnaires in order to detect any difference on the general level of physical activity during daily activities and on mood and motivation. Adherence parameters (i.e. attendance) were measured at at 6 and 12 weeks of training in order to detect differences between caffeine and placebo group. Subjects visited the gym 3 times/week for 12 weeks.

Results:Attendance to the exercise program were significantly different on the weeks 1 to 6 [CAF (12.92 ± 3.75) PLAC (15.92 ± 2.54) $t(23) = -2.99$, $p = 0.030$],

6 to 12 [CAF (4.00 ± 5.34) PLAC (8.67 ± 5.34) t(23) = -4.67, p = 0.040] and all the 12 weeks of training [CAF(16.92 ± 7.98) PLAC (24.58 ± 6.49) t(23) = -7.66, p = 0.040].The mean percentage of time completed was significantly different on the weeks 1 to 6 [CAF(68.01 ± 19.46) PLAC(85.50 ± 14.19) t(23) = -17.49, p = 0. 018] and from week 1 to 12 [CAF(47.01 ± 22.18) PLAC(68.29 ± 18.02) t(23) = -21.28, p = 0.015]. The comparison of mean total time performed for each training was found significantly different at session 12,15, 17, 21, 22, 33, 35 (P<0,05).PSYCHOLOGICAL QUESTIONNAIRES: Results on BRUMS questionnaire did not reveal any significant treatment x time interaction between caf_pre and plac_pre compared to caf_post and plac_post on ANGER (p = 0.700), CONFUSION (p = 0.976), DEPRESSION (p = 0.807), FATIGUE (p = 0.700), TENSION (p = 0.582) and VIGOUR (p = 0.867). Furthermore, there was not found any main effect of time for each tested variable (P>0,05). The EMI2 results did not show any interaction between the pre – post test comparisons between caf and plac groups on AFFILIATION (p = 0.370), APPEARANCE (p = 0.685), ENJOYMENT (p = 0.643), HEALTH PRESSURE (p = 0.855), HILL HEALTH AVOIDANCE (p = 0.394), NIMBLENESS (p = 0.597), POSITIVE HEALTH (p = 0.227), REVITALISATION (p = 0.324), SOCIAL RECOGNITION (p = 0.364), STRENGTH ENDURANCE (p = 0.696) and WEIGHT MANAGEMENT (p = 0.929). Furthermore, there was a significant main effect of time was found on AFFILIATION, POSITIVE HEALTH (P<0.05), whilst for all the other EMI-2 tested variables was not found any main effect of time (P >0.05). The enjoyment of the exercise measured using the PACES scale did not show any significant interaction between caf and plac on PACES SCALE SCORING (p = 0.794). There was not a significant main effect of time (p = 0.095). The IPAQ results presented a significant treatment x workload interaction on the pre-post comparisons between caf and plac on IPAQ HIGH INTENSITY DAYS (p< 0.05, p = 0.028), IPAQ HIGH INTENSITY MINUTES (P< 0.05, p = 0.014). On the other hand, all the other parameters, like IPAQ MODERATE INTENSITY DAYS (p = 0.374), IPAQ MODERATE

INTENSITY MINUTES (p = 0.594), IPAQ WALKING DAYS (p = 0.647), IPAQ WALKING MINUTES (p = 0.898) and IPAQ SEATED MINUTES (p = 0.898) did not present any significant interaction (P>0.05). Main effect of time was found significantly different for IPAQ HIGH INTENSITY DAYS, IPAQ HIGH INTENSITY MINUTES (P<0.05) PHYSIOLOGICAL PARAMETERS: The pre-post test analysis of the PPO did not show a significant treatment x workload interaction between caf and plac (p = 0.683). There was a significant main effect of time (P<0.05) (p = 0.003). Moreover, the ANOVAs performed on VO_2 PEAK and HR pre-post test results did not reveal a significant treatment x workload interaction (VO_2 PEAK (p = 0.462), HR (p = 0.856)). Furthermore, a significant main effect of time for HR was found (p< 0.05, p = 0.001). The data analysis of RPE pre-post data comparing caf and plac groups, did not reveal a significant difference accounted for the effort perceived during each training sessions (p = 0.487). A significant main effect of workload (p< 0.05, p = 0.001) was found. All the data are presented by mean ± SD.

Conclusions: This study demonstrated that there is not any effect of caffeine on perception of effort and adherence improvement in sedentary subjects. From a physiological point of view, due to a reduction in total time of training and percentage of total training completed we do not have demonstrated an improvement in aerobic fitness. On the other hand, adherence for the placebo group is resulted significantly different compared to caffeine.

3.2 INTRODUCTION

Physical activity and an overall active lifestyle are important issues in the reduction of the likelihood related to chronic illness, like type 2 diabetes, cancers and cardiovascular diseases. As indicated by ACSM in their position stand [4] for quantity and quality of exercise for the improvement and maintenance of cardiorespiratory fitness, randomized clinical trials of exercise training and meta-analyses contributed evidence for the causal effects of exercise training for improving physical fitness and biomarkers of chronic disease. Nevertheless the effort of introducing new short bouts high intensities training strategies, a lot of studies and guidelines are based on a downward shift of training intensities in order to try to improve exercise adherence in sedentary individuals [4,30,31,89]. As indicated by Dishman et al. [6], this aspect is of importance in the physical activity domain. A popular theory recently published indicates that the negative effect of high exercise intensity on exercise adherence [22,87] is mediated by the relationship between exercise intensity and affective responses (low and moderate intensity exercise = positive affect; high intensity exercise = negative affective). Therefore, it has also been suggested that people should self-select their own exercise intensity to elicit a positive affective response during exercise [22]. However, to date, there is no evidence that this behavioural intervention improves exercise adherence.

Another mediator of the negative effect of high exercise intensity on exercise adherence is perception of effort [6]. Therefore, an intervention that reduces perception of effort would be likely to increase exercise adherence in previously sedentary people. The easiest way to reduce perception of effort is to reduce exercise intensity. Indeed, most guidelines on physical activity recommend moderate intensity exercise [15,21,23]. Although it is true that moderate intensity exercise confers significant health benefits, vigorous exercise improves health and physical fitness even further [27,28,29]. Therefore, an intervention that reduces perception of effort without reducing exercise intensity would be highly desirable because it may improve adherence to exercise training without reducing its positive effects on health and physical fitness.

One strategy concerns a concentrated dose of caffeine before each training session. Indeed, several studies have shown that pre-exercise caffeine supplementation significantly reduces perception of effort [39,40,79]. Moreover, these studies have included only subjects with a VO_{2max} above 45 $ml^{*}O_2^{*}min^{-1}$, and there is some evidence that the effect of caffeine on perception of effort is positively correlated with VO_{2max}. At the moment, there is a lack of informations on how caffeine behaves in a middle term high intensity aerobic training program with sedentary individuals. Thus, the primary aim of this study was to analyze the effect of caffeine on adherence, physiological and perceptual responses on a 3 d/wk aerobic training program.

3.3 METHODS

Subjects and Ethical issues

Twenty five eligible subjects, 13 addressed in the caffeine group [mean ± SD, age 37.62 ± 12.62 yr, height 1.72 ± 0.12 m, weight 74.48 ± 9.69 kg, peak oxygen uptake (VO_{2peak}) 32.20 ± 6.73 ml · kg^{-1} · min^{-1}, peak power output (PPO) 226.92 ± 74.63 W] and 12 addressed in the placebo group [mean ± SD, age 43.92 ± 10.73 yr, height 1.63 ± 0.04 m, weight 63.76 ± 5.15 kg, peak oxygen uptake (VO_{2peak}) 30.13±3.62 ml · kg^{-1} · min^{-1}, peak power output (PPO) 179.17 ± 27.86W] signed an informed consent form describing the study protocol. The eligibility criteria were being between 18 and 45 for men, and from 18 to 55 for women, being no involved in aerobic activities for more than 3 times per week in the past 3 months, being free of any know illness condition and with a VO_{2peak} below "GOOD" according the Heyward cardiorespiratory fitness classification [27]. All subjects were informed and given written instructions describing all procedures related to the study. Participants believed that the study was conducted on the effect of two different psycho stimulants on the physiological responses during a 12 weeks cycle-ergometer aerobic program for the improvement of their fitness. At the end of the last visit, subjects were clarified on the real aims of the study, thanked for their participations and they were also asked to not talk to the other subjects the real aims of the research.

Study design and procedures

In this study we employed a double - blind, randomized controlled trial design. Subjects visited the laboratory and the training centre 39 times, scheduled in 3 times per week for a total duration of 12 weeks. More precisely, the number of the scheduled training sessions were 36, with a pre, mid, and post test visits. During the pre-test visit were asked to participants to complete the International

Physical Activity Questionnaires (IPAQ) [82] to ensure the absence of any regular physical exercise in the last three months. Moreover, subjects were asked to complete several psychological questionnaires. Specifically, we asked to filled in the EMI-2 questionnaire [90] in order to define their participation motives and willingness to be adherent to a middle term physical activity program. Moreover, we also asked to the subjects to complete the BRUMS questionnaire [91], a useful tool used for the analysis of the mood states. A caffeine questionnaire was also administered in order to obtain informations about the frequency and quantity of any beverage and food containing caffeine in the last three months. Participants were also asked to perform an incremental exercise test (3 min at 50 W + 25 W every 1 min with a pedal frequency from 60 to 100 revolutions/min) in order to determine $VO_{2\ peak}$ and Peak Power Output (PPO) calculated according to the equation of Kuipers et al.[83] on an electromagnetically-braked cycle ergometer (Lode, Groningen, The Netherlands). Tidal volume (V_T), breathing frequency (B_F), minute ventilation (VE), oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were measured using a breath-by-breath gas Analyzer (Metalyzer, Cortex, Netherlands) connected to an oro–(mouth) mask. The highest VO_2 value measured during the test was considered maximal (VO_{2peak}) when any two of the following criteria were met: a respiratory exchange ratio ≥ 1.15; a maximal heart rate higher than the 90% of the subject's age-predicted maximal heart rate; a plateau in the VO_2 with an increase in work rate; leg or chest RPE higher than 18 on the 15 point Borg Scale. The cycle ergometer was regularly checked for accuracy of power output and cadence. Prior to the incremental test the ergometer was adjusted for each subject. Settings were recorded to allow the reproduction in the subsequent visits. Subjects were familiarized and received instructions for the rating of perceived exertion (RPE) using the 15-point scale developed by Borg (Borg, 1998). PPO found during the pre and mid tests visit were after used for the training intensities definitions (see fig. 13).

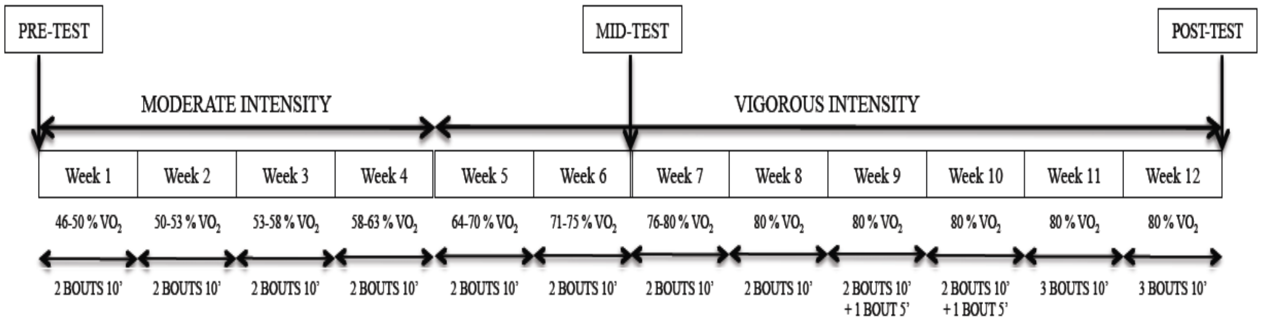


Figure 13. Schematic representation of the training protocol. Testing time points, relative intensities and volume of training are represented.

After randomization (www.randomization.com), subjects taken either a 240 mg caffeine based pills or a placebo pill 1 hour before each training session. Prior to the testing session participant informed consent and informations were provided in order to refrain from caffeine, nicotine, alcohol and any medication in the 3 h prior each training session, as well as to avoid exercise in the 24 h prior the scheduled training [84,85]. At each visit subjects were asked to complete a pre-test checklist to ascertain that they had complied with the instructions given to them. Briefly, the structure of the training program was setup in different phases due to the poor fitness level of the subjects. In fact, the total training volume of each training session was completed in two or more than two bouts interspersed with a passive recovery. We decided to use this strategy also for the other two training periods in order to help subjects coping with the task [27,4] (see training program characteristics for details). Each training session consisted in either 2 or 3 bouts with a defined duration based on the number of the training week. More precisely, each subject was asked to pedal 10 min at a defined percentage of their PPO obtained during the first visit. Each bout was interspersed with a rest of 3 min. HR was continuously measured for the entire duration of each bout. Perceptual responses to the exercise, i.e. RPE, were measured during the last 15 seconds of the 1st, 5th and 10th minute of each bout (see Physiological and perceptual responses to exercise for details). Each subject completed all the training sessions with a time recovery in between of at least 48 h. Environmental conditions during the laboratory were kept constant between 18 and 22 °C for the temperature and 45 and 60 % for humidity.

Treatment

Experimental treatment.

Subjects involved in the study were deceived and they were informed of a testing of the effects of two different psychostimulants on physiological responses during an aerobic training program in order to avoid any changes in perceptual responses due to the different composition of the treatment. Every visit, they were asked to take either a caffeine based pill or a placebo pill. At the end of the data collection subjects were informed of the real nature of the aims of the study and the composition of the treatments. The pills were assumed 60 minutes before each training session. Than, subjects performed the training program scheduled for each visit. The rest interspersed within each bout was 3 minute. *Caffeina 240* ® is a commercial available caffeine-based pill. Its caffeine concentration is 240 mg for any piece. We used a maximal caffeine concentration of 3 $mg*kg^{-1}$. As shown in several studies [40,41,66] subjects rested for 60 minutes after the assumption of the pill and warm up. The reason is the required time that is needed to achieve the maximum caffeine concentration using the pill formulation [40,41]. We decided to use this dosage of caffeine based on studies that demonstrated the absence of any side effect in healthy subjects who assumed caffeine in a range from 3 to 10 $mg*kg^{-1}$ [78].

Control treatment. Control treatment consisted of performing the same protocol as indicated in the "experimental treatment" section. The main difference was related to the pills that subjects were asked to take. They assumed a normal pill with a similar taste compared to caffeine-based pills.

Physiological and perceptual responses

HR was measured every 5 seconds for the entire duration of each bout, using a heart rate monitor (Polar S610i, Polar Electro Oy, Kempele, Finland). Minute ventilation (VE), oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were measured using a breath-by-breath gas Analyzer (Metalyzer, Cortex, Netherlands) connected to an oro–(mouth) mask. This device was calibrated before each test using certified gases of known concentration (11.5% O2 and 5.1% CO2) and a 3.0-liter calibration syringe (series 5530, Hans Rudolph). The measurement of these parameters was performed at pre and post-test. During the last 15 seconds of the 1st, 5th and 10th minute of exercise, subjects were asked to rate their perception of effort using the 15-point Borg Scale [42].

Psychological questionnaires

Mood. Subjects were asked to complete a modified version of the Brunel mood Scale (BRUMS) in order to measure mood states [91]. This questionnaire is based on the Profile of Mood States, contains 24 items divided into six respective sub-scales: anger, confusion, depression, fatigue, tension, and vigor. The items are answered on a 5-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely) and each subacale, with four relevant items, can achieve a row score in the range from 0 to 16.

Participation motives. Willingness to be adherent with the proposed program and exercise motives participation to the sub-maximal aerobic program was measured using the exercise inventory motivation questionnaire developed and validated by Markland et al. [90]. This questionnaire contains 51 items that are to be answered on a 6-point Likert scale (0 = not all true for me, to 5 = very true for me). Therefore, the total scale scores for this questionnaire are based on the means of the appropriate items related to its 14 subscales.

Statistical analysis.

All data are presented as mean ± SD. The effects of treatment on adherence parameters (number of training sessions attended, total time completed for each session, total time completed compared to total time requested by the training program) were tested using unpaired t-test between groups. The effect of treatment and the time of testing on outcome measure parameters (VO_2 – PPO – EMI_2 – IPAQ – BRUMS) were tested by using 2 x 2 mixed ANOVAs. The effects of treatment on physiological (HR) and perceptual responses (RPE) were tested using a 2 x 12 mixed ANOVAs. Due to the high rate of subjects that dropped out we were only able to perform an intention to treat analysis using the so-called “last carry forward method”.

3.4 RESULTS

Effect of caffeine on adherence

As indicated in figure 14 mean attendance on the first 6 weeks of training comparing CAFFEINE (M = 12.92, SD = 3.75, N = 13) and PLACEBO (M = 15.92, SD = 2.54, N = 12) was significantly different t(23) = -2.99, p = 0.030. The mean attendance related to weeks 7 to 12 comparing CAFFEINE (M = 4.00, SD = 5.34, N = 13) and PLACEBO (M = 8.67, SD = 5.34, N = 12) was significantly different t(23) = -4.67 , p = 0.040. The 12 weeks mean attendance comparing CAFFEINE (M = 16.92, SD = 7.98, N = 13) and PLACEBO (M = 24.58, SD = 6.49, N = 12) resulted significantly different t(23) = -7.66, p = 0.040. Data are presented by mean ± SD.

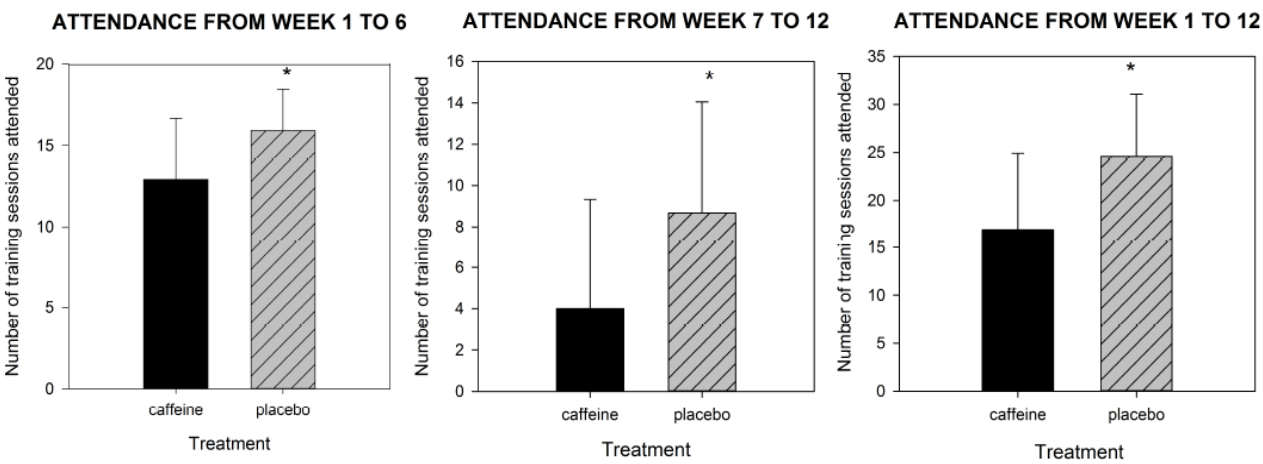


Figure 14 Effect of caffeine on attendance from weeks 1 to 12. ∗ Significant difference (P<0.05). Data are presented as mean ± SD.

The comparisons of the mean percentage of exercise prescribed completed is indicated in figure 15. Indeed, in the first 6 weeks of training the mean percentage of time completed comparing CAFFEINE (M = 68.01, SD = 19.46, N = 13) and PLACEBO (M = 85.50, SD = 14.19, N = 12) was significantly different t(23) = -17.49, p = 0. 018. Comparisons of the mean percentage of time completed during the weeks 7 to 12 comparing CAFFEINE (M = 14.23, SD = 19.40, N = 13) and PLACEBO (M = 28.83, SD = 20.12, N = 12) was not significantly different t(23) = -14.60, p = 0.078 . As indicated in fig. 12 C, the comparison between CAFFEINE (M = 47.01, SD = 22.18, N = 13) and PLACEBO (M = 68.29, SD = 18.02, N = 12) on the mean percentage of exercise prescribed completed between the week 1 and 12 was significantly different t(23) = -21.28, p = 0.015. Data are presented by mean ± SD.

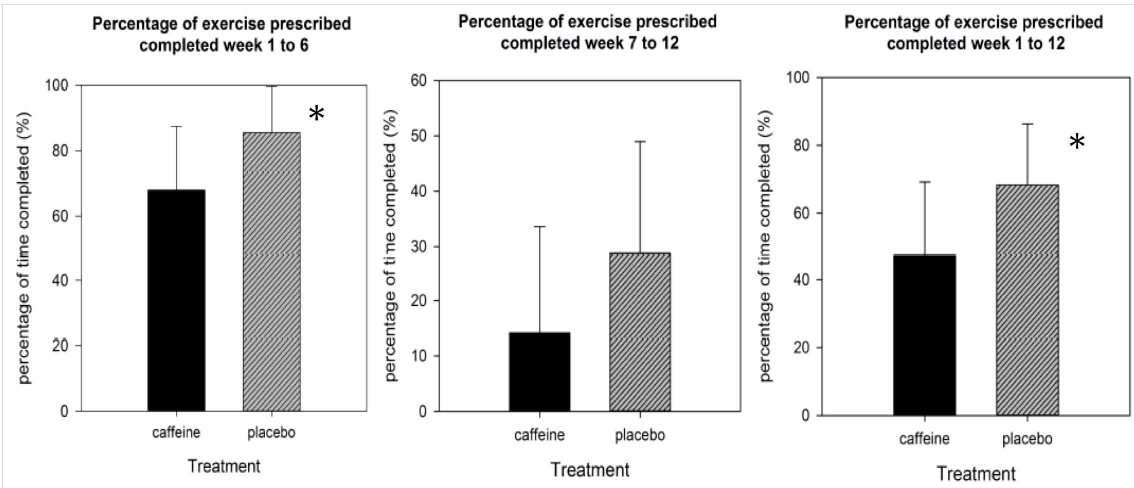


Figure 15. Effect of caffeine on the percentage of exercise prescribed completed from weeks 1 to 12. * significant difference (P<0.05)Data are presented as mean ± SD.

The comparison between caf and plac on the total time completed for each training session was found significantly different on Session 12 CAF (6.15 ± 9.71) PLAC (15.00 ± 9.05) t(23) = -8.846, p = 0.027, session 15 CAF (3.46 ± 7.47) PLAC (11.00 ± 9.94) t(23) = -7.538, p = 0.042, session 17 CAF (7.38 ± 8.86) PLAC (15.00 ± 7.85) t(23) = -7.615, p = 0.033, session 21 CAF (10.77 ± 10.377) PLAC (18.33 ± 5.774) t(23) = -7.564, p = 0.036, session 22 CAF (5.62 ± 7.29) PLAC (11.33 ± 5.82) t(23) = -5.718, p = 0.042, session 33 CAF (0.81 ± 5.46) PLAC (5.33 ± 8.77) t(23) = -4.521, p = 0.027, session 35 CAF (1.00 ± 3.61) PLAC (6.33 ± 7.90) t(23) = -5.333, p = 0.027 (Fig. 16).

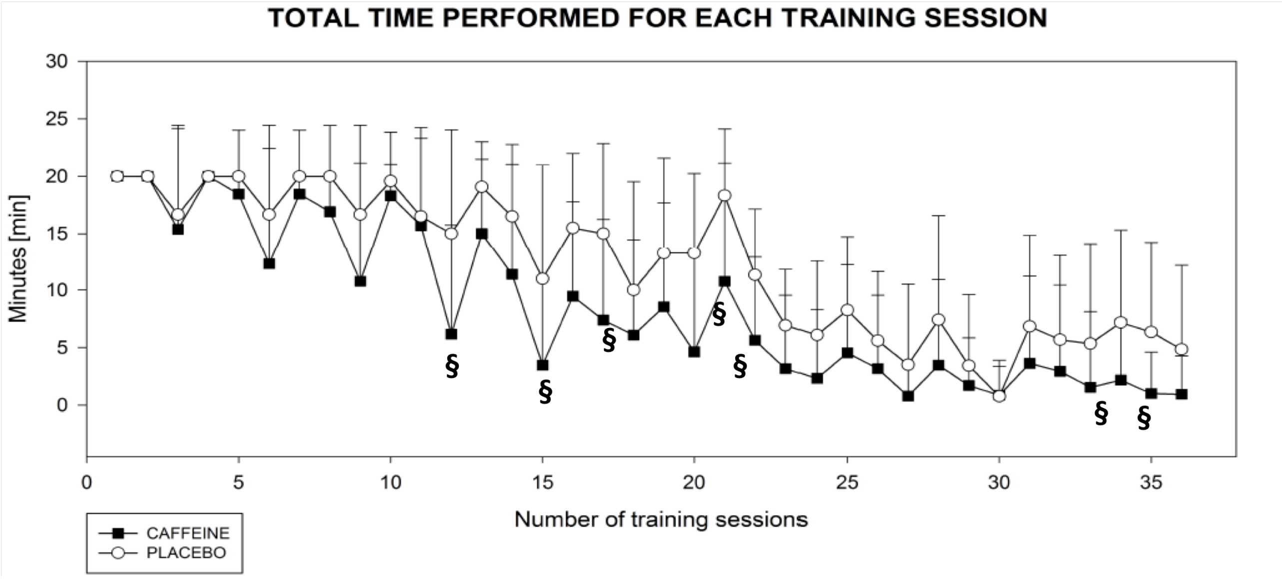
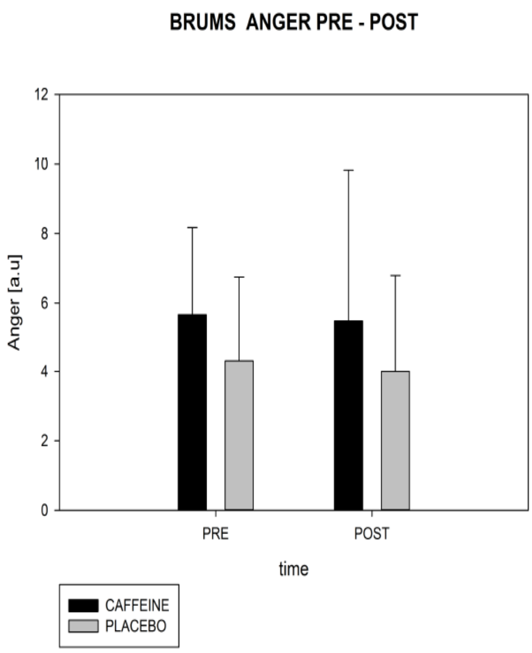


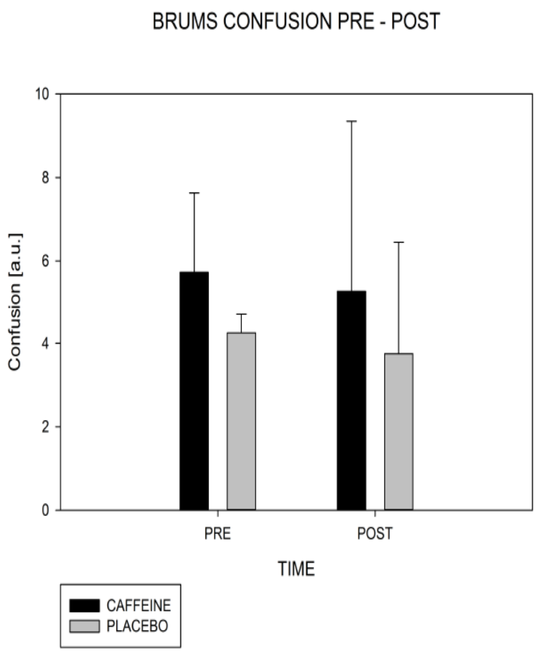
Figure 16. Effect of caffeine on the total time performed for each training session. § Mean total time performed for each training was significantly different. Data are presented by mean ± SD.

Effect of caffeine on psychological questionnaires

Results on BRUMS questionnaire did not reveal any significant treatment x time interaction between caf_pre (5.64 ± 2.54) plac_pre (4.33 ± .888) and caf_post (5.45 ± 4.37) (N=13) plac_post (4.00 ± 2 .80) on ANGER (p = 0.700) There was not significant main effect of time (p = 0.910). There were not significant treatment x time interaction between caf_pre (5.73 ± 1.90) plac_pre (4.25 ± .872) and caf_post (5.27 ± 4.08) plac_post (3.75 ± 2.72) on CONFUSION (p = 0.976) There was not significant main effect of time (p = 0.527). There was not significant treatment x time interaction between caf_pre (5.09 ± 2.21) plac_pre (4.33 ± .461) and caf_post (5.27 ± 3.95) plac_post (3.83 ± 2.86) on DEPRESSION (p = 0.807). There was not significant main effect of time (p = 0.601). There was not significant treatment x time interaction between caf_pre (6.45 ± 2.84) plac_pre (5.08 ± 1.24) and caf_post (7.64 ± 5.22) plac_post (4.67 ± 3.75) on FATIGUE (p = 0.700) There was not significant main effect of time (p = 0.910). There was not significant treatment x time interaction between caf_pre

(5.73 ± 1.90) plac_pre (5.33 ± 1.30) and caf_post (4.82 ± 3.17) (N=13) plac_post (3.67 ± 2.50) on TENSION (p = 0.582) There was not significant main effect of time (p = 0.071). There was not significant treatment x time interaction between caf_pre (10.82 ± 1.89) plac_pre (11.42 ± 2.23) and caf_post (8.55 ± 5.41) (N=13) plac_post (8.67 ± 6.47) on VIGOUR (p = 0.867) There was not significant main effect of time (p = 0.089) (Fig.17). Moreover, none of the six mood dimensions presented any main effect (P>0.05).





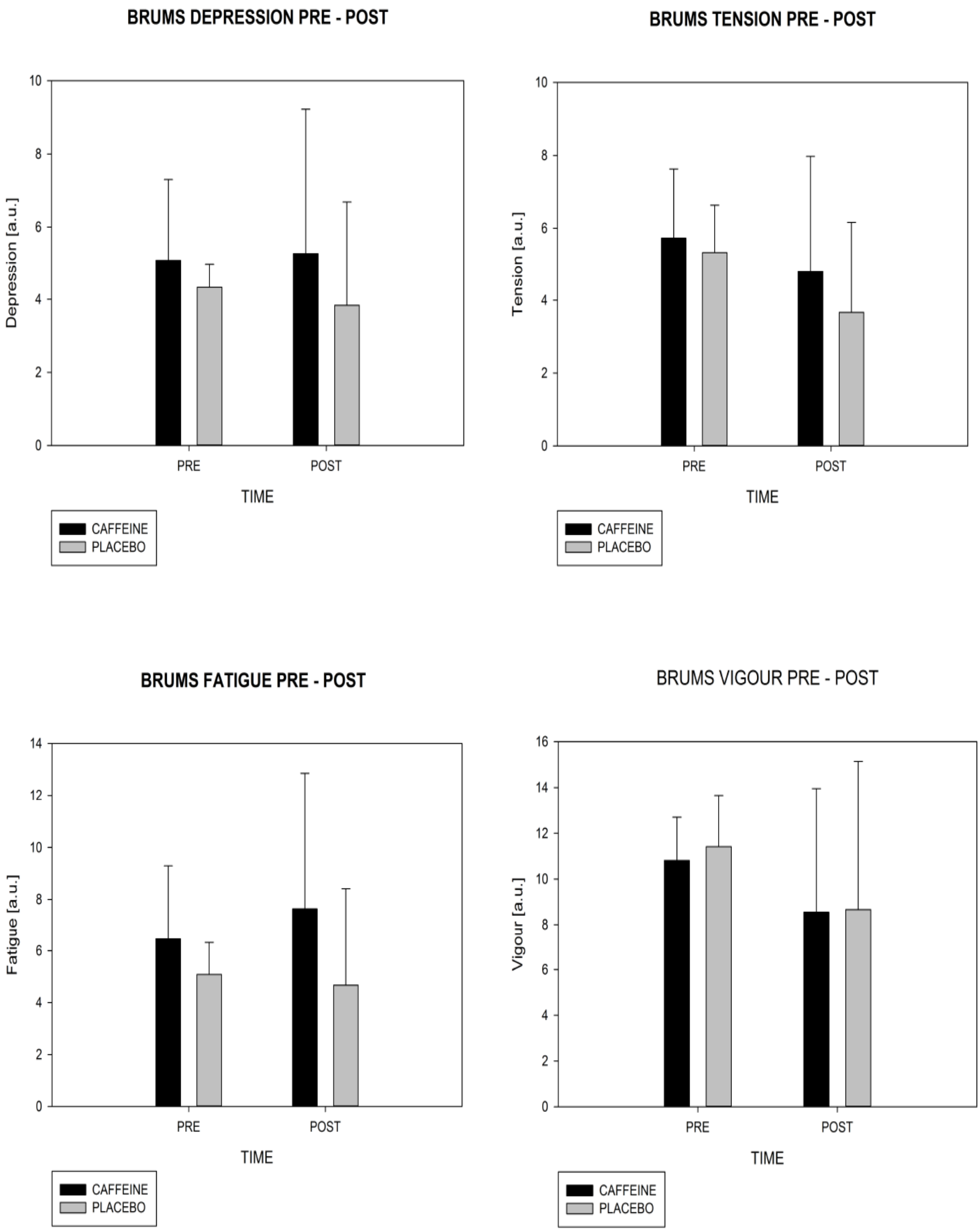
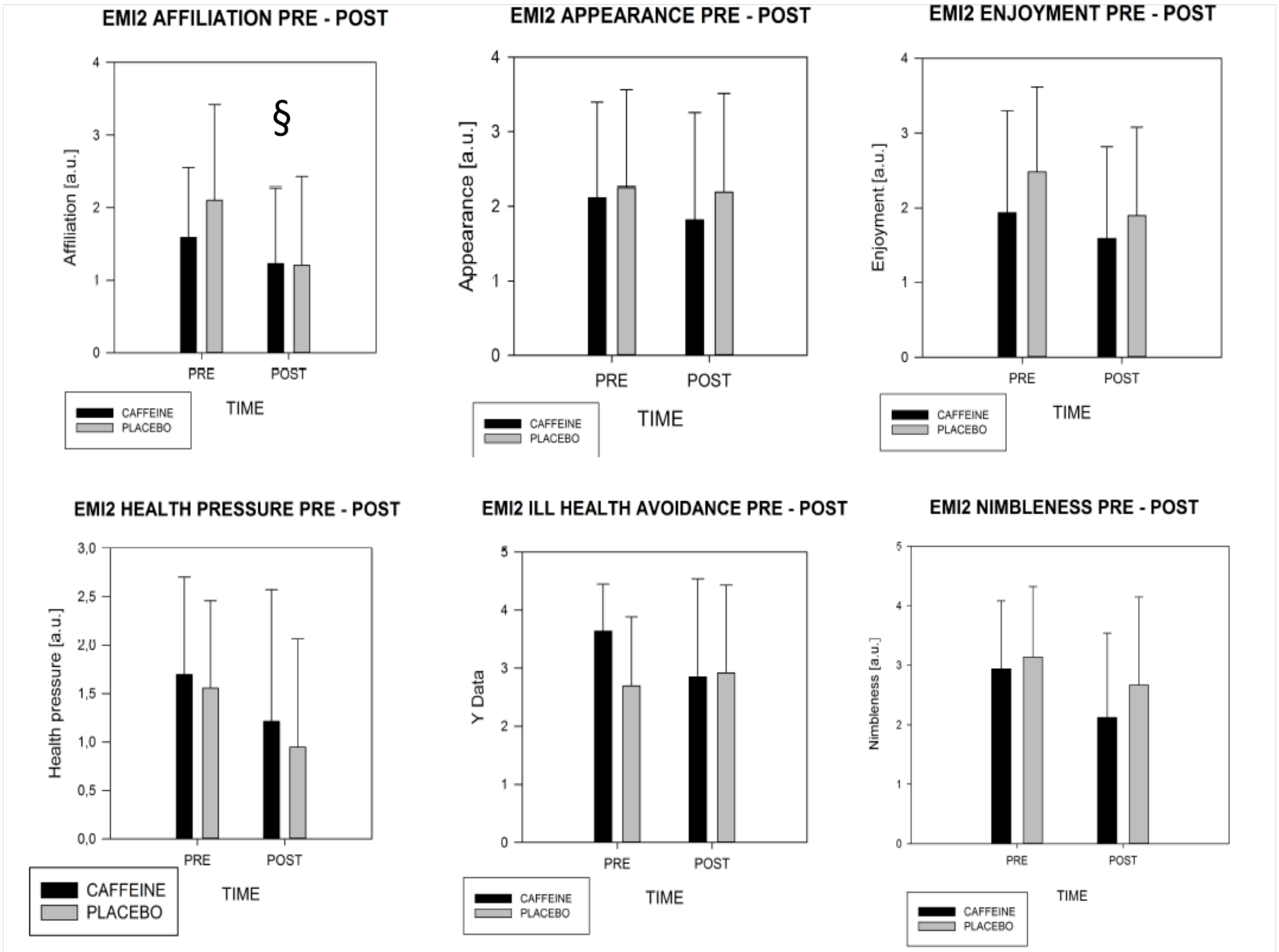


Figure 17. Effect of caffeine on pre-post test Brums questionnaire..A) Anger B) Confusion C) Depression D) Tension E) Fatigue F) Vigour.

The analysis of the exercise motives participation performed by using the EMI2 questionnaire revealed that there were not any interaction in the pre-post test on affiliation caf_pre (1.59 ± .96) plac_pre (2.10 ± 1.32) and caf_post (1.23 ± 1.04) plac_post (1.20 ±1.22) ($p = 0.370$), appearance caffeine_pre (2.11 ± 1.28) plac_pre (2.25 ± 1.31) and caf_post (1.82 ± 1.44) plac_post (2.18 ± 1 .82) ($p = 0.685$), enjoyment caf_pre (2.11 ± 1.28) plac_pre (2.25 ± 1.31) (N=12) and caf_post (1.82 ± 1.44) plac_post (2.18 ± 1 .82) ($p = 0.643$), health pressure caf_pre (2.11 ± 1.28) plac_pre (2.25 ± 1.31) and caf_post (1.82 ± 1.44) plac_post (2.18 ± 1 .82) ($p = 0.855$), hill health avoidance caf_pre (3.64 ± .81) plac_pre (2.69 ± 1.18) and caf_post (2.85 ± 1.69) plac_post (2.92 ± 1.51) ($p = 0.394$), nimbleness caf_pre (2.94 ± 1.14) plac_pre (3.14 ± 1.18) and caffeine_post (2.12 ± 1.42) plac_post (2.66 ± 1 .48) ($p = 0.597$), positive health caf_pre (3.88 ± .934) plac_pre (3.53 ± 1.04) and caf_post (2.72 ± 1.84) plac_post (3.11 ± 1.35) ($p = 0.227$), revitalisation caf_pre (3.15 ± 0.77) plac_pre (3.27 ± 1.13) and caf_post (2.45 ± 1.65) plac_post (3.25 ± 1 .42) ($p = 0.324$), social recognition caf_pre (1.20 ± 1.21) plac_pre (0.85 ± 0.92) and caf_post (0.86 ± 0.93) plac_post (0.75 ± 0.72) ($p = 0.364$), strength and endurance caf_pre (2.61 ± 1.31) plac_pre (2.35 ± 1.27) and caf_post (2.11 ± 1.49) plac_post (2.10 ± 1.33) ($p = 0.696$), weight management caf_pre (2.77 ± 1.24) plac_pre (3.29 ± 1.08) and caf_post (2.18 ± 1.35) plac_post (2.65 ± 1.31) ($p = 0.929$), challenge caf_pre (2.25 ± 1.87) plac_pre (1.92 ± 1.34) and caf_post (1.75 ± 1.65) plac_post (2.22 ± 1.79) ($p = 0.481$), competition caf_pre (1.32 ± 1.28) plac_pre (0.72 ± .71) and caf_post (0.85 ± 0.94) plac_post (0.49 ± 0.76) ($p = 0,442$) and stress management caf_pre (2.65 ± 0.88) plac_pre (1.86 ± 1.24) and caf_post (2.93 ± 1.15) plac_post (2.22 ± 1.34) ($p = 0.819$). Affiliation, positive health, competition and stress management significantly changed over time ($P < 0.05$) (Fig. 18).


EMI2 AFFILIATION PRE - POST
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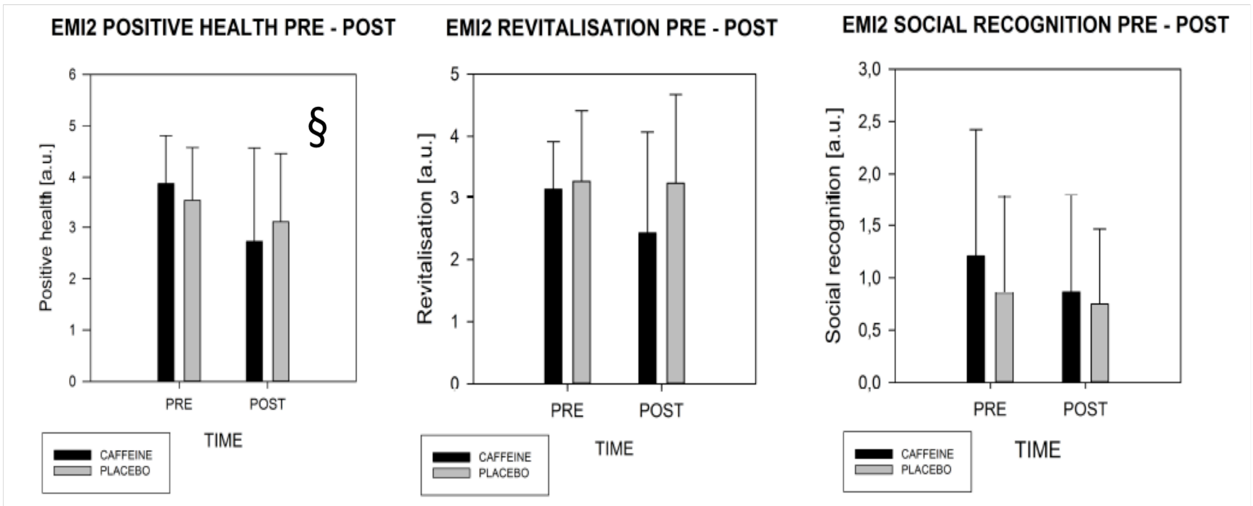
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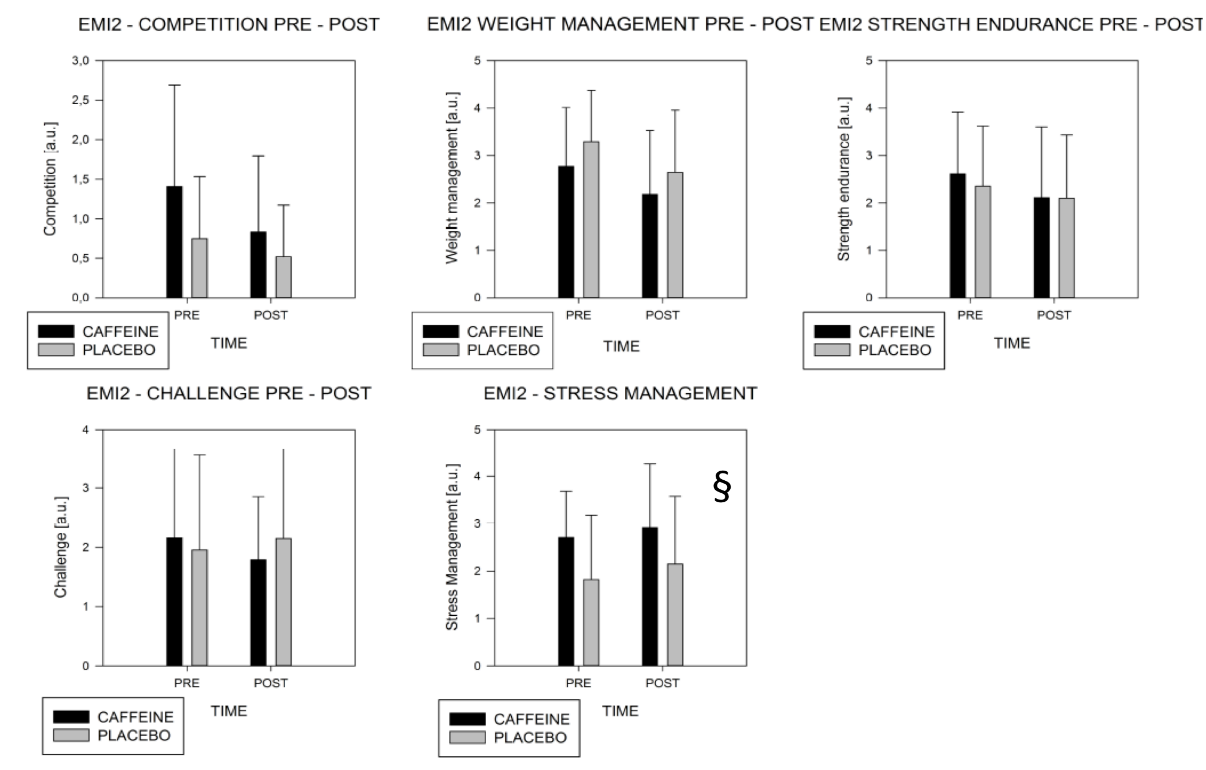
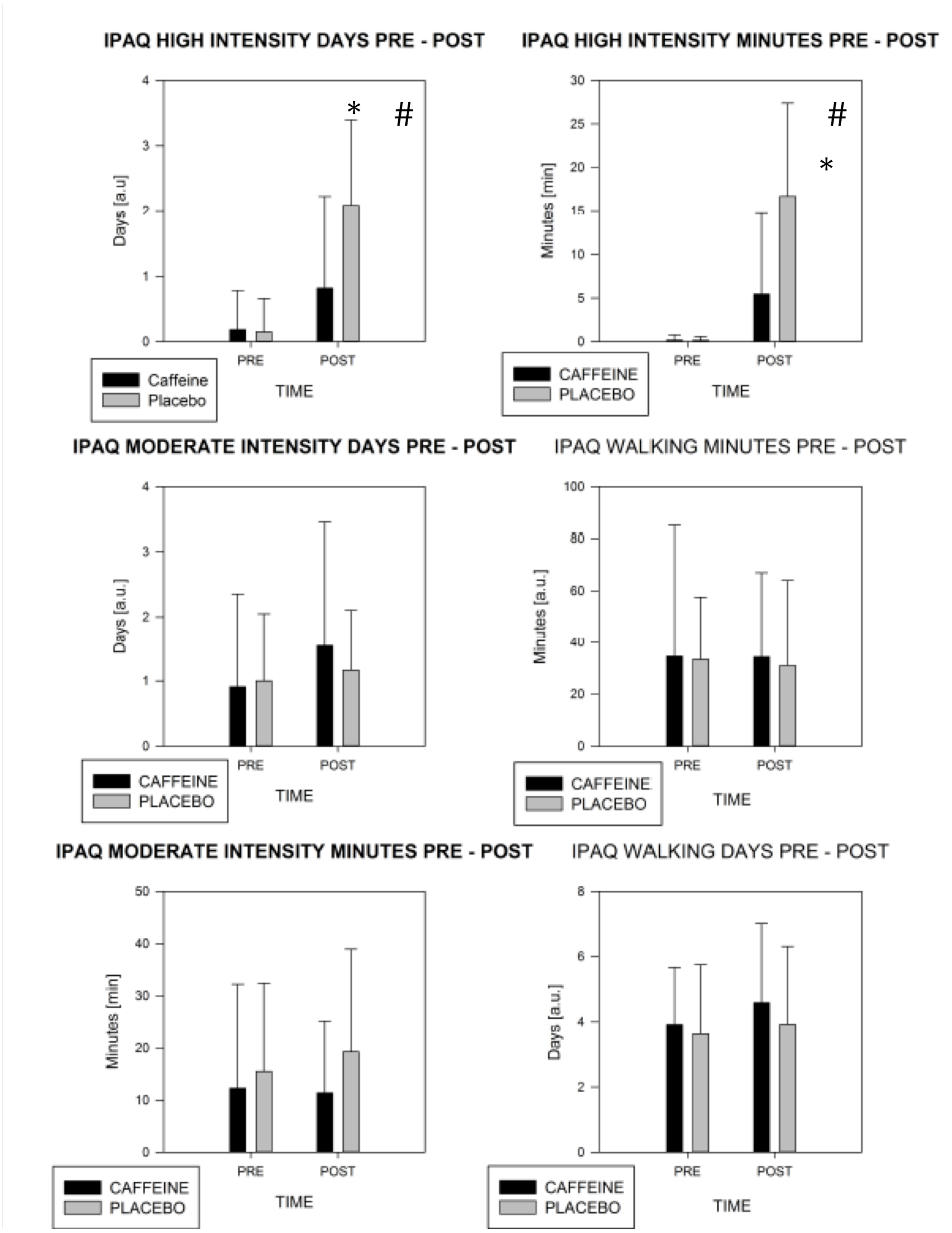


Figure 18. Effect of caffeine on EMI2 questionnaire. **A)** AFFILIATION § **B**) APPEARANCE **C)** ENJOYMENT **D)** HEALTH PRESSURE **E)** HILL HEALTH AVOIDANCE **F)** NIMBLENESS **G)** POSITIVE HEALTH §. **H**) REVITALISATION **I)** SOCIAL RECOGNITION **L)** COMPETITION § **M)** WEIGHT MANAGEMENT. **N)** STRENGTH ENDURANCE **O)** CHALLENGE. **P)** STRESS MANAGEMENT § significant main effect of time ($P<0.05$). Data are presented by mean ± SD

Data analysis on the general level of physical activity measured by the International Physical Activity Questionnaire showed some significant differences comparing the pre – post test values. More precisely, significantly differences were found between caf_pre (0.18 ± 0.60) plac_pre (0.15 ± 0.51) and caf_post (0.82 ± 1.40) plac_post (2.08 ± 1.31) on IPAQ HIGH INTENSITY DAYS ($p< 0.05$, $p = 0.028$), caf_pre (0.18 ± 0.60) plac_pre (0.13 ± 0.31) and caf_post (5.45 ± 9.44) plac_post (16.67 ± 10.73) on IPAQ HIGH INTENSITY MINUTES ($p< 0.05$, $p = 0.014$). These two parameters presented also a significant main effect of time ($p< 0.05$, $p = 0.000$). There was not a significant treatment x time interaction between caf_pre (0.91 ± 1.45) plac_pre (1.00 ± 1.04) and caf_post (1.55 ± 1.92) plac_post (1.17 ± .937) on IPAQ MODERATE

INTENSITY DAYS (p = 0.374) There was not significant main effect of time (p = 0.135). There was not significant treatment x time interaction between caf_pre (12.27 ± 19.92) plac_pre (15.50 ± 16.90) and caf_post (11.36 ± 13.80) plac_post (19.25 ± 19.67) on IPAQ MODERATE INTENSITY MINUTES (p = 0.594) There was not significant main effect of time (p = 0.745). There was not a significant treatment x workload interaction between caf_pre (3.91 ± 1.76) plac_pre (4.58 ± 2.43) and caf_post (3.64 ± 2.11) plac_post (3.92 ± 2.39) on IPAQ WALKING DAYS (p = 0.647). There was not a significant main effect of time (p = 0.281). There was not a significant treatment x workload interaction between caf_pre (34.55 ± 50.86) plac_pre (33.33 ± 24.34) and caf_post (34.27 ± 32.72) plac_post (30.92 ± 33.11) on IPAQ WALKING MINUTES (p = 0.898). There was not a significant main effect of time (p = 0.873). There was not a significant treatment x time interaction between caf_pre (409.09 ± 153.72) plac_pre (345.00 ± 133.25) and caf_post (433.64 ± 141.58) plac_post (365.00 ± 121.24) on IPAQ SEATED MINUTES (p = 0.898). There was not a significant main effect of time (p = 0.217) (Fig.19).


IPAQ HIGH INTENSITY DAYS PRE - POST
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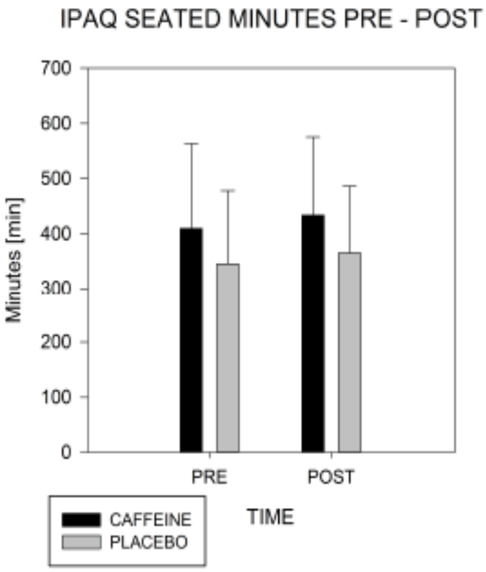

Figure 19. Effect of caffeine on IPAQ. **A)** IPAQ HIGH INTENSITY DAYS. **B)** IPAQ HIGH INTENSITY MINUTES **C)** IPAQ MODERATE INTENSITY DAYS **D)** IPAQ MODERATE INTENSITY MINUTES **E)** IPAQ WALKING DAYS **F)** WALKING MINUTES **G)** IPAQ SEATED MINUTES. # * Significantly different (P<0.05) .Data are presented by mean ± SD.

Effect of caffeine on physiological and perceptual responses

The pre-post test analysis of the PPO did not show a significant treatment x workload interaction between caf_pre (234.09 ± 79.27) plac_pre (179.17 ± 27.87) and caf_post (245.45 ± 86.47) plac_post (193.75 ± 26.38) (p = 0.683). There was a significant main effect of time (P<0.05) (p = 0.003) (Fig. 20a). Moreover, there was not a significant treatment x workload interaction between caf_pre (33.05 ± 7.00) plac_pre (30.13 ± 3.62) and caf_post (34.00 ± 8.86) plac_post (32.22 ± 6.34) on VO_2 PEAK (p = 0.462). There was not a significant main effect of time (p = 0.058) (Fig. 20b).

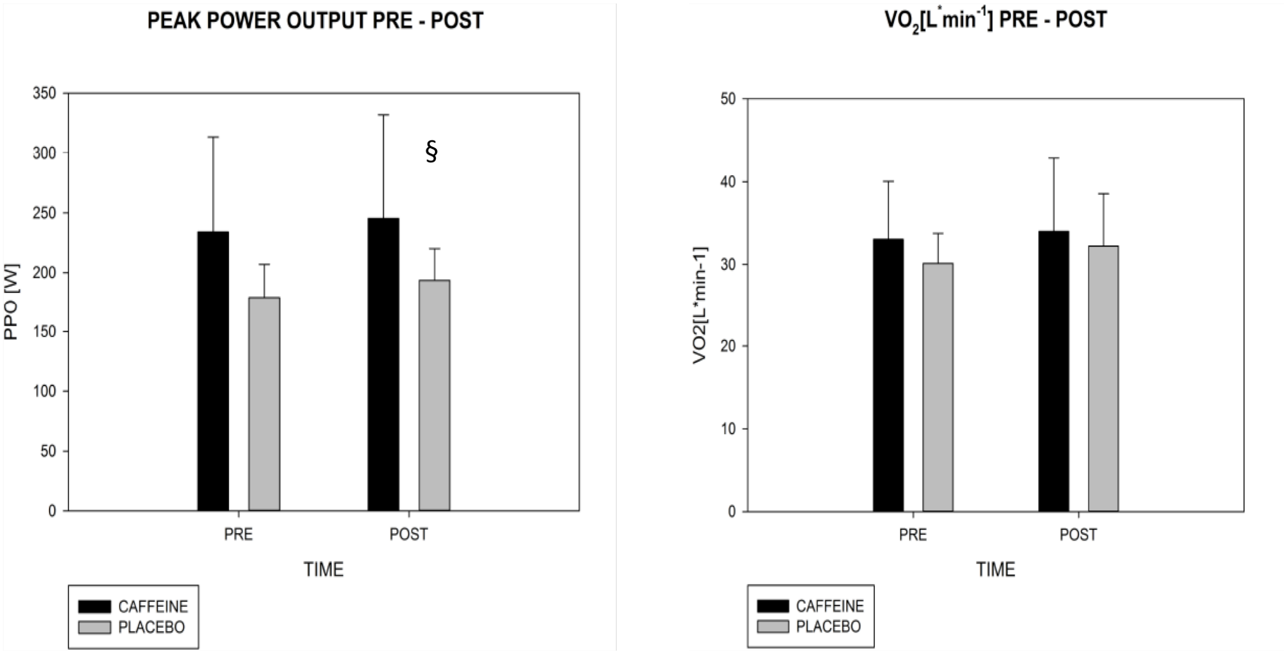

Figure 20. Effect of caffeine on PPO (A) and $VO_{2\ peak}$ (B). § significant main effect of time ($P<0.05$). Data are presented by mean ± SD.

Comparisons of the average HR and RPE obtained on the fifth minute of the first bout of the first session of the week in the two different conditions during the 12 weeks of training did not reveal a significant interaction between treatment and workload respectively on HR ($p = 0.856$) (Fig 21) and RPE ($p = 0.487$) (Fig 22). Significant main effects of time were found on HR and RPE ($P < 0.05$).

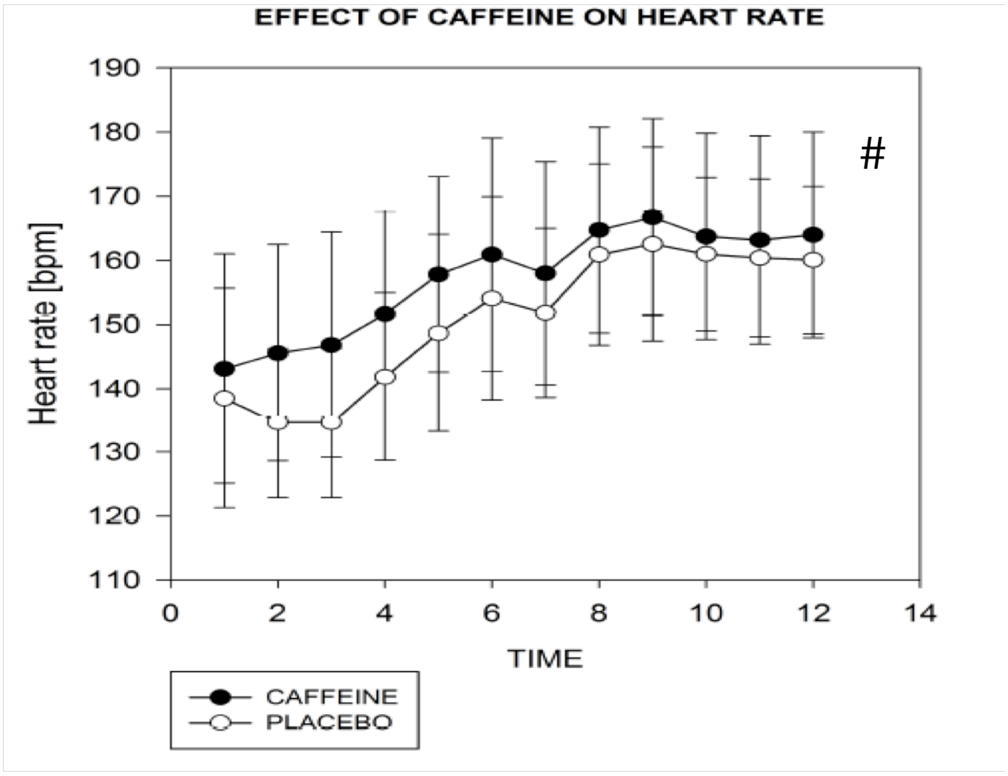

Fig. 21 Effect of caffeine on HR. There was not significant treatment x workload interaction on HR (p = 0.856) ♯significant main effect of workload (p< 0.05, p = 0.001) Data are presented by mean ± SD.

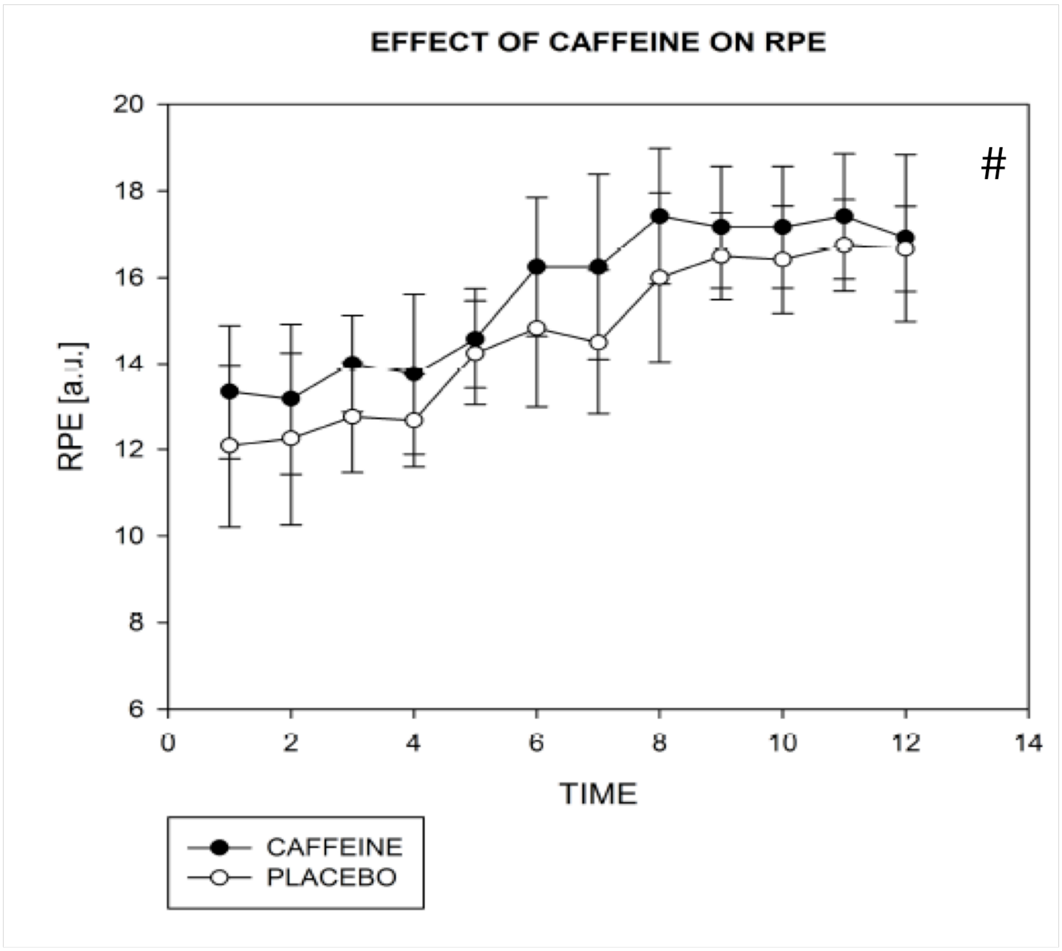

Figure 21 b) There was not significant treatment x workload interaction on RATING OF PERCEIVED EFFORT (p = 0.487) ♯ significant main effect of workload ($p < 0.05$, p = 0.001) Data are presented by mean ± SD

3.5 DISCUSSION

The purpose of the second study was to test the efficacy of a 200 mg caffeine pill administered 60 minutes before an aerobic session in order to reduce RPE and increase exercise adherence in previously sedentary subjects. Due to the poor presence of studies related to this topic, it is difficult to make comparisons between our results and results coming from other researches. One of the reasons is related to the fact that randomized trial with longitudinal data collection are mainly based on behavioral strategies and not on nutritional as we proposed. On the basis of the small number of results published on peer review journals, we have found a really different outcome in terms of RPE. Indeed, while Shrader P. et al. [95] found a significant reduction of perceived exertion over time our results indicated a main effect of workload over time in increasing RPE without any difference between caffeine and placebo. Nevertheless, it is important to note that Shrader P. [95] for his double blind randomized controlled trial used a training protocol based on a running treadmill, and not on a cycle ergometer. Moreover, the relative intensity of the training ranged from 60 to 70 % of the maximal heart rate of the subjects involved in the study, whilst in our study subjects performed more than 50 % of the total time of the planned training program over 80 % of their HR max. For this reason, is still complicated to make comparisons between studies of this methodology. The analysis of HR during the 12 weeks of training showed a significant increase over time due to the increased workload. Adherence to the exercise in this study was measured as attendance, percentage of the exercise prescribed completed and minutes of exercise completed for each session. Overall, all the measured adherence parameters were found significantly better in the subjects that were asked to take placebo rather than caffeine group. Those results are in contrast compared to the little amount of data useful for making some comparisons [31,89,96]. Another study, performed by Judice P.B. et al. [8], showed results in line with what we found in our data collection. Indeed, they found a negative relationship between caffeine and increase of physical activity level. As indicated on the aforementioned lines, it would not be possible to make comparisons between this study and our data collection and results, because of the very short duration of the protocol (4 days) and also for the nature of the training, based on short bout of physical exercise and total energy expenditure during the day. Nevertheless, due to the lack of studies present on this topic, it is possible to declare that the results on caffeine assumption and physical activity increase are in contrast. A possible explanation for the results coming from our randomized controlled trial is based on the likelihood of presence of a type I error. A possible explanation of this error comes from the results of the psychological questionnaires performed on the study. More specifically, it is well known that some psychological aspects like i.e. motivation and exercise motives participation have a clear implication on the initiation and maintenance of an exercise program [90]. The mood dimensions and the exercise participation motives of the two groups were not significantly different between them and also compared to pre-post, thus it is possible to explain an opposite result by the presence of a type I error.

One weakness of this study was that we did not measure the real amount of circulating caffeine at the time of the training session. This could have given the possibility to better understand and to check the participants' states of caffeine withdrawal. Moreover, due to the very poor fitness level of the athletes, also the scheduled training intensities progression could have represented a problem on the attrition of the subjects to the program.

3.6 CONCLUSIONS

This study demonstrated that there is not any effect of caffeine on perception of effort and adherence improvement in sedentary subjects. From a physiological point of view, due to a reduction in total time of training and percentage of total training completed we do not have demonstrated an improvement in aerobic fitness. On the other hand, adherence for the placebo group is resulted significantly different compared to caffeine.

CHAPTER 4

GENERAL CONCLUSIONS

4.1 CONCLUSIONS

Overall, this thesis was performed in order to better understand the psychobiological mechanisms that regulate exercise behaviour in sedentary individuals. To date, our studies are included in the very small number of researches that were performed using a nutritional strategy, i.e. caffeine. One of the most important results of our thesis is represented by the ability to reduce perception of effort independently from the intensity of the exercise. The different results obtained on the second study, indicated in the aforementioned sections need to be assessed as a primary and not definitive outcome on the research process of this area. The higher rate of dropouts and the presence of a type I error are aspects necessary to be taken into account for new study in which will be possible to control also for the weakness that this research demonstrated.
Therefore, the first study demonstrated that either 200 or 400 mg of caffeine administered 15 minutes before the exercise on a chewing gum based formulation were able to significantly reduce RPE at 80 % of PPO compared to placebo. This was the first study demonstrating that is possible to reduce RPE acting centrally on sedentary individuals using a psychoactive drug as caffeine without producing any significant detrimental effect on cardiovascular parameters. Based on exercise guidelines i.e. ACSM [4], this relative intensity is related to a higher rate of drop out, as demonstrated in several studies [31,89]. On the basis of those findings and on the psychobiological model applied to physical activity domain we concluded that caffeine could be used in exercise programs of moderate to long duration as an effective tool for the improvement of exercise adherence in previously sedentary individuals. Unfortunately, the results obtained on the second study were not as expected as claimed on the aims of the study. Indeed, 200 mg of caffeine took 60 minutes before each training session did not reduce perception of effort. Moreover, the comparisons on the analyzed adherence parameters like the percentage of the time completed, the attendance and the total time completed for each session of the planned program,

demonstrated that the placebo group significantly increased all those aspects during the 12 weeks of training whilst the caffeine group did not produce any significant improvement.

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References

1 DISHMAN, R.K. (1994). Prescribing exercise intensity for healthy adults using perceived exertion. Medicine and Science in Sports and Exercise, 26, 1087-1094.

2 Lars Osterberg, M.D., and Terrence Blaschke, M.D. Adherence to medication. N Engl J Med 2005; 353:487-497August 4, 2005

3 Adrian E Bauman, Rodrigo S Reis, James F Sallis, Jonathan C Wells, Ruth JF Loos, Brian W Martin. Correlates of physical activity: why are some people physically active and others not? Lancet 2012; 380: 258–71

4 Garber C.E.et al.. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011 Jul;43(7):1334-59

5 Swift D.L., Johansenn N.M., Lavie C.J., Earnest C.P., Church T.S.. The role of exercise and physical activity in weight loss and maintenance. Prog. Cardiovasc. Dis. 2014 Jan-Feb;56(4):441-7.

6 Dishman R.K., Sallis J.F., Orenstein D.R.. The determinants of physical activity and exercise. Public Health Report. March-April 1985, Vol. 100, No. 2

7 Michelle C Kegler, Cam Escoffery, Iris Alcantara, Denis Ballard, Glanz K. A qualitative examination of home and neighborhood environments for obesity prevention in rural adults International Journal of Behavioral Nutrition and Physical Activity 2008, 5:65

8 Judice P.B. et al., 2013

9 Thompson WD. ACSM's Guidelines for Exercise Testing and Prescription. 8th ed. Philadelphia (PA): Lippincott Williams and Wilkins (2010).

10 Dishman R.K., Buckworth J.. Exercise Psychology. HumanKinetics. 2002

11 Masaaki S, Takegami M., Shibata A., Kuzumaki M., Higashi T., Hayashino Y., Suzukamo Y., MoritaS., Katsuki M., and Fukuhara S.. Effect of feedback in promoting adherence to an exercise programme: a randomized controlled trial. Journal of Evaluation in Clinical Practice 17 (2011) 7–11

12 Bèlisle M., Roskies E. and Levesque JM.. Improving adherence to physical activity. Health Psychology, 1987, 5(2), 159-1

13 Rhodes R.E., Warburton D.E.R., Bredin S.S.D.. Predicting the effect of interactive video bikes on exercise adherence. An efficacy trial. Psychology, health and medicine, 14:6, 631 – 640

14 Anton S.D., Perri M.G., Riley J., Kanasky W.J., Rodrigue J.R., Sears S.F., and Martin A.D. Differential Predictors of Adherence in Exercise Programs With Moderate Versus Higher Levels of Intensity and Frequency. Journal of Sport & Exercise Psychology, 2005, 27, 171-187

15 Warburton D.E.R., Bredin S.S.D., Horita L.T.L., Zbogar D., Scott J.M., Esch B.T.A., Rhodes R.E.. The health benefits of interactive video game exercise. Appl. Physiol. Nutr. Metab. 32: 655–663 (2007)

16 Johnson R.A., Meadows R.L.. Dog walking: motivation for adherence to a walking program. Clin Nurs Res 2010 19(4) 387-402

17 Jacobsen D.J., Donnelly J.E., Snideer Hilan K., Livingston K.. Adherence and attrition with intermittent exercise and continuous exercise in overweight women. Int J Sports Med 2003; 24: 459-464

18 Andersson E.K., Moss T.P.. Imagery and implementation intention. A randomized controlled trial of interventions to increase exercise behaviour in the general population. Psychology of Sport and Exercise 12 (2011) 63-70

19 Nancy Humpel, BPsyc, Neville Owen, PhD, Eva Leslie, MHN. Environmental Factors Associated with Adults' Participation in Physical Activity: A Review. Am J Prev Med. (2002);22(3):188-199

20 Cunningham GO, Michael YL. Concepts Guiding the Study of the Impact of the Built Environment on Physical Activity for Older Adults: A Review of the Literature. Am J Health Promot . 2004 ; 18(6): 435–443.

21 Buckworth J., Dishman R.K., O'Connor P.J., Tomporowski P.D.. Exercise psychology. Second Edition. Human Kinetics (2007).

22 Ekkekakis P. Let them roam free? Physiological and psychological evidence for the potential of self-selected exercise intensity in public health. Sports Med. 2009;39(10):857-88

23 Berlin J.A. and Colditz G.A.. A meta-analysis of physical activity in the prevention of coronary heart disease.*Am. J. Epidemiol.* (1990) 132 (4): 612-628

24 HASKELL, WILLIAM L.; LEON, ARTHUR S.; CASPERSEN, CARL J.; FROELICHER, VICTOR F.; HAGBERG, JAMES M.; HARLAN, WILLIAM; HOLLOSZY, JOHN O.; REGENSTEINER, JUDITH G.; THOMPSON, PAUL D.; WASHBURN, RICHARD A.; WILSON, PETER W. F.. Cardiovascular benefits and assessment of physical activity and physical fitness in adults. Med. Sci. Sports Exerc.1992 vol:24 iss:6 Suppl pag:S201 -20

25 Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. Journal of the American Medical Association 1995;273:402–407.

26 Hardy C.J., Rejeski W.J.. Not what, but how one feels: the measurement of affect during exercise. Journal of sport and exercise psychology. 1989,11, 304-317

27 Heyward, V.H.. Advanced Fitness Assessment and Exercise Prescription. Human Kinetics, 2010

28 American College of Sports Medicine. ACSM's Guidelines For Exercise Testing and Prescription.Eight edition (2009)

29 Warburton DER,Nicol CW,Bredin SSD. Health benefits of physical activity: the evidence. CMAJ.2006;174:801–9

30 Pollock M.L., Gaesser G.A., Butcher J.D., Despres, J.P., Dishman R.K., Franklin B.A., Garber C.E.. ACMS Position Stand: The Recommended Quantity and Quality of Exercise for Developing and Maintaining Cardiorespiratory and Muscular Fitness, and Flexibility in Healthy Adults. Med. Sci.Sports Exerc. Vo. 30 No.6, pp. 975-991, 1998.

31 Perri M.G., Anton S.D., Durning P.E., Ketterson T.U., Sydeman S.J., Berlant N.E., Kanasky W.F.,Newton R.L., Limacher M.G., Martn A.D.. Adherence to exercise prescriptions: Effects of

prescribing moderate versus higher levels of intensity and frequency. Health Psychology 2002, Vol. 21, No. 5,452– 458

32 Sallis, JF; Haskell, WL; Wood, PD; et al.. Vigorous physical activity and cardiovascular risk factorsin young adults. JOURNAL OF CHRONIC DISEASES. 1986;(39), 115-120

33 Bartlett JD, Close GL, MacLaren DP, Gregson W, Drust B, Morton JP. High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: Implication for exercise adherence. J Sports Sci. 2011 Mar;29(6):547-53

34 Annessi J.J., Mazas J.. Effects of virtual reality-enhanced exercise equipment on adherence and exercise-induced feeling states. Perecpt Mot Skills 1997 Dec;85(3 Pt 1):835-44.

35 Maddison R., Mhurchu C.N. et al. Active video games: the mediating effect of aerobic fitness on body composition. International Journal of Behavioral Nutrition and Physical Activity 2012, 9:54

36 Ryan E. Rhodes, Darren E.R. Warburton and Shannon S.D. Bredin. Predicting the effect of interactive video bikes on exercise adherence: An efficacy trial. Psychology, Health & Medicine. Vol. 14, No. 6, December 2009, 631–640

37 Icek AJZEN. The theory of planned behavior. Organizational Behavior Aand Human Decision Processes. 50, 179-211 (1991)

38 Rodhes R.E., Blanchard C.M., and Mathseon D.H. A multicomponent model of the theory of planned behaviour. Br J. Health Psychol. 2006: Feb;11(Pt 1):119-37.

39 M. Doherty, P.M. Smith, Hughes M.G., Davison R.C. Caffeine lowers perceptual responses and increases power output during high-intensity cycling. J Sports Sci. 2004 Jul;22(7):637-43.

40 M. Doherty, P.M. Smith. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. Scand J Med Sci Sports 2005: 15: 69-78

41 Marcora S. Perception of effort during exercise is independent of afferent feedback from muscles, heart, and lungs. J Appl Physiol. 106: 2060 – 2062, 2009.

42 Borg GA. *Borg's Perceived Exertion and Pain Scales*. Champaign, IL: Human Kinetics, 1998.

43 Noble RJ., Robertson RJ. Perceived exertion. Champaign, Illinois: Human Kinetics, 1996

44 Taylor JL & Gandevia SC (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. J Appl Physiol 104, 542–550

45 FERNANDES, A., GALBO, H., KJAER, M., MITCHELL, J. H., SECHER, N. H. & THOMAS, S. N. (1990) Cardiovascular and ventilatory responses to dynamic exercise during epidural anaesthesia in man. *J Physiol,* 420**,** 281-93.

46 KJAER, M., SECHER, N. H., BANGSBO, J., PERKO, G., HORN, A., MOHR, T. & GALBO, H. (1996) Hormonal and metabolic responses to electrically induced cycling during epidural anesthesia in humans. *J Appl Physiol,* 80**,** 2156-62.

47 Samuele M Marcora, Andrea Bosio, Helma M de Morree. Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense cycling exercise independently from metabolic stress. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 294,3, 874-883, 2009.

48 Wright, R. A., Dill, J. C., Geen, R. G., & Anderson, C. A. (1998). Social evaluation influence on cardiovascular response to a fixed behavioral challenge: Effects across a range of difficulty levels. Annals of Behavioral Medicine, 20, 277–285.

49 Aadahl M, Kjær M, Jørgensen T. Perceived exertion of physical activity: negative association with self-rated fitness. Scandinavian Journal of Public Health 2007; 35(4):403-9

50 Lee I.M., Relative Intensity of Physical Activity and Risk of Coronary Heart Disease. Circulation.2003; 107: 1110-1116

51 Prochaska J.O. and Marcus B.H.. (1994) The Transtheoretical Model: Applications to exercise. In Dishman R.K.. Advances In Exercise Adherence (pp. 161 – 180). Champaign, IL: Human Kinetics.

52 Stuart J.H. Biddle and Nanette Mutrie. Psychology of Physical Activity. Determinants, Well-Being & Interventions. 2nd edition. Routledge, New York. 2008

53 Dishman R.K. (1994) Advances in exercise adherence. Human Kinetics

54 Buckworth J., Dishman R.K., O'Connor P.J., Tomporowski P.. Exercise Psychology-2nd edition (2007)

55 Brehm, J.W., Self, E., 1989. The intensity of motivation. In: Rozenweig, M.R., Porter, L.W.(Eds.), Annual Review of Psychology. Annual Reviews, Inc., Palo Alto, pp. 109–131

56 Ach N. Analysis of the will. In E. Abderhalden (Ed.), Handbuch der biologischen Arbeitsmethoden. Berlin, Germany: Urban & Schwarzenberg. (1935)

57 Hillgruber, A. (1912). Fortlaufende Arbeit und Willensbeta¨tigung [Continuous work and will performance]. Untersuchungen zur Psychologie und Philosophie, [Studies on the Psychology and Philosophy], 1..

58 Wright, R. A., & Franklin, J. (2004). Ability perception determinants of effort-related cardiovascular response: Mood, optimism, and performance resources. In R. A. Wright, J. Greenberg, & S. S. Brehm (Eds.), Motivational Analyses of Social Behavior: Building on Jack Brehm's Contributions to Psychology. Hillsdale, NJ: Erlbaum.

59 Wright, R.A. Refining the prediction of Effort: Brehm's Distinction between Potential Motivation and Motivation Intensity. Social and Personality Psychology Compass 2/2 (2008):682-701.

60 Gendolla, G. H. E., & Wright, R. A. (2005). Motivation in social settings: Studies of effort-related cardiovascular arousal. In J. P. Forgas, K. Williams & B. von Hippel (Eds.), Social Motivation: Conscious and Nonconscious Processes. New York, NY: Cambridge University Press.

61 Ekkekakis, P. (2003). Pleasure and displeasure from the body: Perspectives from exercise. Cognition and Emotion, 17 (2), 213-239

62 Ekkekakis, P., & Petruzzello, S.J. (2000). Analysis of the affect measurement conundrum in exercise psychology: I. Fundamental issues. Psychology of Sport and Exercise, 1 (2), 71-88

63 Ekkekakis, P., & Petruzzello, S.J. (2002). Analysis of the affect measurement conundrum in exercise psychology: IV. A conceptual case for the affect circumplex. Psychology of Sport and Exercise, 3 (1), 35-63.

64 Carol Ewing Garber, Ph.D., FACSM, (Chair); Bryan Blissmer, Ph.D.; Michael R. Deschenes, Ph.D., FACSM; BarryA. Franklin, Ph.D., FACSM; Michael J. Lamonte, Ph.D., FACSM; I-Min Lee, M.D., Sc.D., FACSM; David C. Nieman, Ph.D., FACSM; andDavid P. Swain, Ph.D., FACSM. Quantity and Quality of Exercise for Developing and Maintaining Cardiorespiratory,Musculoskeletal, and Neuromotor Fitness in Apparently Healthy Adults: Guidance for Prescribing Exercise. Medicine & Science in Sports & Exercise, 43 (7), 1334-1349.

65 Marcora S.M., Staiano W.. The limit to exercise tolerance in Humans: Mind over muscles? EJAP. 2010,109:4,763-770.

66 Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference tofactors that contribute to its widespread use. Pharmacol Rev 1999: 51: 83–133.

67 Juliano, LM; Griffiths, RR. A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. Psychopharmacology 2004:176(1):1-29.

68 Gastin PB, Misner JE, Boileau RA, Slaughter MH. Failure of caffeine to enhance exercise performance in incremental treadmill running. Aust J Sci Med Sport 1990: 22: 23–27.

69 Graham, T., Hibbert, E., & Sathasivam, P. (1998). Metabolic and exercise endurance effects of coffee and caffeine ingestion. Journal of Applied Physiology, 85, 883–889.

70 Fredholm BB. Adenosine, adenosine receptors and the actions of caffeine.Pharmacol Toxicol 1995: 76: 93–101

71 Fredholm, Bertil B. Targeting adenosine receptors. ACTA PHARMACOLOGICA SINICA Volume: 27 Supplement: 1 Pages: 26-26 Published: JUL 2006

72 SM Marcora, W Staiano, V Manning. Mental fatigue impairs physical performance in humans. Journal of Applied Physiology 2009:106 (3), 857-864

73 Samuele M Marcora. Role of feedback from Group III and IV muscle afferents in perception of effort, muscle pain, and discomfort. Journal of Applied Physiology 2011:110 (5), 1499-1499

74 O’Connor PJ, Motl RW, Broglio SP, Ely MR. Dose-dependent effect of caffeine on reducing leg muscle pain during cycling exercise is unrelated to sistoli blood pressure. Pain 2004: 109:291–298.

75 LeBlanc J, Jobin M, Cote J, Samson P,Labrie A. Enhanced metabolic response to caffeine in exercise-trained human subjects. J. Appl Physiol 1985: 59:832–837.

76 Wallman, K., Goh, J., & Guelfi, K. (2010). Effects of caffeine on exercise performance in sedentary females. Journal of Sports Science and Medicine, 9, 183–189.

77 George Laurence , Karen Wallman & Kym Guelfi (2012): Effects of caffeine on time trial performance in sedentary men, Journal of Sports Sciences, 30:12, 1235-1240

78 Glade M.J.. Caffeine – Not just a stimulant. Nutrition (2010), 26: 932-938

79 Graham TE. Caffeine and exercise:metabolism, endurance and performance. Sports Med 2001b: 31:785–807.

80 Turley, K. R., Desisso, T., Gerst, J. W.. Effects of caffeine on physiological responses to exercise: boys versus men. Pediatr Exerc Sci. 2007: 19(4): 481-92.

81 Davis JM, Zhao Z, Stock HS, Mehl KA, Buggy J, Hand GA. Central nervous system effects of

caffeine and adenosine on fatigue. Am J Physiol Regul Integr Comp Physiol 2003: 284: R399–R404.

82 Craig C.L. et al. (2003). " International physical activity questionnaire : 12-country reliability and validity. " Med Sci Sports Exerc. 35: 1381-95.

83 Kuipers H, Verstappen FT, Keizer HA, Geurten P, van Kranenburg G (1985) Variability of aerobic performance in the laboratory and its physiologic correlates. Int J Sports Med 6(4):197–20.

84 Syed S.A., Kamimori G.H., Kelly W. And Eddington N.D.. Multiple doses Pharmacokinetics of Caffeine Administered in Chewing Gum to Normal Healthy Volunteers. Biopharm. Drug Dispos. 26: 403-409 (2005).

85 Kevin Currell and Asker E. Jeukendrup. Validity, Reliability and Sensitivity of Measures of Sporting Performance. Sports Med 2008; 38 (4): 297-316.

86 Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald-Mammosser M, Mettauer B, Geny B, Lonsdorfer J. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at restand during exercise: comparison with the "direct" Fick method. Eur J Appl Physiol 82: 313–320, 2000

87 Hardy C.J., Rejeski W.J. Not what, but how one feels: the measurement of affect during exercise. Journal of sport and exercise psychology. 1989: 11, 304-317.

88 Dishman R.K.. Advances in exercise adherence 1994 Human Kinetics.

89 Anton S.D., Perri M.G., Riley J., Kanasky W.J., Rodrigue J.R., Sears S.F., and Martin A.D..Differential Predictors of Adherence in Exercise Programs With Moderate Versus Higher Levels of Intensity and Frequency. Journal of Sport & Exercise Psychology, 2005, 27, 171-187.

90 Markland, D. & Ingledew, D.K. (1997). The measurement of exercise motives: Factorial validity and invariance across gender of a revised Exercise Motivations Inventory. British Journal of Health Psychology, 2, 361-376.

91 Terry P.C., Lane M.A., & Fogarty, G.J (2003). Construct validity of the POMS-A for use with adults. Psychology of Sport and Exercise, 4, 125-139

92 Faidon Magkos and Stavros A. Kavouras. Caffeine and Ephedrine: physiological, metabolic and performance-enhancing effects. Sports Med 2004; 34 (13): 871-889.

93 Bell DG, Jacobs I, Zamecnik J. Effects of caffeine, ephedrine 424-9 and their combination on time to exhaustion during high intensity exercise. Eur J Appl Physiol Occup Physiol 1998; 77

94 Ronald J. Maughan PhDBart Roelands andRomain Meeusen. Nutritional Effects on Central Fatigue. The Encyclopaedia of Sports Medicine: An IOC Medical Commission Publication, Volume 19, 2013.

95 Shrader P., Panek L.M., Temple J.L.. Acute and chronic caffeine administration increases physical activity in seentary adults. Nutrition research 33(2013)457 - 463.