Università degli studi di Verona

DEPARTMENT OF NEUROSCIENCES, BIOMEDICINE AND MOVEMENT SCIENCES

PhD SCHOOL OF HEALTH AND LIFE SCIENCES

PhD IN NEUROSCIENCE, PSYCHOLOGY AND PSYCHIATRY

XXXI CYCLE /YEAR 2015

Effects of moderate and low frequency recreational football on cardiovascular risk: a dose-response study

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Tesi di Dottorato

Verona, 17 Maggio 2019

1 Abstract

Introduction. Physical inactivity ranks fourth as a risk factor for global mortality. World Health Organisation (WHO) physical activity guidelines recommend at least 150 min/wk of moderate or 75 min/wk of vigorous intensity aerobic physical activity for "substantial health benefits". Lack of time and motivation are often a barrier to meeting these recommendations.

Aims. There is abundant evidence that recreational football (RF) at moderate and high frequency is effective to improve cardiovascular health, however, data are scarce regarding the effects of low frequency training (once a week).The aims of the present study were to: 1) compare the effects of low and moderate RF training volume (1 and 2 times a week for 12 weeks) on cardiovascular risk factors, 2) assess changes in inflammatory status after RF training, and 3) determine what cardiac and peripheral adaptations occur. The study population was 40 healthy, sedentary men (age range 35 - 55 years).

Study 1. We compared the outcome of 12 weeks RF training in three groups: moderate frequency group (MFG, training twice a week), low frequency group (LFG, training once a week), and control group (CG, no training). As compared with the CG, the fat mass was decreased in both the LFG and the MFG, whereas body mass and body-mass index (BMI, weight in kg divided by height in meters squared) were decreased only in the MFG. Maximal oxygen consumption was higher in both the LFG and the MFG. Arterial blood pressure and blood lipid profile remained unchanged.

Study 2. Endothelial function impairment and atherosclerosis are precursors of many cardiovascular events. Despite the consistent body of literature on inflammatory markers and their relationship with physical exercise, it is not clear how RF influences them. We measured inflammatory markers white blood count (WBC), neutrophil-lymphocyte ratio (NLR), and C-reactive protein (CRP). No differences among the three groups were found at 12 weeks. Comparison of CG with the merged LFG and MFG groups (FG), revealed differences only for WBC. This difference probably had no remarkable meaning.

Study 3. To better understand the adaptations that lead to improvement in cardiovascular risk factors, we evaluated microvascular responsiveness by near-infrared spectroscopy (NIRS) and cardiac function and structure by echocardiography. At 12 weeks we recorded changes in area under the curve (AUC) and increased hyperaemia reserve in the MFG and the LFG, respectively, versus the CG. This increase was also noted when the FG was compared versus the CG, suggesting a possible improvement in hyperaemic response. Echocardiography showed an increase in cardiac dimensions in both the LFG and the MFG versus the CG. Cardiac function parameters remained unchanged, except for a difference in right ventricular systolic function between the FG and the CG.

Conclusion. The main, novel finding of these studies is that low frequency RF training produces beneficial effects on some cardiovascular risk factors in sedentary, healthy, and untrained middle-aged men. No beneficial effects on inflammatory conditions (CRP, WBC, NLR) were noted. In addition, echocardiographic assessment showed ventricular remodelling in the FG, as demonstrated by increased left and right ventricular diameters and left ventricular mass. Diastolic function remained unchanged, indicating that low frequency RF training for 12 weeks was not sufficient to further improve normal diastolic function in this healthy sample. Finally, a small positive effect on endothelial function was detected, but further investigations are needed to explain this observation.

Sommario

Introduzione. La sedentarietà è il quarto fattore di rischio di mortalità. L'Organizzazione Mondiale della Sanità (OMS) nelle sue linee guida raccomanda almeno 150 min/settimana di esercizio moderato o 75 minuti di esercizio vigoroso. La mancanza di tempo e la scarsa motivazione sono però molto spesso degli ostacoli a queste raccomandazioni.

Obiettivi. L'evidenza scientifica ci dice che il calcio ricreativo (CR) giocato con una moderata o alta frequenza è capace di migliorare la salute cardiovascolare, mentre i dati sugli effetti di questa attività svolta con una bassa frequenza (una volta a settimana), sono scarsi. Gli obiettivi del presente studio erano: 1) confrontare gli

effetti di un volume basso e moderato di CR (1 o 2 volte a settimana per 12 settimane) sui fattori di rischio cardiovascolare, 2) valutare cambiamenti nello stato infiammatorio dopo questo tipo di allenamento, e 3) determinare quali adattamenti avvengono a livello cardiaco e vascolare periferico. La popolazione di studio era composta da 40 maschi sani e sedentari (età 35 – 55 anni).

Studio 1. Abbiamo confrontato gli effetti di 12 settimane di CR in tre gruppi: gruppo frequenza moderata (MFG, 2 sessioni a settimana), frequenza bassa (LFG, 1 sessione a settimana) e gruppo di controllo (CG, nessun allenamento). Se confrontato con CG, la massa grassa è diminuita sia in LFG che MFG, mentre il peso e l'indice di massa corporea (BMI, peso in kg diviso la statura in metri al quadrato), sono diminuiti solo in MFG. Il massimo consumo d'ossigeno è aumentato sia il LFG che in MFG, mentre pressione arteriosa e profilo lipidico sono rimaste inalterate.

Studio 2. Una funzione endoteliale non ottimale e l'aterosclerosi sono precursori di molti eventi cardiovascolari. Nonostante la consistente evidenza scientifica riguardo la relazione tra marker infiammatori ed esercizio fisico, non è ancora ben chiaro come un'attività come il CR possa influenzare questi fattori. Abbiamo misurato, quali marker infiammatori, la conta leucocitaria totale (WBC), il rapporto neutrofili-linfociti (NLR), e la Proteina C-reattiva (CRP). Non abbiamo riscontrato nessun cambiamento nei tre marker tra i gruppi dopo le 12 settimane di allenamento. Il confronto tra tutto il gruppo che ha svolto CR a dispetto della frequenza, con il CG ha mostrato differenze solo per WBC. Queste differenze non hanno probabilmente un significato rilevante.

Studio 3. Per approfondire gli adattamenti che portano a miglioramenti nei fattori di rischio cardiovascolare abbiamo valutato la responsività microvascolare attraverso la spettroscopia a raggi infrarossi (NIRS) e struttura e funzione cardiaca con un'ecocardiografia. Dopo 12 settimane di CR abbiamo registrato aumenti nell'area sotto la curva (AUC) e nella riserva iperemica sia in LFG che MFG confrontati con CG. Questo incremento è confermato quando i gruppi LFG e MFG vengono fusi in uno unico (FG) e confrontati con CG, suggerendo un possibile miglioramento nella risposta iperemica. L'ecocardiografia ha mostrato un

incremento delle dimensioni cardiache in entrambi i gruppi sperimentali. I parametri di funzionalità cardiaca sono rimasti inalterati, ad eccezione di un aumento della funzione sistolica del ventricolo destro in FG confrontato con CG.

Conclusioni. Il principale risultato degli studi è rappresentato dal fatto che una bassa frequenza di CR produce benefici su alcuni fattori di rischio cardiovascolare in uomini adulti, sani e sedentari. Nessun effetto su fattori infiammatori (CRP, WBC, NLR) è stato evidenziato. Inoltre, la valutazione ecocardiografica ha mostrato un rimodellamento ventricolare dopo 12 settimane di CR. La funzionalità diastolica è rimasta invariata, indicando che una bassa frequenza di CR per 12 settimane non è sufficiente per migliorare ulteriormente una condizione comunque di normalità. Infine, un piccolo effetto positivo è stato trovato per quanto riguarda la funzione endoteliale, ma ulteriori ricerche sono necessarie per chiarire questi aspetti.

2 Index

3 General Introduction

3.1 Background

Physical inactivity ranks as the fourth leading risk factor for global mortality and is responsible for 6 to 10% of non-communicable diseases such as coronary heart disease, type 2 diabetes, and breast and colon cancers. The World Health Organization (WHO) has stated that sedentary lifestyle is the cause of 9% of premature mortality, accounting for more than 5.3 million deaths in 2008 alone.[1] Lee et al. have demonstrated that if physical inactivity were eliminated, about 6% of cardiovascular heart diseases, 7% of type 2 diabetes, 10% of breast cancer, and 10% of colon cancer could be cancelled. While total elimination of physical inactivity is a utopian scenario, the implementation of effective national and international strategies to promote physical activity could reduce sedentariness by 10% or 25%, saving between 533,000 and 1.3 million lives per year worldwide.[2]

A well-established relationship between physical activity and good health dates back to ancient times when Chinese physicians in 2600 BC and Hippocrates around 400 BC praised the benefits of physical activity for a healthy life. In 1953 James Morris conducted the first methodologically rigorous epidemiological study investigating the relationship between physical inactivity and cardiovascular diseases in the UK. Assessing different worker categories, he and his colleagues found a lower incidence of coronary heart diseases (CHD) in more physically active workers than in sedentary ones: specifically, the mortality rate from cardiovascular disease (CVD) and CHD among double-decker bus drivers in London was roughly twice that of the more active bus conductors. Their later studies involving British government workers showed that physically active postal service workers were more protected against CVD/CHD than less physically active government employees. They also demonstrated that when a more active worker developed a CVD/CHD, it was less severe and happened later in life.[3]

Since then, studies investigating the negative impact of sedentariness and physical inactivity on health have highlighted the many positive effects of an active lifestyle (Table 3.1).[2,4–6] The literature is clear on the fact that higher levels of physical

activity and cardiorespiratory fitness are positively associated with a reduction not only in the risk for CVD/CHD but also for certain types of cancer, hypertension, and type 2 diabetes. In addition, regular physical activity has been associated with reduced disability and depression, improved bone health, and increased longevity.[7–9]

More recently, studies have assessed the dose-response relationship between physical activity and CVD/CHD incidence. Coherent with the 2008 Physical Activity Guidelines Advisory Committee Report of the US Department of Health and Human Services, studies have shown that women and men who practice physical activity at high and moderate volume or intensity have, on average, a reduced risk of ≈ 30% to 35% and ≈ 20% to 25% respectively, for developing CVD/CHD as compared to less active people.[4] The "moderate" and "high" amount of physical activity are not simple and clear to define but are consistent with those proposed in the 2008 Guidelines.[9] The Guidelines recommend at least 150 min/wk of moderate or 75 min/wk of vigorous intensity aerobic physical activity for "substantial health benefits" and 300 min/wk of moderate or 150 min/wk of vigorous intensity aerobic physical activity for "additional and more extensive health benefits".[10] Other studies, published after the 2008 Guidelines were issued, have confirmed these beneficial effects, but showed no clear evidence for homogeneity in dose-response relationship based on gender, age category, and ethnic group.[11] Furthermore, the recommended amount of physical activity (150 min/week of moderate or 75 min/week of vigorous intensity) should not be considered the base minimum to gain beneficial effects. A pooled analysis and a meta-analysis reported an inverse association between leisure-time physical activity and CHD, with lower relative risk in adults who were active below the level suggested by the 2008 Guidelines, as compared with inactive people, supporting their statement ("All adults should avoid inactivity. Some physical activity is better than none, and adults who participate in any amount of activity gain some health benefits.").[12,13]

The minimal effective dose may be influenced by an individual's genetic characteristics and cardiorespiratory fitness. Studies have demonstrated a close relationship between cardiorespiratory fitness (dose) and the mortality risk decrease

(response), showing a reduction between 10% and 25% in mortality risk for every 1-MET increase in exercise capacity in both men and women.[7,9,14] Cardiorespiratory fitness is usually measured as maximal oxygen consumption (VO2max). Genetic studies have demonstrated that after aerobic training about 40-50% individual variation in this index is influenced by genetic factors. [15,16] This is another reason why a minimal level of physical activity should not be taken as a goal to improve health. The common aim should be "to nudge people into engaging in whatever kind and amount of physical activity they are capable of doing."

Despite these well-known health benefits and national and international programs to increase physical activity in the adult population, too many people still have a sedentary lifestyle [17]. The most recent epidemiological data show that more than 25% of the adult world population engages in less physical activity than recommended by the WHO guidelines. Furthermore, there is wide variability between countries in level of development, cultural and social characteristics, and availability of facilities and infrastructures that facilitate access to physical activity. It is not surprising to find physical inactivity rates of over 70% in several of the developed countries[1]. One of the most common barriers to engaging in regular physical activity is lack of time, especially in the developed countries. As stated in the Global Action Plan of Physical Activity 2018-2030 proposed by the WHO[18], physical activity should be integrated into the environment where people live, work and play. Walking and cycling are both optimal means of transport and ways to do physical activity saving precious time. Sport and recreational activities can give a relevant contribution to promote physical activity for people of all ages and abilities.

To be successful, strategies to get people engaged in physical activity should stimulate intrinsic motivation. Football (soccer) is the world's most popular sport, with millions of people playing it for recreation. Indeed, it is estimated that there are at least 400-500 million active football players worldwide[19], around 200 million of which are affiliated with the Federation Internationale de Football Association (FIFA), and 200-300 million are non-FIFA-affiliated players. This popularity of football can be exploited to increase intrinsic motivation and hence adherence to physical activity programs. Accordingly, this team sport has the potential to be an effective exercise strategy to enhance aerobic fitness and reduce cardiovascular risk

factors. Furthermore, recreational football has been associated with positive psychosocial interactions, including increased social capital, improved quality of life, general well-being, and motivational status[20–22].

During the last decade, studies on the effects of recreational football as a health-promoting activity have consistently shown that participants experienced a wide range of positive physiological effects after training that were sometimes even better than those obtained through other recreational activities such as running, interval running, and fitness training. The majority of these studies examined the positive relationship between playing recreational football and improvement in cardiovascular risk factors. [19,23,24]

3.2 Aims

There is ample evidence that recreational football at moderate and high frequency is effective to improve cardiovascular risk parameters, however, data on the effects of low-frequency training are lacking. Low and moderate frequency training for 1 h for 1 or 2 times per week is more ecological than high training volume (2 or 3 h per week) since people usually play recreational football 1 or 2 times per week for approximately 1 h per session, which is also the time for which the pitch is rented. The aims of this dissertation were to: 1) compare the effects of low and moderate recreational football training volume (1 and 2 times per week for 12 weeks), performed as small-sided games, on cardiovascular risk factors; 2) assess changes in inflammatory status after 12 weeks of training; and 3) determine what cardiac and peripheral adaptations occur after low and moderate frequency training. The study subjects (age range 35-55 years) were selected from a healthy, sedentary male population.

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3.4 Tables

Strong evidence for reduced rates of:
• All-cause mortality • Coronary heart disease • High blood pressure • Stroke • Metabolic syndrome • Type 2 diabetes • Breast cancer • Colon cancer • Depression • Falling
Strong evidence for:
• Increased cardiorespiratory and muscular fitness • Healthier body mass and composition • Improved bone health • Increased functional health • Improved cognitive function

Table 3.1 – Health benefits of physical activity in adults (modified from Lee et al. 2012)

4 General protocol overview

4.1 Methods

4.1.1 Subjects

For this study 47 untrained middle-aged men were recruited. Their characteristics are shown in Table 4.1. Recruitment was done via public advertisement (local newspapers, websites, local radio station, and flyers). Men interested in taking part in the study were interviewed by one of the researchers (R.M.) to evaluate eligibility. The inclusion criteria were age between 35 and 55 years, no regular physical activity performed during the previous year, and willingness to participate in the training and undergo a test battery. Exclusion criteria were female gender, presence of history or symptoms of chronic diseases such as cardiovascular diseases, hypertension, diabetes, and cancer. Seven men were excluded because they either did not meet the inclusion criteria or met at least one exclusion criterion.

	Age (years)	**Weight (kg)**	**Height (cm)**
Mean	44.3	81.9	177.4
95% Confidence Limits	42.4 – 46.2	78.3 – 85.5	175.1 – 179.7

Table 4.1 - Main baseline characteristics of study subjects.

The subjects underwent complete cardiovascular screening, including medical history, physical examination, resting and exercise electrocardiogram, to exclude the presence of cardiomyopathy, ischaemic heart disease, and major exercise-related arrhythmias. Exercise ECG was conducted on a cycle ergometer (Technogym). From an initial power output of 90 W, work rate was increased by 40 W every 90 s till volitional exhaustion. During the exercise test, ECG was

continuously recorded (Quark C12x, Cosmed, Rome, Italy), and blood pressure was measured at each step (ERKA, Bad Tölz, Germany).

Medical examination disclosed no pathological conditions or contraindications to physical activity. Forty subjects were enrolled and administered the International Physical Activity Questionnaire Long form (IPAQ-L) to verify their sedentary lifestyle [1]. We defined sedentary lifestyle as less than at least 20 min of physical activity 3 times per week; this definition is more conservative than that proposed by the WHO[2].

Before data collection, participants were informed about the study methods and procedures. Written, informed consent for taking part in the study was obtained. The institutional ethics committee of the Department of Neurosciences, Biomedicine and Movement Sciences of the University of Verona approved the study methods and procedures (n. 241127, 15th September 2016), according to the ethical principles established in the 2013 revision of the Helsinki Declaration for experimentation involving human subjects.

4.1.2 Design

The study has a case-crossover design nested in a randomized control trial. Subjects were randomly assigned to either a control (n = 20) or an experimental (n = 20) group. The control group (CG) performed the evaluation tests before and after the 12-week study period during which they were asked not to change their usual lifestyle habits (control period). In a second randomization of all subjects (n = 40) one half was assigned to the low frequency group (n = 20) and the other half to the moderate frequency group (n = 20). The randomization scheme was generated using a balanced restricted randomization procedure available at the Web site Randomization.com ⟨http://www.randomization.com⟩. The low frequency group (LFG) and the moderate frequency group (MFG) performed 1 h of recreational football once and twice a week, respectively, for 12 weeks. This design ensured that no participant was excluded from the benefit of physical activity (ethically desirable) and provided greater statistical power of the study. Three participants of the MFG dropped out for different reasons: one because of a hamstring strain injury, one because of ankle sprain, and one for personal reasons unrelated to the study.

The study design is illustrated in Figure 4.1 and the composition and main characteristics of the three groups at baseline are given in Table 4.2.

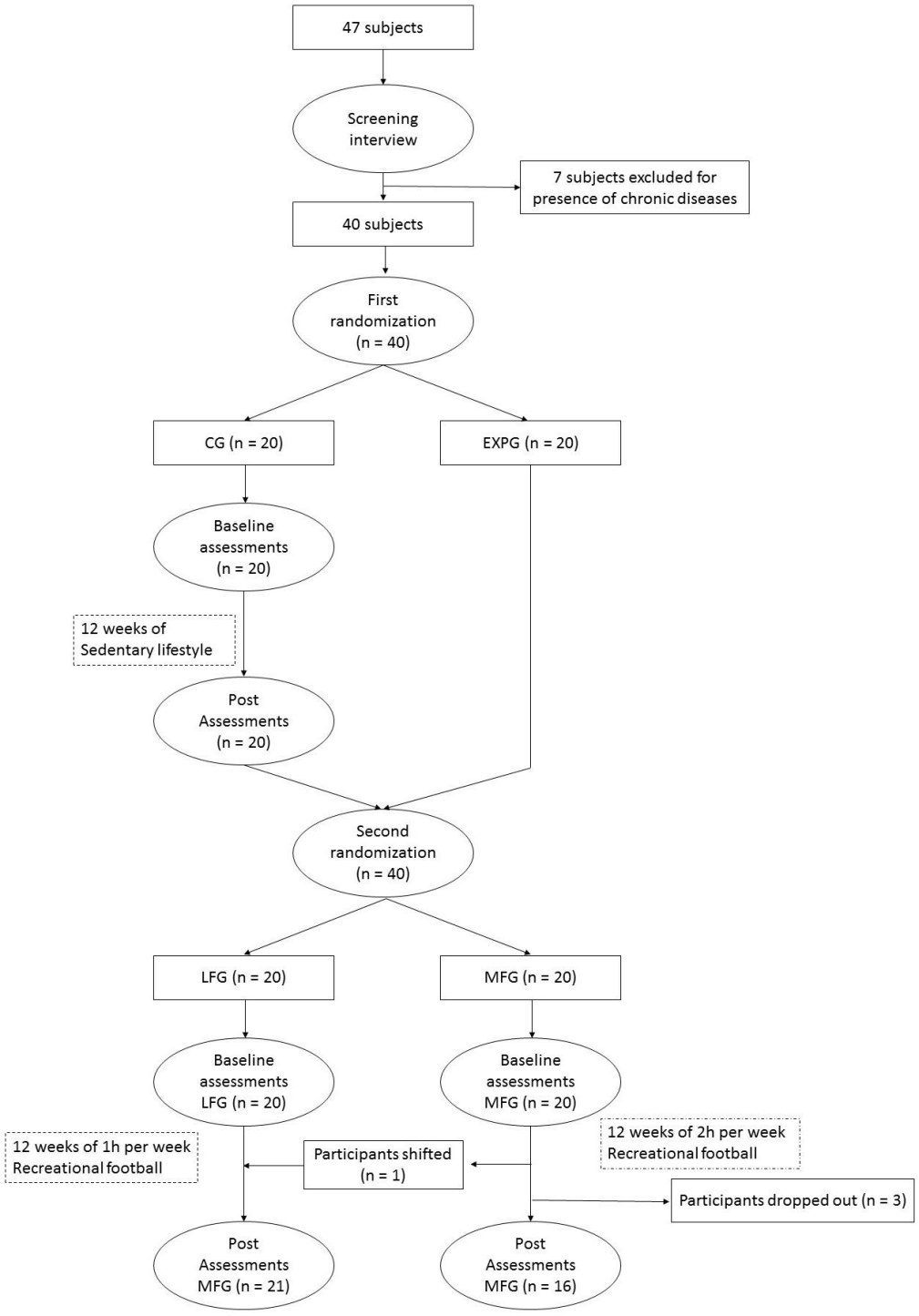

Figure 4.1 - Study design

	Age (years)	Weight (kg)	Height (cm)
	Mean [95% Confidence Limits]		
CG	44.68 [41.85 – 47.51]	83.59 [78.75 – 88.45]	177.57 [173.71 –
LFG	43.81 [41.40 – 46.21]	80.28 [78.75 – 88.45]	177.13 [174.12 –
MF	45.08 [41.75 – 48.41]	84.45 [79.43 – 89.46]	177.71 [173.75 –

Table 4.2 Group characteristics at baseline. CG denotes control group, LFG low frequency group, MFG moderate frequency group.

4.1.3 Statistical analysis

Statistical analysis was performed using IBM® SPSS® (version 21.0.0, IBM Corp., Somers, NY, USA) and statistical spreadsheets developed by W.G. Hopkins.[3,4] The Shapiro-Wilk test showed that the assumption of normality was not met for some parameters, which were therefore log transformed before analysis. Subsequent repetition of the Shapiro-Wilk test on the log-transformed data showed that some data were still not normally distributed. Some outliers were removed from the parameters. Leven's test was used to check for the homogeneity of variance. The comparison between groups, of internal training load during training session was performed by means One-Way ANOVA. The analysis of covariance for the group effect was performed using a linear mixed model, where the independent variables were the post-intervention values for each outcome and the pre-intervention values were the covariate and fixed factor. Bonferroni-adjusted pairwise comparison between-groups was performed on the estimated means. The effect size was also calculated as standardized Cohen's difference (d) and the magnitude of this effect was evaluated as: 0.2 – 0.59 small effect, 0.6 – 1.19 moderate effect, 1.2 – 1.99 large effect, and > 2.0 very large effect.[3] All descriptive statistics are shown in the text and figures as mean values and 95% of confidence limits [lower limit – upper limit].

4.1.4 Training

The participants played recreational football games for 12 weeks in sessions typically consisting of 2 x 25 minutes of 5-a-side football match with 1 min

recovery; subjects took turns as goalkeeper for 5 min in each half of the match. Sessions started with a standardized warm-up lasting 8 min (First part of FIFA 11+) to reduce the risk of injury and to prepare the body for the match. The warm-up routine consisted of 6 exercises carried out in pairs in two 30-m long rows marked by six cones; the rows were 6 m apart. The routine included two sets of jogging, dynamic stretching, lateral steps, jumping with body contact, acceleration and backward exercises [5,6]. The sessions were carried out between 8 and 10 pm on a 38 x 16 m artificial turf field. The target attendance frequencies were once and twice a per week for the LFG and MFG, respectively, yielding a total attendance at 12 and 24 sessions, respectively, for the study. Because of personal commitments and minor injuries, some subjects had to skip the training session, but no one was absent for more than two consecutive sessions. Attendance frequency at the end of the study was (mean [95% confidence limits]) 0.94 [0.89 – 0.99] and 1.74 [1.64 – 1.84] for the LFG and the MFG, respectively.

4.1.5 Training session monitoring

Subjects were monitored for heart rate (HR) and time-motion characteristics using a heart rate monitoring system (Polar, Finland) connected to a GPS system (Viper Statsports, Ireland). Before starting the session, the GPS devices were turned on and positioned for at least 15 min in the middle of the field to ensure correct acquisition of satellite signals[7]. Just before warm-up, the subjects donned an elastic thoracic belt for heart rate monitoring and a tight vest with a pocket located on the back between the scapula for the GPS device. At the end of the training session, each subject provided a rate of his own perceived effort based on the CR-100 Borg Scale[8,9].

Internal training load during the training sessions was assessed by means of two heart rate-based methods, Edwards Training Load (EdwTL)[10] and Training Impulse (TRIMP)[11], plus session-rate of perceived exertion (RPE) based on the CR100 Borg scale[8,9]. EdwTL, proposed by Edwards, was also used by Foster et al.[12] in interval training monitoring. The accumulated training duration (min) in 5 HR zones is multiplied by a coefficient relative to each zone : zone 1, 50 - 60% of HR_{max}; zone 2, 60 - 70% of HR_{max} ; zone 3, 70 - 80% of HR_{max} ; zone 4, 80 - 90% of HR_{max};

zone 5,, 90 - 100% of HR_{max}); the sum of the five products represents the internal training load as determined by the EdwTL method. TRIMP was calculated using the formula proposed by Banister:

$$TD \cdot HR_R \cdot 0.64 \cdot e^{1.92 \cdot HR_R}$$

where TD is the training session duration (min) and HR_R is determined as follows:

$$\left[(HR_{avg} - HR_{rest})/(HR_{max} - HR_{rest})\right]$$

where HR_{avg} is the average HR measured during the training session and HR_{rest} is the HR measured at rest. Session-RPE was calculated by multiplying the training session duration times the RPE given by the subjects at the end of each session and then divided by 10.

$$\lceil TD\ (minutes) \cdot RPE \rceil\ /\ 10$$

4.1.6 Internal Training Load

We found differences between the low-frequency and the moderate-frequency groups for three internal training load parameters ($p < 0.001$); the mean and 95% interval of confidence are presented in Tables 4.3 and 4.4 and Figures 4.2 and 4.3. When the internal training load indices were normalized for number of sessions, there were no differences between the two groups ($p = 0.196$, $p = 0.203$, $p = 0.976$ and $p = 0.491$ for TLEdw, TRIMP, Session-RPE and RPE, respectively). Based on these results we can say that the internal training load of the single session is not related to the frequency of playing once or twice a week. The main difference between the groups seems to be the volume of activity, because frequency doesn't seem to affect the intensity of the training session.

	LFG	MFG	p	Cohen's d
	mean [95 interval confidence]			
TLEdw [AU]	2508.37 [2288.62 - 2728.12]	4563.08 [3932.78 - 5193.38]	< 0.001	1.54 - large
TRIMP [AU]	1229.25 [1097.61 - 1360.9]	2182.02 [1821.71 - 2542.33]	< 0.001	1.4 - large
Session-RPE [AU]	3281.59 [2924.34 - 3638.84]	6079.51 [5457.73 - 6701.28]	< 0.001	1.65 - large

Table 4.3 – Accumulated Internal Training Load of LFG (low frequency group) and MFG moderate frequency group)

	LFG	MFG	p	Cohen's d
	mean [95 interval confidence]			
TLEdw [AU]	220.46 [207.10 - 233.83]	207.37 [191.06 - 223.68]	0.196	0.43 - small
TRIMP [AU]	108.36 [98.59 - 118.14]	98.93 [86.95 - 110.91]	0.203	0.43 - small
Session-RPE [AU]	289.35 [261.80 - 316.91]	290.10 [241.07 - 339.13]	0.976	0.01 - trivial
RPE [AU]	50.16 [45.66 - 54.65]	48.06 [43.81 - 52.30]	0.491	0.23 - small

Table 4.4 – Relative Internal Training Load (normalized for number of sessions played) of LFG (low frequency group) and MFG moderate frequency group).

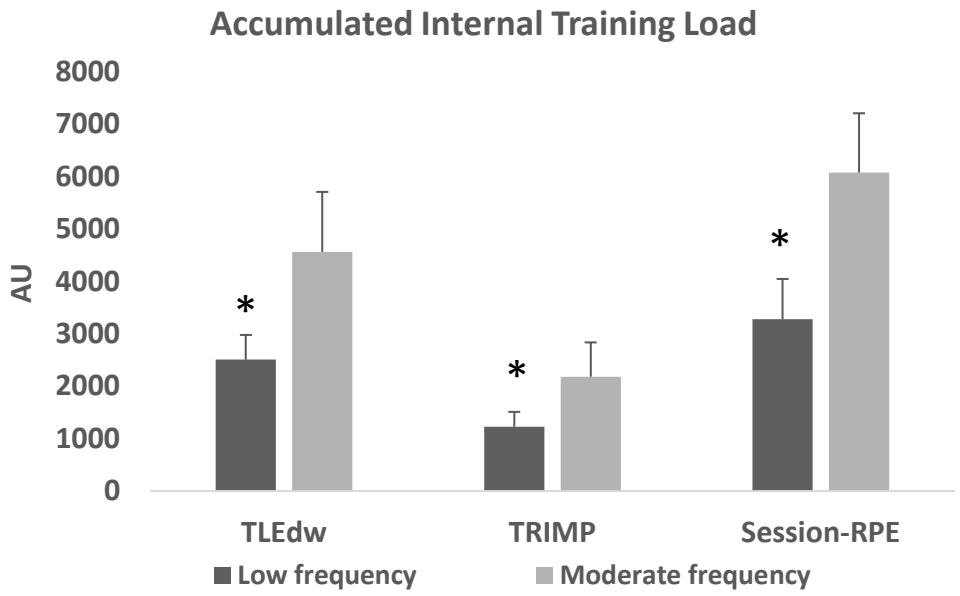

*Figure 4.2 – Accumulated Internal Training Load parameters; comparison between low frequency and moderate frequency groups. * p < 0.001 vs Moderate frequency group*

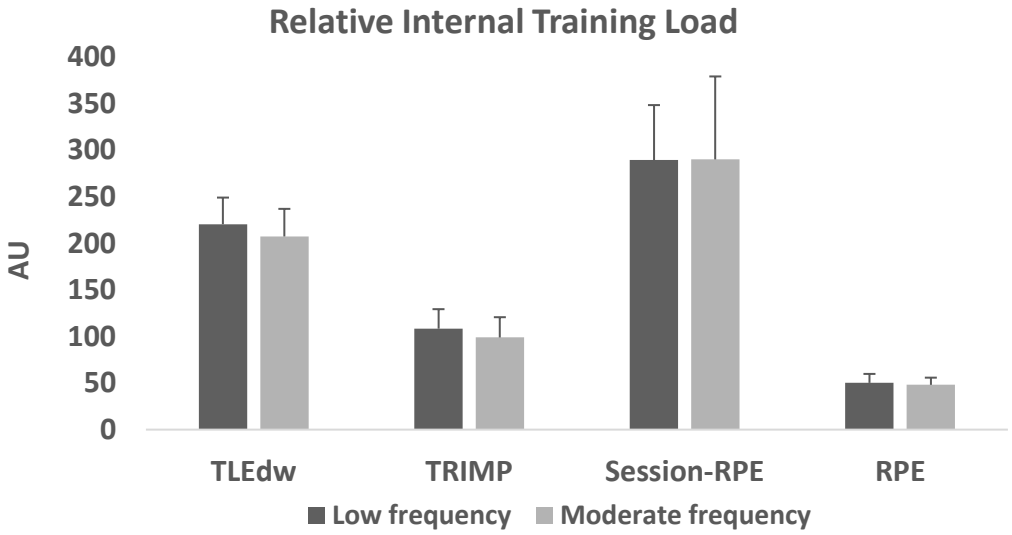

Figure 4.3 - Relative Internal Training Load (normalized for number of sessions played); comparison between low frequency and moderate frequency groups

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5 Study 1 – Can a low dose of recreational football improve cardiovascular risk factors?

5.1 Introduction

As briefly presented in the general introduction, several studies have focused their attention on the relationship between recreational soccer and chronic diseases. Most have analysed whether recreational soccer is effective for improving cardiovascular health in healthy and unhealthy people. The studies have used various different parameters to assess cardiovascular health: resting blood pressure and heart rate, body composition, waist-to-hip ratio, blood lipid profile (LDL and total cholesterol principally), and maximal oxygen consumption.

Krustrup et al.[1] carried out one of the first studies that highlighted the efficacy of recreational soccer to improve cardiovascular risk factor in healthy adults. They trained 25 sedentary men (age range 20-43 years) in 1-h sessions 3 times a week for 12 weeks. They compared a group assigned to playing soccer (SO, 5-, 6- or 7-a-side soccer, n = 13) with a group assigned to continuous running (RU, n = 12). The average intensity of the training sessions was the same in both groups (about 82% of HR_{max}) but the time spent above 90% of HR_{max} was higher in the SO than in the RU (20% ± 4 and 1% ± 1, respectively). As compared with a sedentary control group (CO), improvement was noted in several CVD risk factors in both treatment groups: maximal oxygen consumption was increased from 39.6 ± 1.1 mL/min/kg to 44.6 ± 1.1 mL/min/kg and from 39.3 ± 2.5 mL/min/kg to 42.2 ± 1.8 mL/min/kg in the SO and the RU group, respectively. Resting heart rate was lowered by 6 ± 1 bpm in the SO and by 6 ± 2 bpm in the RU. An improvement in blood pressure was also found: systolic and diastolic blood pressure decreased by 8 and 5 mm Hg in the SO and the RU group, respectively. Furthermore, the LDL cholesterol concentration was reduced (2.3 ± 0.2 vs 2.7 ± 0.2 mmol/l) only in the SO, while no change in the other lipoproteins (HDL cholesterol and total cholesterol) was noted. Benefits were also found in fat mass, with a decrease of 3% ± 0.6 and 1.8% ± 0.4 in the SO and the RU respectively. Recently, also Milanovic et al. [2] reported improvements in maximal oxygen consumption and body composition in a group of healthy untrained men (age range 20-40 years) after 12

weeks of recreational soccer. The 60-min training sessions were performed 3 times a week. At 12 weeks, the maximal oxygen consumption related to body mass was increased by about 24% in the soccer group, to some extent due to a large reduction in body mass (- 5.9 kg). The fat-free mass, as measured with a Tanita Body Composition Analyser, was increased by roughly 6%.

Similar findings have been reported in older population as well. In a study of Schmidt and colleagues[3], nine healthy and sedentary men aged 65-75 years have performed recreational soccer, 2 h per weeks, for 4 months and 3 h per a week for another 8 months. As compared with baseline, maximal oxygen uptake was 16% after 4 and 18% higher after 12 months of training, respectively. Systolic and diastolic blood pressure remained unchanged, whereas the resting heart rate was reduced by 6 bpm after 4 months and 8 bpm after 12 months from baseline.

Other interesting results have been reported by Randers et al.[4] who assessed body composition, maximal oxygen consumption, blood lipid profile, as well as resting heart rate and blood pressure before and after 12 weeks of recreational soccer performed between 2 and 3 times per week in a group of 10 healthy, sedentary young men (age range 20-43 years). In addition, the subjects continued to play for 1 year after the study period but at a lower frequency (about once a week). After the first 12 weeks of training, improvement was noted in maximal oxygen uptake (+ 3.2 mlO_2/min/kg), resting heart rate (-7 bpm), systolic blood pressure (-9 mm Hg), fat mass percentage (-2.1%), and fasting LDL cholesterol (-0.4 mM). Moreover, the study showed that a low training frequency (1.3 h per week) was enough to maintain the beneficial effects gained during the more intensive part of the training program.

Evidence to date shows that recreational football is a valid tool to stimulate musculoskeletal, metabolic, and cardiovascular adaptations and improve maximal oxygen consumption (VO_{2max}), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate at rest (RHR), as well as anthropometric parameters such as body weight, fat and lean body mass. Each of these improvements concurs to lower the risk of CVD when football is played 2 or 3 times per week. Nevertheless, few and partially controversial findings show its capacity to improve blood lipid

profile parameters: total cholesterol, HDL cholesterol, LDL cholesterol, and fasting blood glucose in CVD risk.[1,4] Furthermore, to our best knowledge, no studies have shown whether a training frequency less than 2 times per week of recreational soccer may be enough to promote positive adaptations to cardiovascular health.

Among the few studies examining the effect of low frequency exercise on aerobic fitness[5–7], Nakahara et al.[7] reported that even once-a-week high-intensity training can induce improvements in maximal oxygen consumption in healthy and unhealthy people of different ages. Specifically, they analysed the adaptations of physical fitness in 7 subjects performing a high-intensity interval training program once a week for 3 months; after 3 months the absolute and relative maximal oxygen consumption was increased by 11% and 13%, respectively. Although the 2008 guidelines suggest at least 150 min/week of physical activity to obtain health gains, there is evidence that even less can be beneficial and has a remarkable value. According to the literature[8,9], lack of time is a common barrier to wider engagement in physical activity.

Soccer is one of the world's most popular sports and is played by people of all ages, but the frequency of practice is usually low (1 or 2 times a week). A typical session lasts about 1 h, which is the amount of time the pitch is rented. For this reason, it is relevant from a practical point of view to investigate the adaptations following a more ecological (low frequency) training plan. To this end, we compared the effects of low and moderate recreational soccer training volume (1 h once and twice a week) performed as small-sided games (i.e., futsal) and analysed the effects on several cardiovascular risk factors, including aerobic fitness, resting rate, and blood pressure, blood lipid profile, body composition, and waist circumference.

5.1.1 Testing

The evaluation protocol required four visits to the laboratory before and after the control and the experimental period. The relevant outcomes of the first study were assessed during the first two evaluation sessions. A description of the procedures in these two sessions is given below.

5.1.1.1 Session 1

The subjects came to the laboratory between 7 am and 9 am after an overnight fast and were seated for 10 min under controlled environmental conditions before starting the assessments.

Resting heart rate (RHR) and blood pressure (RBP). RHR and RBP were measured at least five times with the subject sitting, separated by a 1- min interval, by means of an automatic upper arm blood pressure monitor (HEM-709; OMRON, Hoffman Estates, IL, USA), and the average was calculated. The outcomes were systolic (SBP) and diastolic (DBP) blood pressure and mean arterial pressure (MAP); the MAP was calculated as 1/3 SBP + 2/3 DBP.[10]

Blood analyses. Blood lipoprotein and hemochrome indices were also evaluated during this session. Blood was drawn in primary blood tubes containing K2EDTA (Terumo Europe N.V., Leuven, Belgium). The whole blood samples were immediately transported to the local laboratory under controlled conditions of temperature and humidity, where a complete blood cell count (CBC) was performed on an Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The analysis included measurement of haematocrit (HMC), haemoglobin (Hb), red blood cell (RBC) count, mean corpuscular volume (MCV), platelet count, mean platelet volume (MPV), white blood cell (WBC) count, and differential, as well as blood glucose. The plasma lipoprotein profile was also assessed: total cholesterol (TChol), low-density lipoprotein cholesterol (LDL chol), non-high-density lipoprotein cholesterol (nHDL chol), and triglycerides. In addition, some inflammatory markers related to cardiovascular risk were analysed and will be discussed in Study 2. Analysis of blood specimens was concluded within 3 h after sample collection. Lipoprotein analysis was performed using enzymatic kits (Roche Diagnostics, Penzberg, Germany).

Weight and Height. Weight was measured within 0.1 kg on an electronic scale at least three times, and a fourth time if there was a difference greater than 0.2 kg between the previous ones. Height was measured within 0.1 cm three times using a stadiometer. Both weight and height were measured according to a standardized reference protocol[11]. The values were averaged for statistical analysis.

5.1.1.2 Session 2

During the second evaluation, participants were assessed for body composition and aerobic fitness. Pre and Post sessions were carried out at the same time of the day. Subjects were asked to have a light meal at least 2 h before the test and refrain from any kind of physical activity the day before the test. They could drink water at libitum before and during the session.

Body composition. One of the main outcomes of Study 1 is body composition; to this end we measured eight skinfold sites (chest, subscapular, midaxilla, triceps, suprailiac, abdomen, thigh, and medial calf) and four circumferences (waist, hip, thigh, and arm). Skinfold and circumference measurements were performed by an expert technician according to published standardized procedures and sites to maximize reliability[11–13]. Measurements were taken with the subjects standing, and the technician marked the skinfold and circumference sites using an appropriate pencil. Skinfold thickness was measured to within 0.2 mm using a high-quality metal caliper. Two measurements were taken at each site in rotational order on the right side of the body; measurement was repeated if the first two values varied from each other by more than ±10%. An average of two values within ±10% of each other was used in the analysis. Circumference was measured with a tape measure to within 0.1 mm. The tape measure had a spring-loaded handle that allowed constant tension to be applied to the end of the tape during measurement. The technician measured circumference twice at each site in rotational order, applying the correct tension to the tape so that it fit tightly around the body part but did not compress the skin or the subcutaneous tissue. Each pair of measurements was averaged and then used in the analysis. Among the anthropometric and body composition characteristics related to cardiovascular health, we selected the following outcomes: body mass (BM), body-mass index (BMI), waist-to-hip circumference ratio (W:H), the sum of each skinfold gauged (∑8SKF), and the fat mass percentage (FM%) as estimated with the lean waist-triceps equation[12].

Aerobic fitness assessment. Following body composition evaluation, we assessed the cardiorespiratory characteristics in three different conditions: 6 min basal condition assessment (Basal), 6 min submaximal running (SubMax), and a maximal

incremental test (Max). Cardiorespiratory measurements were collected continuously using a breath-by-breath method on an automated open-circuit gas analysis system (Quark PFT Ergo, Cosmed). Before each test, the gas analysers were calibrated using ambient air, assumed to contain 20.93% oxygen and 0.03% carbon dioxide, and certified standard gases containing 16.0% (± 0.02%) oxygen and 5.0% (± 0.02%) carbon dioxide. The turbine flow meter for sampling respired air flow was calibrated with a 3-l calibration syringe (Cosmed Srl) according to the manufacturer's instructions. The heart rate was recorded using an elastic thoracic belt (T31, Polar, Finland) connected to the gas analysis system. The running trials were performed on a motorized treadmill (RL3500E; Rodby, Södertalje, Sweden) equipped with a safety harness connected to an emergency brake. An overview of the entire protocol is described in Figure 5.1.

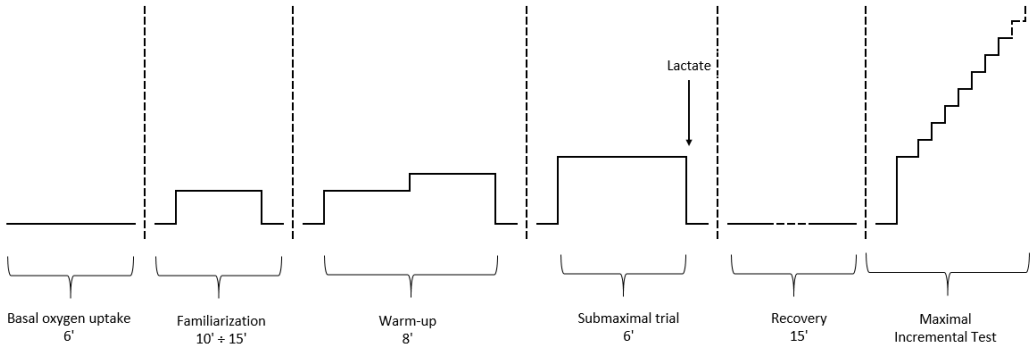

Figure 5.1 - Progression of the aerobic fitness protocol.

Basal oxygen consumption was measured for 6 min; the last 30 s of the more relevant indices (oxygen consumption, respiratory exchange ratio, and heart rate) were averaged and used for analysis.[1] Participants were asked to stand quietly without moving and to breathe normally. After familiarization with the treadmill, the subjects warmed-up for 8 min, with 4 min walking at 5 km/h and 4 min running at 6 km/h. The SubMax trial was performed at 6.5 km/h with a 1% slope. An earlobe blood sample was then immediately collected for lactate concentration analysis. The calibration and collection procedures were performed according to the

manufacturer's manual of the lactate analyser (Biosen C-line, EKF Diagnostics GmbH, Barleben, Germany). An average of the last 3 min of the respiratory exchange ratio (RER), oxygen consumption (VO_2), and submaximal heart rate (subHR) were entered in the analysis. The subjects had a 15-min rest interval before starting the maximal incremental test[1]. During the maximal incremental trial, the treadmill slope was set at 3% and the initial speed of 6.5 km/h was maintained for 3 min then increased by 0.5 km/h every 30 s until volitional exhaustion. The maximal oxygen consumption and the maximal heart rate was calculated as the 30-s and 15-s averaged values, respectively, during the last part of the test[1].

5.2 Results

For each parameter assessed, we present the estimated means with the p value, the covariate values, and the change in the mean shown as mean [95% confidence interval] (Tables 5.3, 5.4, and 5.5).

5.2.1 Anthropometric measures and body composition

As compared with the CG, changes in weight and body-mass index (BMI) were noted for the MFG (p = 0.002 and p = 0.014) but not for the LFG (p = 0.155 and p = 0.118) (Figures 5.3 and 5.4). As compared with the CG, fat mass percentage (FM) was decreased in both the LFG and the MFG (p = 0.028 and p = 0.001) (Figure 5.5). No differences in weight, BMI, and FM (p = 0.263, p = 0.88 and p = 0.373) were noted between the LFG and the MFG. Finally, no changes between groups were found for waist-to-hip ratio (W:H) (p = 0.301, p = 0.113, and p = 1 for LFG vs. CG, MFG vs. CG, and LFG vs. MFG, respectively) (Figure 5.6).

5.2.2 Maximal oxygen consumption (VO_2max)

As compared with the CG, a clear improvement in maximal oxygen consumption was noted for both the LFG and the MFG (p = < 0.001 and p < 0.001) but no differences were found between the two experimental groups (p = 0.433) (Figure 5.7).

5.2.3 Blood pressure

No changes in systolic blood pressure (SBP) (p = 0.453, p = 1, and p = 1) (Figure 5.8), diastolic blood pressure (DBP) (p = 0.313, p = 1, and p = 1) (Figure 4.9), and mean blood pressure (MBP) (p = 0.271, p = 1, and p = 1) were noted on comparison between CG vs. LFG, CG vs. MFG, and LFG vs. MFG, respectively (Figure 5.10).

5.2.4 Blood lipid profile

Blood lipid profile analysis revealed no differences in CG vs. LFG, CG vs. MFG, and LFG vs. MFG. Total cholesterol (p = 0.110, p =0.107, and p = 1) (Figure 5.11), low-density protein (p = 0.368, p =0.263, and p = 1) (Figure 5.12), non-high-density

protein (p = 1, p =0.265, and p = 1) (Figure 5.13), and triglycerides (p = 1, p = 1, and p = 1) (Figure 5.14) remained unchanged.

5.3 Discussion

The main aim of this study was to evaluate the effect of recreational football, played either 1 h or 2 h a week, on the principal cardiovascular factors in healthy, sedentary middle-aged men. Beneficial effects on some parameters were recorded for both groups, especially aerobic fitness and body composition

5.3.1 Anthropometric characteristics and body composition

After 12 weeks of recreational football, the body mass was reduced, on average, by 2.15 kg in the MFG as compared with the CG, whereas the decrease did not reach statistical significance for the LFG. Also, the BMI was decreased by 0.68 $kg•(m^2)^{-1}$ in the MFG as compared with the CG. In addition, the fat mass percentage was lower in both groups, with a reduction of 2.48% (~ 2 kg) in the LFG and 3.46% (~ 2.9 kg) in the MFG. These data are consistent with those reported by other studies in which the participants carried out an activity similar to our study 2 or 3 times a week for between 3 and 16 months and without any dietary intervention[1,2,4,14,15].

Elevated BMI and fat mass are two important cardiovascular risk factors and their reduction is associated with a decrease in the risk of cardiovascular events.[16,17] Although these changes in BMI and fat mass did not reach the clinically important change related to risk lowering reported in the literature[17], they could, together with the improvement in other factors, contribute to reducing cardiovascular risk, nonetheless. Waist-to-hip ratio is another useful anthropometric parameter related to the incidence of cardiovascular diseases [17,18]. Although the confidence intervals of different means for both the LFG and the MFG were nearly below zero, the reduction did not reach statistical significance.

5.3.2 Maximal oxygen consumption (VO_2max)

Maximal oxygen consumption (VO_2max) is a relevant marker for the incidence of cardiovascular diseases and mortality.[19–21] We found an improvement in both groups after 12 weeks of recreational football: the VO_2max increased, on average,

by 5.32 ml•kg^{-1}•min^{-1} in the group that played once a week and by 7.11 ml•kg^{-1}•min^{-1} in the group that played twice a week. We know that an increment of 3.5 ml•kg^{-1}•min^{-1}, equivalent to 1 MET, leads to a significant improvement in cardiovascular disease risk and mortality in apparently healthy people.[22] Our results showed average increments more than 1 MET in the LFG and more than 2 METs in the MFG, demonstrating the efficacy of recreational football for improving VO_2max, as found previously.[23] These results are shared by those of other similar studies, where increments of 10-15% VO2max were seen after recreational football training 2-3 times a week.[2,4,14]

Moreover, the novel and important finding of our study is that playing recreational football once a week for 1 h yields a beneficial gain in VO2max and reduces the cardiovascular diseases risk. According to Aspenes et al.[19], 44.2 ml•kg^{-1}•min^{-1} represents a cut-off value in apparently healthy men. They demonstrated that the odds ratio for cardiovascular disease risk was 7.9 for men with a VO_2max below that value. At the beginning of our study, only 4 (2 in the LFG and 2 in the MFG) out 40 subjects (10%) had a VO2max above 44.2 ml•kg^{-1}•min^{-1}, confirming their lack of aerobic fitness and the presence of this risk factor in the majority. By the end of the study, however, considerable improvement in VO_2max (above 44.2 ml•kg^{-1}•min^{-1}) was seen in 6 (37%) subjects in the LFG and in 16 (76%) subjects in the MFG.

5.3.3 Arterial blood pressure

Arterial blood pressure is one of the most critical risk factors related to cardiovascular diseases[24]. There is evidence for a linear association between elevated arterial blood pressure and the incidence of cardiovascular heart diseases and mortality throughout middle age, when systolic pressure is above 115 mm Hg and diastolic pressure is above 75 mm Hg.[24] The latest international guidelines issued by the American Heart Association and other international institutions now set the upper limit to 120/80 mm Hg for normal arterial blood pressure and to 130/90 mm Hg for a diagnosis of hypertension.[25] In our study, the average systolic and diastolic pressure at baseline was 117 mm Hg and 80 mm Hg, respectively, in the LFG and 115 mm Hg and 79 mm Hg, respectively, in the MFG, which can be

defined as normal values. At 12 weeks, the systolic, diastolic, and mean blood pressure are lowered by 3.5 mm Hg, 2.5 mm Hg, and 2.9 mm Hg, respectively, in the LFG and 1.7 mm Hg, 1.6 mm Hg, and 1.6 mm Hg, respectively, in the MFG but did not reach statistical significance ($p > 0.05$). In other similar studies involving normotensive[1,4] and hypertensive[26,27] subjects, the researchers found larger improvements in arterial blood pressure. The main reason for this difference could be the difference present at baseline: the average systolic blood pressure values were higher in the studies by Krustrup et al. (130 mm Hg) and Randers et al. (125 mm Hg) than in ours; higher values at baseline could have led to greater improvement during the training period.

5.3.4 Blood lipid profile

We found no changes in blood lipid profile, total cholesterol, LDL cholesterol, non-HDL cholesterol, and triglycerides at the end of the study. Other studies using either aerobic training or football training produced controversial results: total cholesterol and triglycerides were generally not responsive to training[1,26]. Basically, LDL cholesterol is the most responsive parameter to football training but not in our sample. Given the different and controversial responses of blood lipid profile parameters to aerobic and recreational football intervention reported in the literature[28], we think that a dietary intervention is needed to improve the efficacy of recreational football on these cardiovascular risk factors.

5.4 Conclusion

The main, novel finding of our study is that low frequency o recreational football training, played by healthy, untrained men, yields beneficial effects on some cardiovascular risk factors. We compared training sessions of 1 h of 5-side football matches at two different frequencies to determine whether a possible dose-response relationship existed. Many people play recreational football in their leisure time, and most of them do it just once a week. Although studies have shown the efficacy of this kind of activity for improving cardiovascular health, no data are available to date about a low-frequency recreational football effect. As described above, the

body composition was improved, and the weight and BMI lowered in the MFG, while fat mass was reduced in both the LFG and the MFG.

Maximal oxygen consumption is one of the most important cardiovascular risk factors. A relevant increment in this parameter was found in both groups, better in the MFG than the LFG but the difference was not statistically significant. An increment of more than 1 MET in both the LFG and the MFG means that the incidence risk for cardiovascular events was decreased at the 12-week assessment after training.

Summarising, our results show that, although 1 h of recreational football is less than the dose of physical activity recommended by the WHO guidelines, it still produced a gain in beneficial effects on cardiovascular health.

5.5 Tables

	Weight [kg]	BMI [kg•(m²)⁻¹]	Fat mass [%]	Waist-Hip ratio
	Mean [95% CL]			
GC	82.85 [74.00 - 91.71]	26.46 [26.15 - 26.77]	31.79 [30.80 - 32.77]	0.955 [0.948 - 0.962]
LFG	81.70 [73.40 - 90.01]	26.13 [25.82 - 26.43]	39.30 [28.33 - 30.27]	0.945 [0.939 - 0.951]
MFG	80.70 [69.81 - 91.58]	25.78 [25.45 - 26.11]	28.32 [27.20 - 29.45]	0.944 [0.936 - 0.951]
Covariate	*82.23*	*26.26*	*30.35*	*0.955*
	Change in the mean [95% CL]			
LFG - CG	-1.15 [-2.48 - 0.19] *p = 0.155* *ES 0.64 - moderate*	-0.33 [-0.75 - 0.08] *p = 0.118* *ES 0.70 - moderate*	-2.48 [-4.19 - -0.78] *p = 0.028* *ES 0.76 - moderate*	-0.010 [-0.021 - 0.001] *p = 0.301* *ES 0.69 - moderate*
MFG -CG	-2.15 [-3.58 - -0.73] *p = 0.002* *ES 1.29 - large*	-0.68 [-1.13 - -0.24] *p = 0.014* *ES 0.24 - small*	-3.46 [-5.31 - -1.61] *p = 0.001* *ES 1.57 - large*	-0.011 [-0.024 - 0.001] *p = 0.113* *ES 1.39 - large*
MFG - LFG	-1.00 [-2.42 - 0.41] *p = 0.263* *ES 0.51 - small*	-0.35 [-0.79 - 0.09] *p = 0.88* *ES 0.35 - small*	-0.98 [-2.81 - 0.85] *p = 0.373* *ES 0.85 - moderate*	-0.001 [-0.013 - 0.010] *p = 1* *ES 0.16 - trivial*

Table 5.5 - Anthropometric parameters comparisons. Weight, BMI, Waist-to-Hip ratio and Fat mass. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

	VO2max [ml•kg⁻¹•min⁻¹]	Systolic Blood Pressure [mmHg]	Diastolic Blood Pressure [mmHg]	Mean Blood Pressure [mmHg]
	Mean [95% CL]			
GC	37.39 [35.64 - 39.14]	116.16 [112.43 - 119.90]	79.41 [76.97 - 81.84]	91.664 [88.965 - 94.363]
LFG	42.71 [41.11 - 44.32]	112.68 [108.84 - 116.53]	76.86 [74.35 - 79.36]	88.738 [85.952 - 91.525]
MFG	44.50 [42.73 - 46.27]	114.49 [110.32 - 118.67]	77.79 [75.07 - 80.50]	90.029 [87.017 - 93.041]
Covariate	*39.72*	*116.09*	*79.33*	*91.580*
	Change in the mean [95% CL]			
LFG - CG	5.32 [2.39 - 8.25] *p < 0.001* *ES 2.14 - large*	-3.48 [-10.09 - 3.13] *p = 0.453* *ES 0.57 - small*	-2.55 [-6.86 - 1.76] *p = 0.313* *ES 0.61 - moderate*	-2.926 [-7.711 - 1.859] *p = 0.271* *ES 0.69 - moderate*
MFG -CG	7.11 [4.04 - 10.18] *p < 0.001* *ES 2.64 - large*	-1.68 [-8.58 - 5.22] *p = 1* *ES 0.27 - small*	-1.62 [-6.11 - 2.88] *p = 1* *ES 0.36 - small*	-1.635 [-6.625 - 3.354] *p = 1* *ES 0.37 - small*
MFG - LFG	1.79 [-1.16 - 4.73] *p = 0.433* *ES 0.52 - small*	1.80 [-5.19 - 8.80] *p = 1* *ES 0.20 - small*	0.93 [-3.63 - 5.49] *p = 1* *ES 0.17 - trivial*	1.291 [-3.772 - 6.353] *p = 1* *ES 0.20 - small*

Table 5.6 Maximal oxygen consumption, diastolic blood pressure, systolic blood pressure, and mean blood pressure. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

	Total-Cholesterol [mg•dL^{-1}]	LDL-Cholesterol [mg•dL^{-1}]	NON-HDL-Cholesterol [mg•dL^{-1}]	Triglycerides [mg•dL^{-1}]
	Mean [95% CL]			
GC	208.06 [201.41 - 214.71]	132.52 [125.98 - 139.06]	155.21 [146.81 - 163.60]	119.62 [98.06 - 141.18]
LFG	193.43 [185.97 - 200.90]	123.97 [116.02 - 131.91]	150.70 [139.58 - 161.82]	122.72 [99.61 - 145.83]
MFG	192.00 [184.08 - 199.91]	123.68 [115.77 - 131.58]	145.75 [135.42 - 156.08]	125.21 [100.41 - 150.01]
Covariate	*206.98*	*136.62*	*158.17*	*116.96*
	Change in the mean [95% CL]			
LFG - CG	-14.63 [-27.69 - -1.56] *p = 0.110* *ES 0.96 - moderate*	-8.55 [-21.28 - 4.18] *p = 0.368* *ES 0.57 - small*	-4.51 [-21.73 - 12.71] *p = 1* *ES 0.39 - small*	3.10 [-35.91 - 42.11] *p = 1* *ES 0.06 - trivial*
MFG -CG	-16.06 [-29.57 - -2.54] *p = 0.107* *ES 0.85 - moderate*	-8.85 [-21.54 - 3.85] *p = 0.263* *ES 0.58 - small*	-9.45 [-25.91 - 7.00] *p = 0.265* *ES 0.43 - small*	5.59 [-34.97 - 46.15] *p = 1* *ES 0.01 - trivial*
MFG - LFG	-1.43 [-15.66 - 12.79] *p = 1* *ES 0.03 - trivial*	-0.29 [-14.15 - 13.57] *p = 1* *ES 0.11 - trivial*	-4.95 [-23.71 - 13.82] *p = 1* *ES 0.49 - small*	2.49 [-39.35 - 44.34] *p = 1* *ES 0.03 - trivial*

Table 5.7 Blood lipid profile. Total cholesterol: Total cholesterol, LDL cholesterol: Low density lipoprotein, NON-HDL-cholesterol: NON-high-density-cholesterol, and triglycerides. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group, and CG = Control group.

5.6 Charts

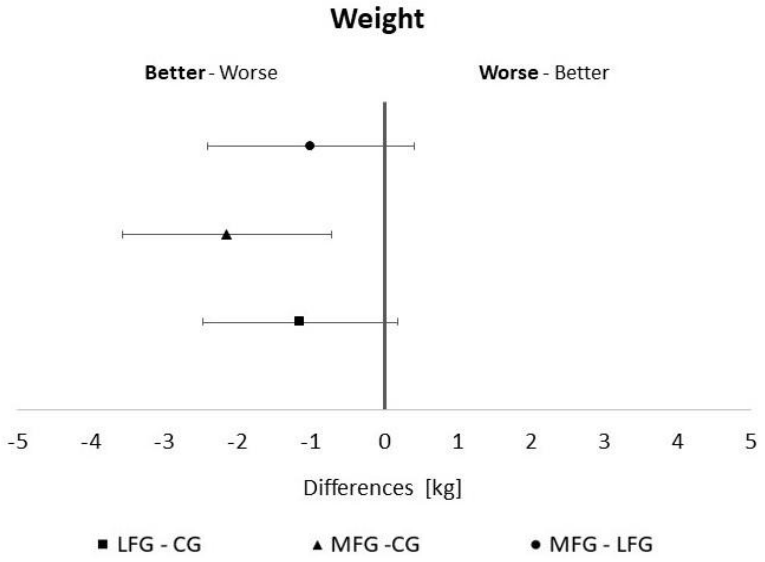

Figure 5.4 Weight. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

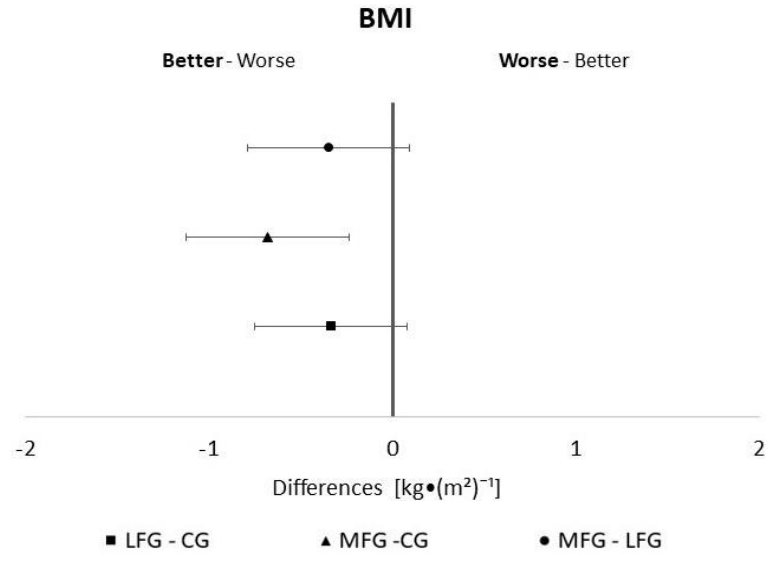

Figure 5.4 Body-Mass Index. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

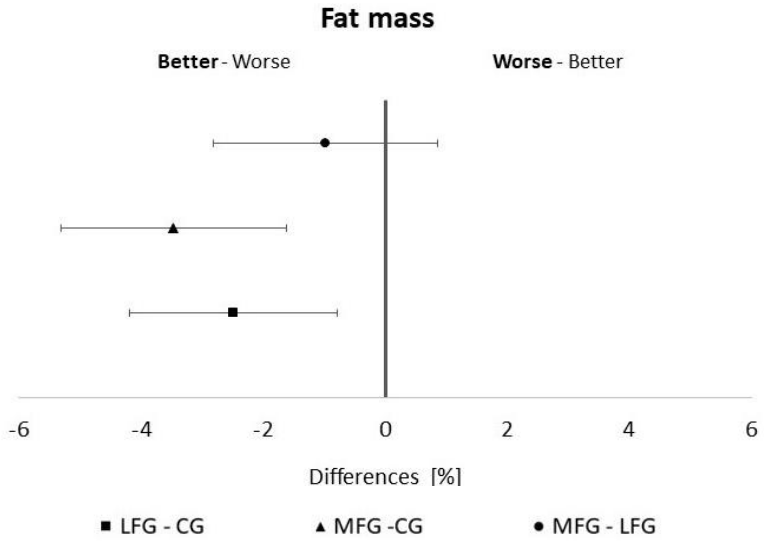

Figure 5.5 Fat mass. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

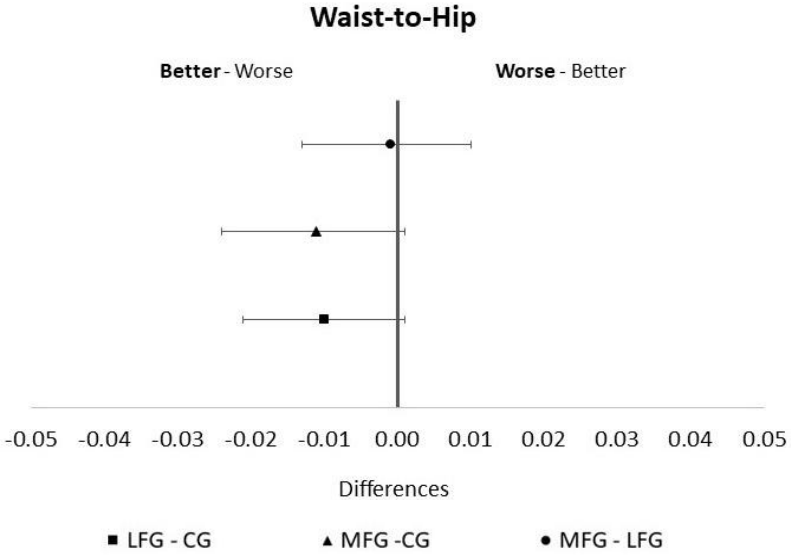

Figure 5.6 Waist-to-hip ratio. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

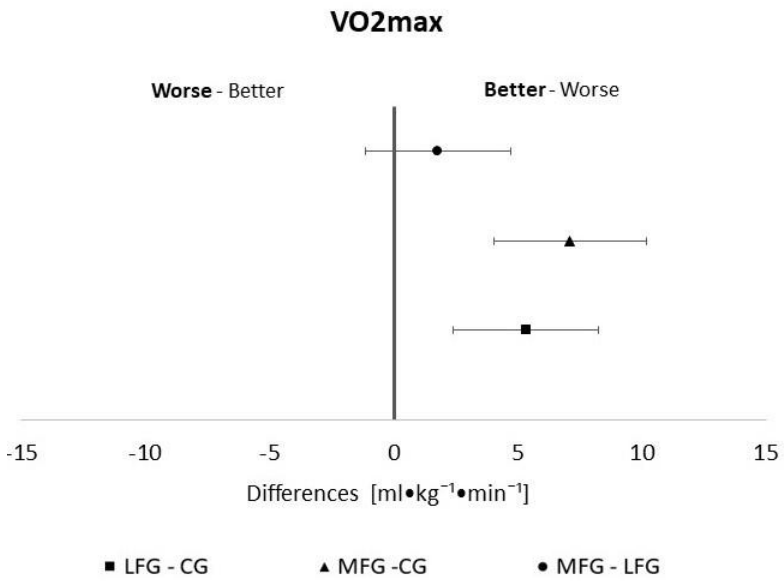

Figure 5.7 Maximal oxygen consumption. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

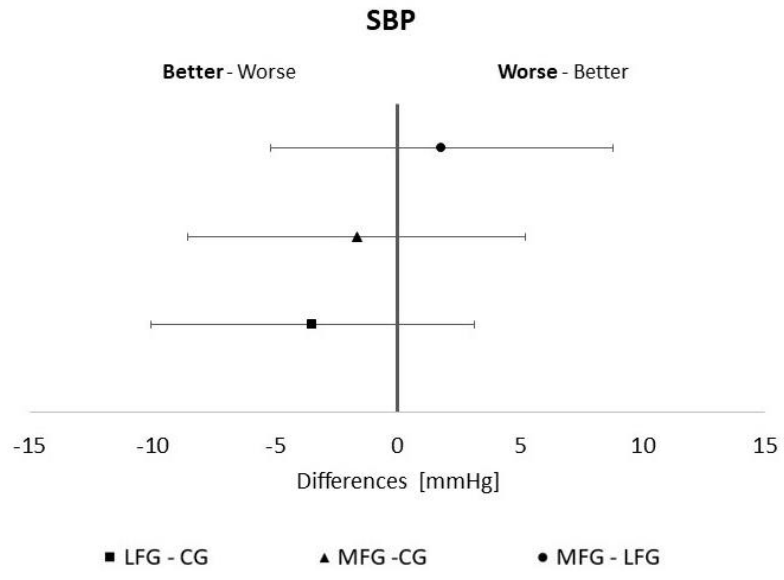

Figure 5.8 Systolic blood pressure. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

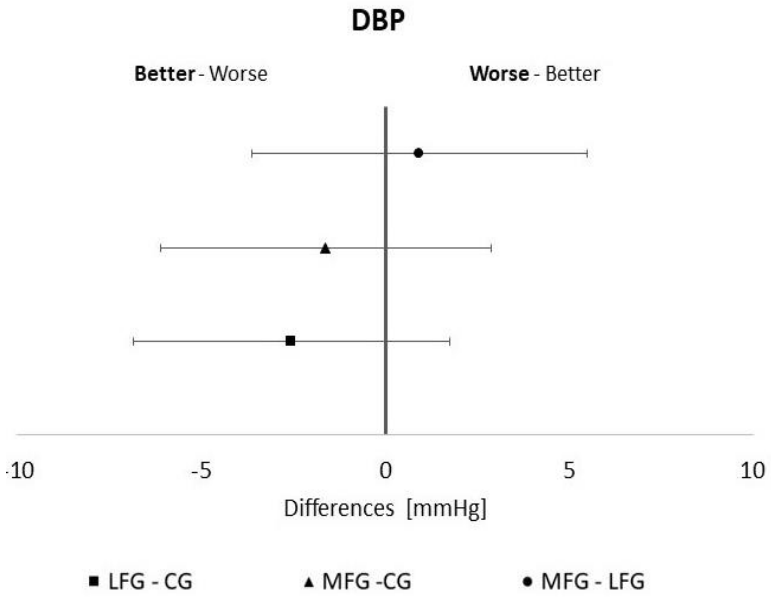

Figure 5.9 Diastolic blood pressure. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

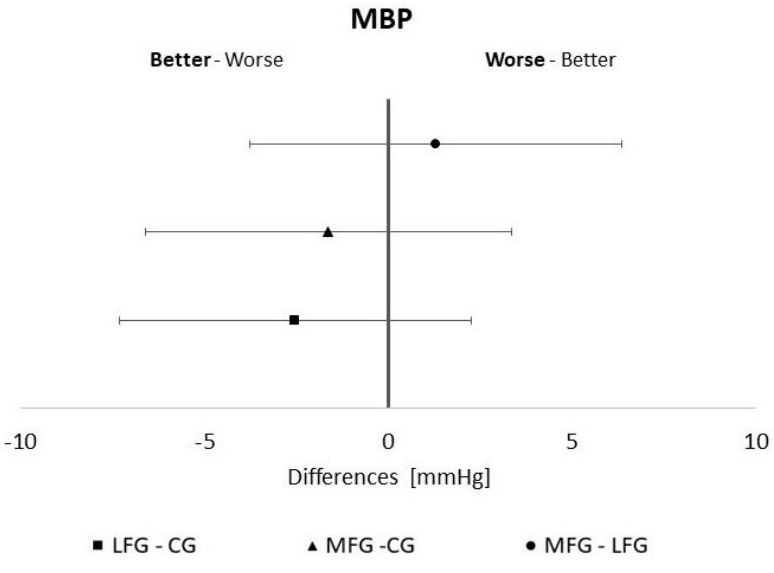

Figure 5.10 Mean blood pressure. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

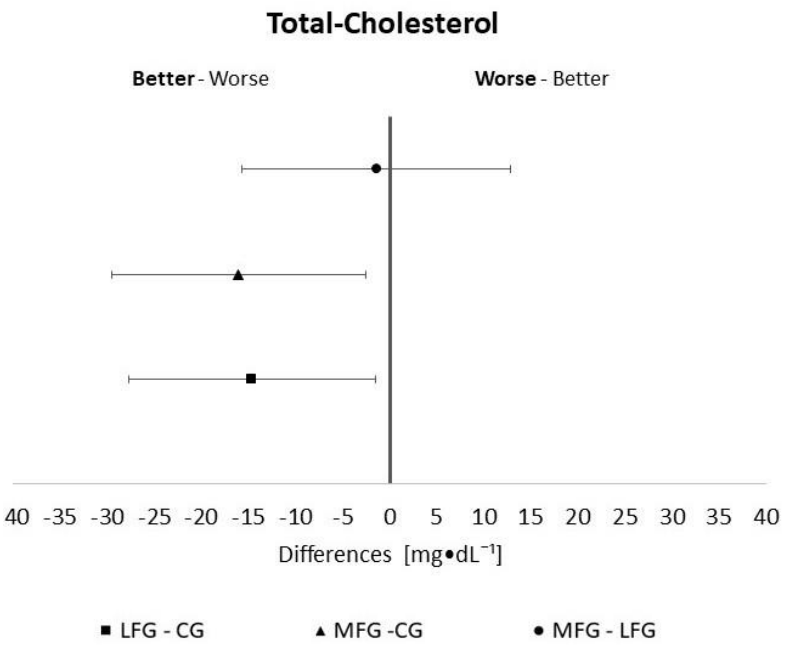

Figure 5.11 Blood lipid profile comparison. Total cholesterol. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group.

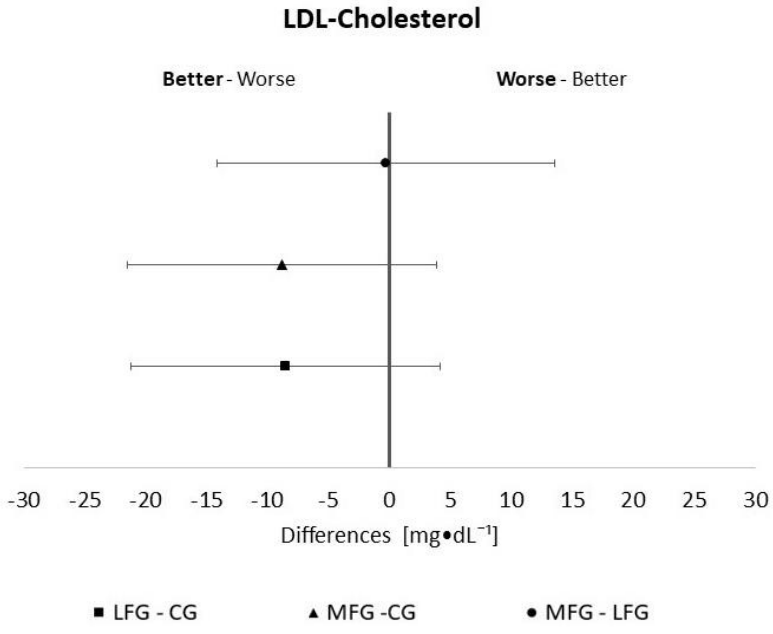

Figure 5.12 Blood lipid profile comparison. LDL cholesterol. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group.

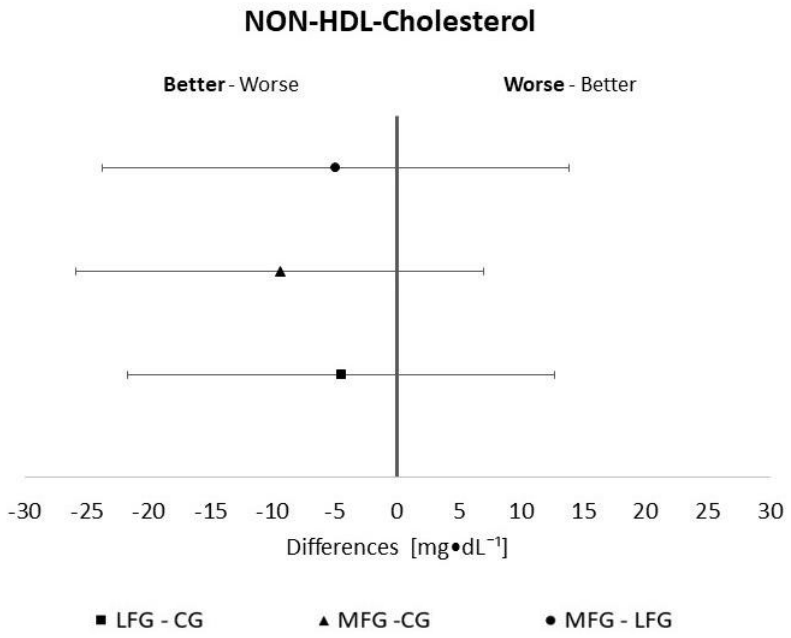

Figure 5.13 Blood lipid profile comparison. NON-HDL cholesterol. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group.

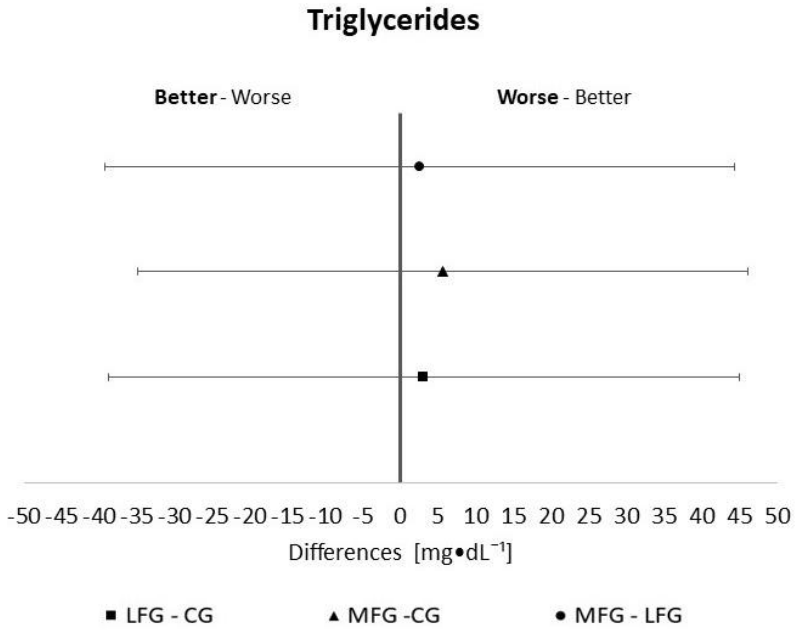

Figure 5.14 Blood lipid profile comparison. Triglycerides Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group.

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6 Study 2 - Changes in inflammatory biomarkers following recreational football activity

6.1 Introduction

Atherosclerosis is responsible for adverse cardiovascular events, such as stroke, coronary artery disease, and peripheral artery disease, which figure among the major causes of cardiovascular morbidity and mortality. Atherosclerosis has been documented since ancient times[1–3]. Lesions found in Egyptian mummies resemble those often observed in vascular surgery and pathological histology. It wasn't until the 19th century that vascular alterations came under medical research scrutiny. The surgeon-pathologist Jean Lobstein introduced the term "arteriosclerosis" in 1829. [4] Later, the pathologist Carl von Rokitansky in Vienna and Rudolf Virchow in Berlin proposed two different theories for the cellular inflammatory changes they observed in atherosclerotic vessel walls.[5,6] von Rokitansky saw these changes as secondary to the development of the disease process, whereas Virchow attributed them a primary role. Accordingly, Virchow[6] coined the term "cellular pathology" to describe the primary role of cellular inflammation in the genesis of atherosclerosis. Later theories advanced during the 20th century include the idea by Ross et al. that mechanical injury, toxins, and oxygen radicals lead primarily to endothelial dysfunction. An alternative hypothesis related to lipoproteins posits that low-density lipoproteins (LDL) play a key role. For instance, Navab[7] demonstrated that LDL is transported into the intima where it is modified and acts as a chemoattractant between monocytes and smooth cells, leading to foam cell formation ("retention of modified LDL hypothesis"). Therefore, it was already clear at the end of the last century that lipoproteins are involved in the genesis of atherosclerosis. Since then, atherosclerosis has been discovered a much more complex mechanism than a simple lipid settlement. We can now describe atherosclerosis as an inflammatory condition characterized by an abnormal lipid metabolism and as a problematic adaptive inflammatory response. This process usually starts with a lesion in the endothelium that leads to endothelial cell activation and recruitment of inflammatory cells to the vessel wall. Activation of endothelial cells facilitates the retention of LDLs in the intima where they are

susceptible to oxidative modification by reactive oxygen species (ROS) and enzymes released by inflammatory cells.[8] Figure 6.1 shows the initial phase of atherosclerosis in normal conditions, when the intima is composed of a single layer of endothelial cells overlying a subendothelial matrix that contains occasional resident smooth muscle cells. The underlying tunica media, separated from the intima by the internal elastic lamina, contains multiple layers of vascular smooth muscle cells.

Usually, circulating leukocytes have low affinity with the endothelium under physiological conditions. In an inflammatory condition, however, several factors (e.g., diet high in saturated fat, hypercholesterolemia, obesity, hyperglycemia, insulin resistance, hypertension, and smoking) can trigger the endothelial expression of adhesion molecules (e.g., P-selectin and vascular cell adhesion molecule-1 [VCAM-1]) that mediate the attachment of circulating monocytes and lymphocytes. Selectins mediate a loose, rolling interaction of leukocytes with the inflammatorily activated endothelial cells. Integrins mediate firm attachment. Chemokines expressed within atheroma provide a chemotactic stimulus to the adherent leukocytes, directing their diapedesis and migration into the intima, where they take residence and divide. Figure 6.1 illustrates these steps in a left-to-right chronological sequence.[9,10]

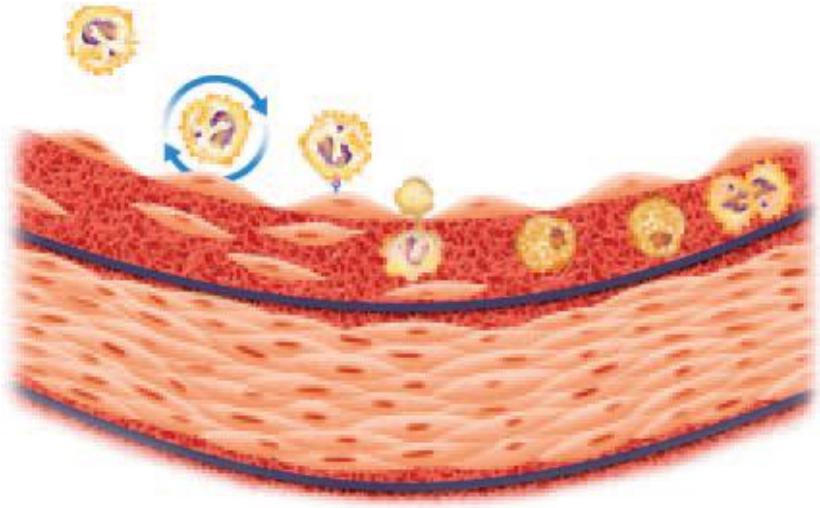

Figure 6.1 - Atherosclerosis, initiation phase – From Libby et al. 2002

Immune response is both innate and adaptive: the action of monocytes and macrophages and the continuous accumulation of lipoprotein activate the apoptosis of foam cells promoted by the endoplasmic reticulum. This scenario is characterized by a deficit of pro-apoptotic factors, which prevents cell apoptosis from contributing to progression of atherosclerosis.[8]

In response to platelet-derived growth factor released by activated macrophages and endothelial cells, smooth muscle cells (SMCs) migrate from the tunica media into the intima, where they proliferate under the influence of various growth factors and secrete extracellular matrix protein (Figure 6.2). This process causes the lesion to evolve from a lipid-rich plaque to a fibrotic and, ultimately, a calcified plaque that

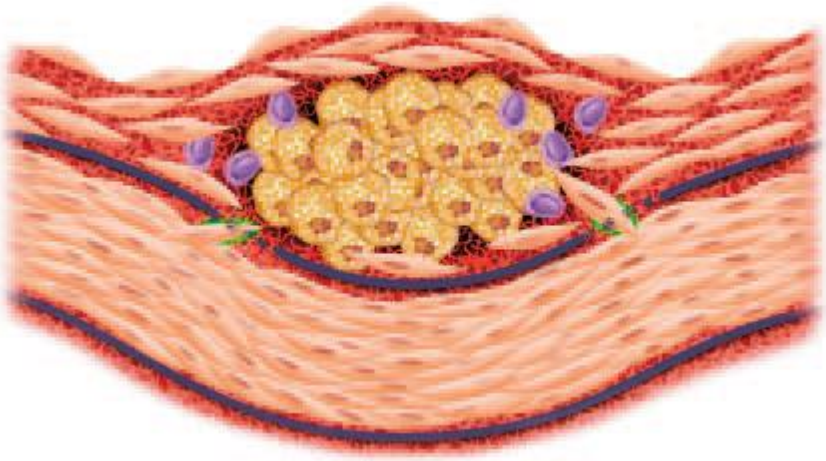

Figure 6.2 –Atherosclerosis progression - From Libby et al. 2002

may create a stenosis.[10] The scene is even more complicated in advanced atherosclerosis. While an acute inflammation is generally self-limiting, atherosclerosis is clearly an unresolved inflammation owing to the lack of mediators that promote the switch from pro-inflammation to anti-inflammation mediators.[8] Plaque rupture and consequent thrombosis set the stage for the most dangerous acute complications of atherosclerosis (Figure 6.3); a physical disruption of the fibrous cap allows the blood to mix with the thrombogenic material in the lipid core or the subendothelial region of the intima.[11] This initiates the formation of a

thrombus, which can lead to a sudden and dramatic obstruction of blood flow through the affected artery. If the thrombus is non-occlusive or transient, it may be either clinically silent or cause symptoms characteristic of an acute coronary syndrome. In summary, inflammation plays a key role in every phase of atherosclerosis (i.e., initiation, progression and destabilization) and it regulates both the "solid-state" thrombotic potential in the plaque itself and the prothrombotic and antifibrinolytic capacity of blood in the fluid phase.[10,12]

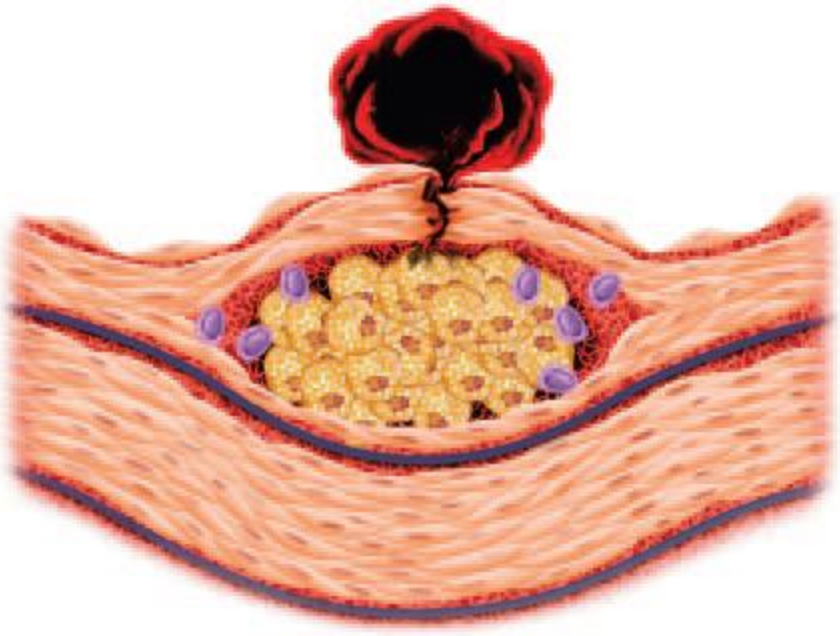

Figure 6.3 - Atherosclerosis Destabilization - From Libby et al. 2002

Building on these findings about the inflammatory pathogenesis of atherosclerosis, researchers have tried to clarify the role of the multiple biomarkers involved (Figure 6.4).[13]

Several inflammatory markers have studied for a relationship between elevated levels and future cardiovascular events in apparently healthy women and men. Prospective epidemiological studies have demonstrated increased vascular risk in association with increased basal levels of cytokines (e.g., IL-6 and TNF-α)[14–17] and cell adhesion molecules (e.g., soluble ICAM-1, P-selectin, and E-selectin).[18–20]

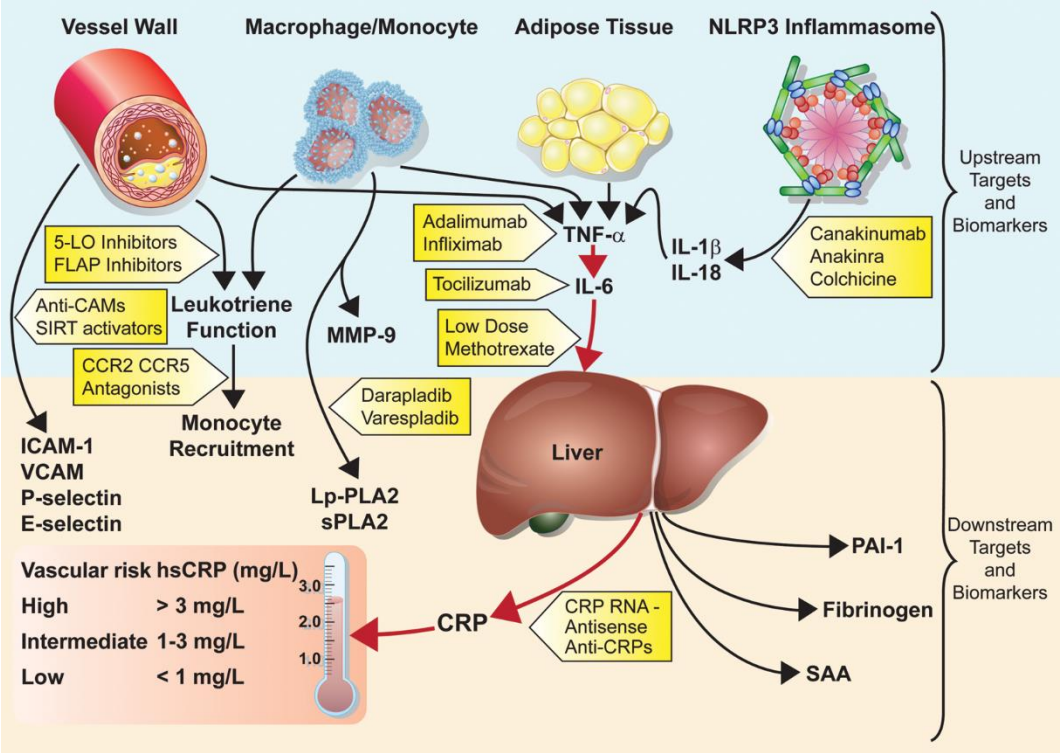

Figure 6.4 - Targeting inflammatory pathways for the treatment of cardiovascular disease. From Ridker 2014

Downstream acute-phase reactants such as C-reactive protein (CRP), fibrinogen, and serum amyloid-A also have a predictive role in asymptomatic people.[14,21,22]

Physical activity can contribute to preventing and reducing the incidence of atherosclerosis and other cardiovascular complications.[23–25] This positive action involves anti-inflammatory effects such as the reduction in inflammatory biomarkers[26] (Figure 6.5).

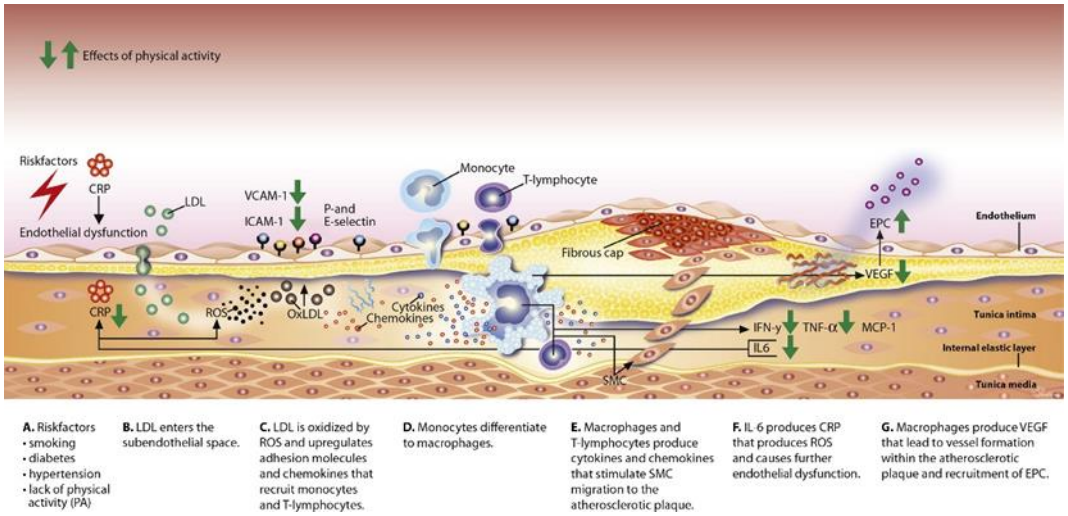

Figure 6.5 - An overview over the effect of physical activity/exercise on key factors in the atherosclerotic process. The green arrows show the effect of physical activity/exercise. Reprinted from Palmefors et al. 2014

C-reactive protein

C-reactive protein (CRP) is a non-specific marker of systemic inflammation and one of the most commonly used markers of cardiovascular risk. CRP is produced primarily by the hepatocytes in the liver as part of the acute phase response mainly upon stimulation by inteleukine-6.[27] Studies have revealed its relationship with the risk of future events in apparently healthy people and with disease severity in patients with CVD.[28] In their meta-analysis, Musunuru et al.[29] highlighted an important association between elevated high sensibility C-reactive protein (hsCRP) levels and elevated risk of future coronary events in different cohorts. Though the relationship between cardiovascular risk and hsCRP levels was fairly linear, two thresholds to stratify patients and three risk groups have been defined: low (< 1 mg/L), moderate (1-3 mg/L), and high (> 3 mg/L). In addition, another meta-

analysis[30] showed a close association between CRP concentration and subsequent risk of CVD in individuals without initial vascular disease (Figure 6.6).

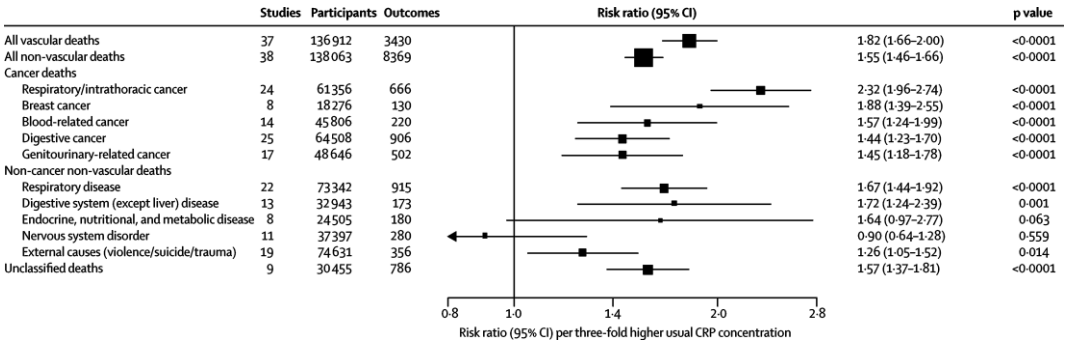

Figure 6.6 - *Age-adjusted and sex-adjusted risk ratios for mortality from vascular and non-vascular diseases per three-fold higher usual C-reactive protein (CRP) concentration. Reprinted from The Lancet 2010*

Lymphocytes and Neutrophils

In the last two decades, studies have focused on the role of white blood cell (WBC) count in clinical practice. Balta et al. (2013) showed an association between the neutrophil-lymphocyte ratio (NLR) and several inflammatory conditions.[31] The NLR is the ratio between the neutrophil and the lymphocyte count.[32] This index, used as an inflammatory marker, was demonstrated to have a predictive function for death, myocardial infarction, and high risk of CAD.[33–35] Research has

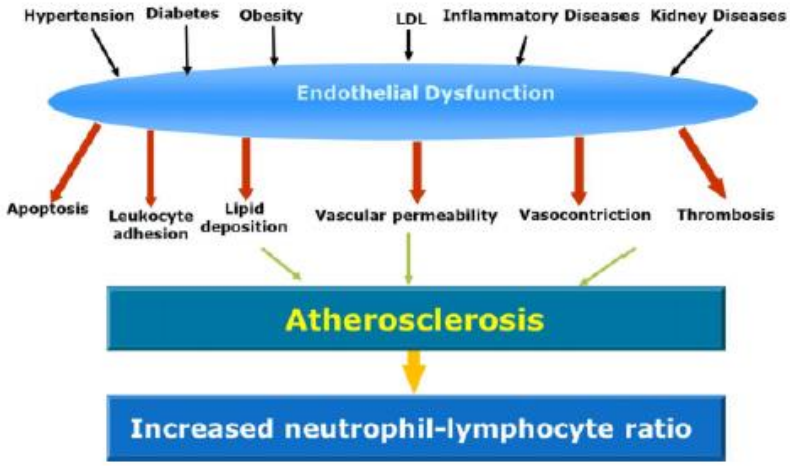

Figure 6.7 - Endothelial dysfunction can be observed in several diseases Reprinted from Balta et al. 2016

highlighted that a high NLR, even with a normal WBC count, is related to risk factors such as diabetes mellitus, hypertension, metabolic syndrome, obesity, hyperlipidaemia, lifestyle habits, and endothelial dysfunction (Figure 6.7).[36,37] In addition, metabolic syndrome presents a condition of inflammation [38] and there is evidence that it may be an early marker of developing CVD.[39] Accordingly, NLR has been proposed as a useful biomarker to predict CV risk.[40]

Physical activity

The recent literature has analysed the anti-inflammatory role of regular physical activity. As described in the previous chapter, physical activity has beneficial effects on the major cardiovascular risk factors. Some of these positive effects may derive from an improvement in key atherosclerotic and angiogenic factors related to the progression of atherosclerosis. Research has shown a clear role of physical activity, particularly leisure-time physical activity (LTPA), in improving endothelial function and key biomarkers of atherosclerosis, such as inflammatory markers and adipokines.

A recent review[41] of randomized controlled trials (RCTs) described the influence of different levels of LTPA on inflammatory markers such as CRP and IL-6. The analysis included 13 studies involving male and female adults engaged in different kinds of LTPA, frequency, and volume. Four out of 7 studies showed a beneficial effect of LTPA on CRP, suggesting a more consistent effect in older women who are still active for longer periods (> 24 weeks). Greater inconsistency was noted among male participants. Five studies investigating the effects on IL-6 reported inconsistent results. One of these studies was particularly relevant for our study. Mendham et al.[42] demonstrated a reduction in both CRP and IL-6 in a group of middle-aged, sedentary men after 8 weeks of rugby touch small-sided games played 3 times a week. Despite these conflicting results, there are indications for a beneficial effect of regular physical activity on CRP and IL-6.[43] As described above, WBC and NLR are two useful markers of inflammation. Although many studies have explained the role of physical activity on the immune system and its effects on WBC, lymphocytes and their subtype expression, few studies have

assessed their relationship with regular physical activity in healthy people from an inflammatory point of view in relation to CVD.

Aerobic exercise performed by sedentary, young men for 30 min 3 times a week, was demonstrated been effective in improving NLR thanks to an increase in WBC.[44] More recently, Momesso dos Santos et al.[45] demonstrated changes in lymphocyte proliferation and gene expression in overweight children after physical exercise performed twice a week.

In this second study, we analysed the effect of two different frequencies and volumes of recreational football on the major inflammatory biomarkers that are normally used in risk stratification and clinical evaluation.

6.2 Methods

The study design, subjects, and training intervention have been described in the previous chapter. Briefly, we compared the outcome of 12 weeks of three different physical activity conditions in 40 healthy, sedentary men (age 44.3 [42.4 – 46.2] years): 1 h of recreational football once a week, 1 h twice a week, and no physical activity. Each training session lasted 1 h and consisted mainly in a 5-side football match; the internal and external training load was monitored using a heart rate monitor and global position system devices.

6.2.1 Inflammatory marker analysis

The subjects arrived at the laboratory between 7 am and 9 am after an overnight fast and were then seated for 10 min under controlled environment conditions before starting the assessments. Blood was drawn in primary blood tubes containing K2EDTA (Terumo Europe N.V., Leuven, Belgium). The whole blood samples were immediately transported to the local laboratory under controlled conditions of temperature and humidity, where a complete blood cell count (CBC) and C-Reactive Protein (CRP) analysis were performed on an Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown NY, USA). For this study, only the changes in WBC, lymphocytes, neutrophils and CRP were taken into account.

6.3 Results

The data are presented as estimated mean and change in the mean with respective 95% CI (Tables 6.1 and 6.2). No differences were found in WBC (p = 0.146, p = 0.329, and p = 1) (Figure 6.8), NLR (p = 1, p = 0.308, and p = 0.601) (Figure 6.9), CRP (p = 1, p = 0.965, and p = 0.297) (Figure 6.10) when we compared LFG vs CG, MFG vs CG, and MFG vs LFG. Comparison between the merged training group (FG) and the CG showed no differences in NLR (p = 0.296) (Figure 6.12), CRP (p = 0.993) (Figure 6.13), whereas WBC was higher in the FG than in the CG (p = 0.039) (Figure 6.11).

6.4 Discussion

As mentioned before, there is evidence that regular physical activity plays an important role in fighting the negative effects of oxidative stress and in improving antioxidant defences and immune system responses. This chronic positive effect derives from a balance between the oxidative stress essentially caused by ROS and the adaptive response to exercise.[46] Some studies have demonstrated an inverse association between LTPA and level of inflammation, as assessed by CRP, WBC, and cytokines such as IL-6,[26,47,48] while others showed opposite results.[49,50] The reasons for these controversial effects could be several, including different types of training and duration, as well as individual differences in adaptation.

To the best of our knowledge, few studies to date have focused on the potential effect of recreational football on inflammation in relation to CVD and only two studies involved men. [51–53] Andersen et al.[51] reported no differences in CRP after 3 months of recreational football training played 2 h/week by a group of hypertensive middle-aged men. No changes in CRP were found by Krustrup et al.[52] in another group of hypertensive men after 6 months of recreational football played twice a week. In contrast, Mendham et al.[42] found a decrease in CRP, IL-6, and leptin in a group of sedentary men after 8 weeks of small-sided rugby played 3 times a week.

Our results are shared by those of Andersen et al. and Krustrup et al. We found no changes in CRP, WBC, and NLR in the LFG, MFG, and CG at 12 weeks. The

higher WBC in the FG versus the CG might represent an inflammation increase, but the lack of differences in CRP and NLR do not support this hypothesis. The lack of improvement in inflammation markers in our study may be explained by the baseline values. According to the consensus statement on metabolic syndrome[54], only one of our subjects met the criteria for this diagnosis. Furthermore, the baseline CRP values can be defined as low risk for more than 50% of the participants.[55] According to Mendham et al., a greater volume of recreational football or similar (i.e., rugby) might lead to an improvement in inflammatory conditions, although a further decrease in inflammatory markers in a healthy population would be difficult to obtain.

6.5 Conclusion

The main aim of this study was to determine whether a low dose of recreational football can improve inflammatory markers related to atherosclerosis. The results are in line with previous studies. It seems that a low or a moderate frequency (once or twice a week) of recreational football does not lead to beneficial effects on inflammatory conditions (CRP, WBC, and NLR) in sedentary, healthy middle-aged men.

6.6 Tables

	WBC [g/L]	NLR	CRP [mg/L]
	Mean [95% CL]		
GC	5.73 [5.33 - 6.14]	1.75 [1.57 - 1.93]	1.91 [1.16 - 2.65]
LFG	6.31 [5.90 - 6.73]	1.73 [1.55 - 1.90]	1.54 [0.87 - 2.21]
MFG	6.17 [5.73 - 6.61]	1.56 [1.36 - 1.76]	1.98 [1.19 - 2.77]
Covariate	*5.94*	*26.26*	*1.52*
	Change in the mean [95% CL]		
LFG - CG	0.58 [-0.14 - 1.29] *p = 0.146* *ES 0.80 - moderate*	-0.03 [-0.33 - 0.28] *p = 1* *ES 0.07- trivial*	-0.37 [-1.61 - 0.88] *p = 1* *ES 0.27 - small*
MFG -CG	0.44 [-0.29 - 1.17] *p = 0.329* *ES 0.61 - moderate*	-0.19 [-0.52 - 0.14] *p = 0.308* *ES 0.53 - small*	0.08 [-1.27 - 1.42] *p = 1* *ES 0.03 - trivial*
MFG - LFG	-0.14 [-0.88 - 0.61] *p = 1* *ES 0.14 - trivial*	-0.16 [-0.49 - 0.17] *p = 0.601* *ES 0.42 - small*	0.44 [-0.84 - 1.72] *p = 0.6.42* *ES 0.20 - small*

Table 6.1 – Inflammatory markers. WBC = white blood count, NLR = Neutrophil: lymphocyte ratio, CRP = C-reactive protein. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

	WBC [g/L]	NLR	CRP [mg/L]
	Mean [95% CL]		
GC	5.73 [5.33 - 6.13]	1.75 [1.58 - 1.93]	1.91 [1.13 - 2.68]
FG	6.24 [5.94 - 6.53]	1.66 [1.53 - 1.78]	1.71 [1.18 - 2.24]
Covariate	*5.94*	*1.68*	*1.52*
	Change in the mean [95% CL]		
FG - CG	0.50 [0.01 - 1.00] *p = 0.039* *ES 0.70 - moderate*	-0.09 [-0.31 - 0.12] *p = 0.296* *ES 0.26 - small*	-0.20 [-1.13 - 0.74] *p = 0.897* *ES 0.18 - trivial*

Table 6.2 - Inflammatory markers. WBC = white blood count, NLR = Neutrophil: lymphocyte ratio, CRP = C-reactive protein. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

6.7 Charts

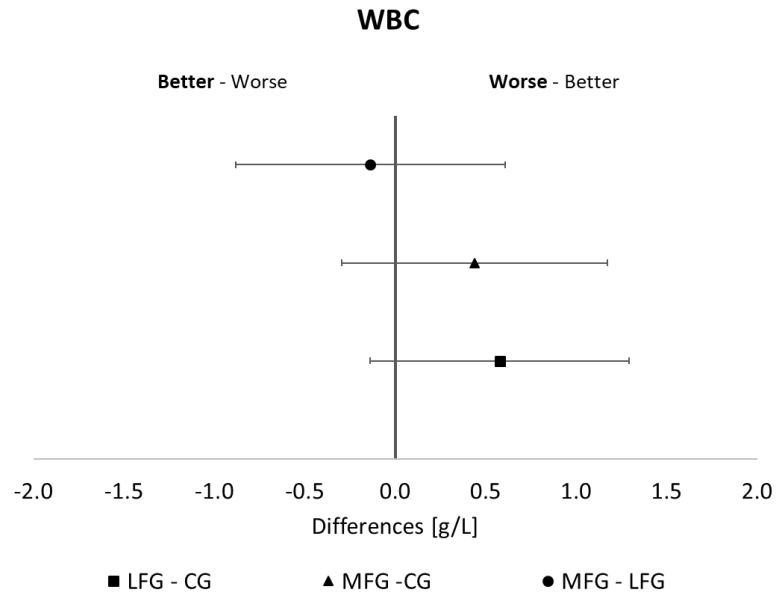

Figure 6.8 – White blood count (WBC) concentration. LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

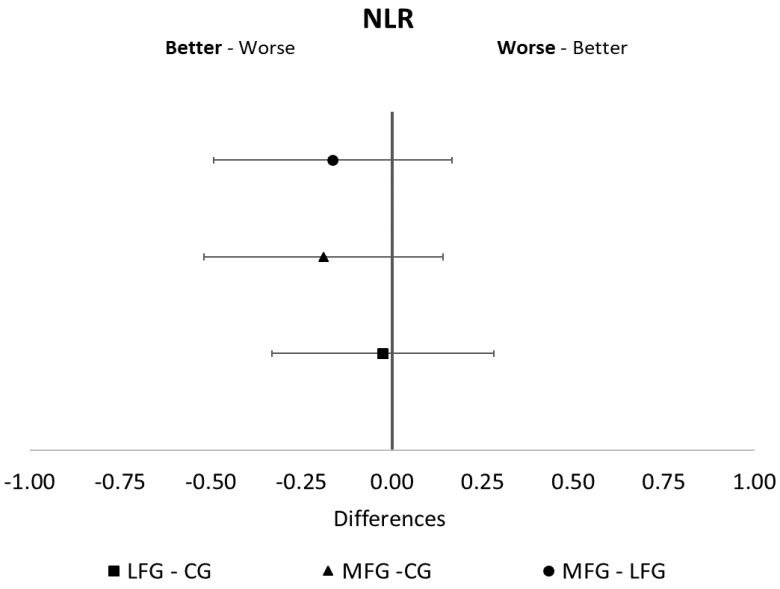

Figure 6.9 – Neutrophil: lymphocyte ratio (NLR). LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

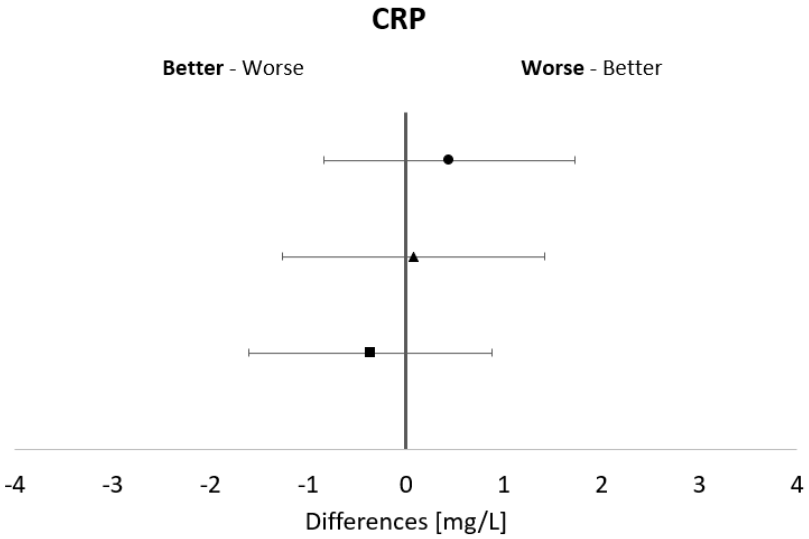

Figure 6.10 – C-reactive protein (CRP) concentration. LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

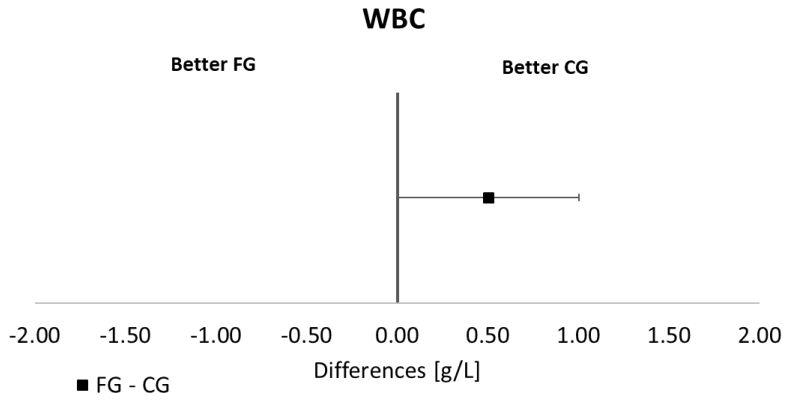

Figure 6.11 - White blood count (WBC) concentration. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

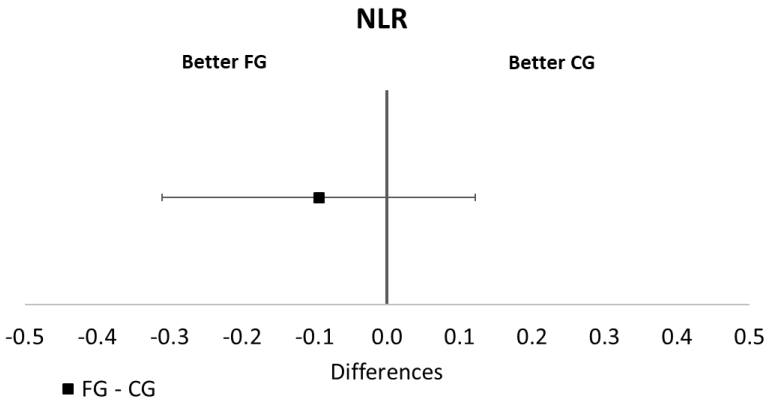

Figure 6.12 – Neutrophil: lymphocyte ratio (NLR). Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI.

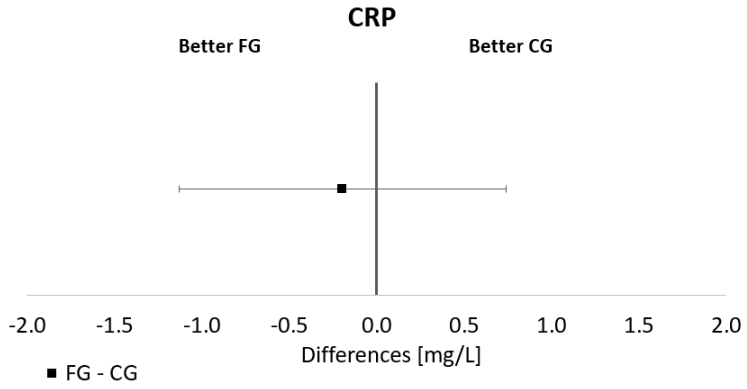

Figure 6.13 - C-reactive protein (CRP) concentration. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

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7 Study 3 – Microvascular responsiveness and cardiac function adaptations

7.1 Introduction

7.1.1 Microvascular responsiveness

Nowadays it is universally accepted that physical exercise has multifaceted beneficial effects on health. Physical activity is considered a powerful tool in preventive and therapeutic protocols for cardiovascular diseases.[1] Numbering among the positive effects are improvements in endothelial function, which is impaired in the presence of atherosclerosis. The endothelium (Figure 7.1) is the largest organ in the human body; it consists of a single-cell lining covering the internal surface of blood vessels and other organs. The main role of endothelium is to regulate the balance between vasoconstriction and vasodilation via its autocrine and paracrine functions. The endothelium perceives changes in haemodynamic forces and blood-borne signals and reacts to them by producing vasoactive substances (Figure 7.2) that in healthy conditions contribute to maintain vascular homeostasis.[3]

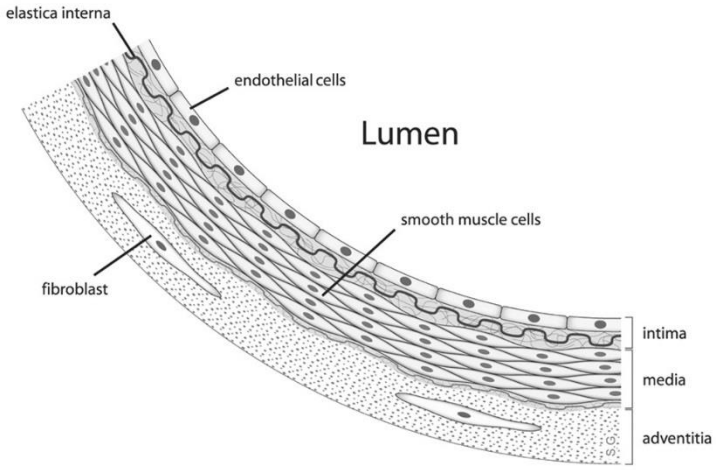

Figure 7.1 - Endhotelium - From Wikipedia website

Vasodilators Nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor, bradykinin, adrenomedullin, C-natriuretic peptide	**Vasoconstrictors** Endothelin-1, angiotensin-II, thromboxane A_2, oxidant radicals, prostaglandin H_2
Antiproliferative Nitric oxide, prostacyclin, transforming growth factor-β, heparan sulphate	**Pro-proliferative** Endothelin-1, angiotensin-II, oxidant radicals, platelet-derived growth factor, basic fibroblast growth factor, insulin-like growth factor, interleukins
Antithrombotic Nitric oxide, prostacyclin, plasminogen-activator, protein C, tissue factor inhibitor, von Willebrand factor	**Prothrombotic** Endothelin-1, oxidant radicals, plasminogen activator inhibitor-1, thromboxane A_2, fibrinogen, tissue factor
Angiogenesis Vascular endothelial growth factor	**Inflammatory markers** Cell adhesion molecules (P- and E-selectin, ICAM, VCAM) chemokines, nuclear factor-κB

ICAM = intercellular adhesion molecule; **VCAM** = vascular cell adhesion molecule.

Figure 7.2 - Endothelial vasoactive substances – From Di Francescomarino et al. 2009

Many cardiovascular risk factors impair endothelial function, leading to endothelial dysfunction. Endothelial dysfunction is characterized by an altered production of vasoactive substances that reduces nitric oxide (NO) bioavailability. Low NO bioavailability is associated with chronic diseases such as hypertension, type 2 diabetes, and atherosclerosis.[4] This relationship suggests the involvement of endothelial dysfunction in such pathological conditions. Several studies have described the beneficial effects of physical exercise as a therapeutic treatment for chronic diseases.[5] Increased NO bioavailability is probably the most important effect of regular exercise in relation to endothelial function. Regular physical activity exerts this positive effect mainly in two ways: 1) the shear stress induced by exercise stimulates the production and release of NO; 2) its anti-inflammatory action reduces reactive oxygen species (ROS) activity, resulting in improved NO efficacy.[6,7]

Studies investigating the effect of exercise on endothelial function in healthy people have shown that exercise is able to restore (or decelerate) the natural aged-related decline[8,9]. However, studies disagree on the beneficial effects of exercise training

on endothelium-dependent vasodilation in healthy people. Some have documented improvements,[9,10] others no change[11] or impairment when exercise intensity is too high.[12,13] There are basically two explanations for these differences: 1) a kind of plateau exists for vascular function in healthy conditions; and 2) oxidative stress induced by very heavy exercise or overtraining can lead to endothelial dysfunction. Exercise intensity has a remarkable relevance and must be taken into account. A recent meta-analysis showed that high-intensity training is more effective than moderate-intensity training to improve vascular function.[14] This is why a high intensity activity like recreational football, performed at low and moderate frequency, permits adequate recovery and may enhance endothelial function in untrained adults.

Protocols and tools to assess endothelial function have progressed from an invasive technique like plethysmography-measurement of blood flow to simpler non-invasive methods such as flow mediated dilation (FMD) and reactive hyperaemia-peripheral arterial tonometry (RH-PAT).[15] Reactive hyperaemia and flow-mediated dilation are two phenomena measured with a vascular occlusion test (VOT), the usual procedure that assesses both artery vascular and microvascular reactivity.[16,17] VOT entails the brief occlusion of a conduit artery, usually 5 min, to dilate downstream arterioles; cuff release, due to the decrease in peripheral resistance, results in a hyperaemia response that, in turn, increases the shear rate in the artery leading the FMD.

Another non-invasive technique demonstrated useful to assess microvascular function relies on near-infrared spectroscopy (NIRS).NIRS has been applied to measure the response of tissue oxygen saturation (StO_2),[18–20] and its concurrent validity was assessed by comparing it with ultrasound FMD.[18,21] Furthermore, it has been demonstrated to have good reliability.[22] To the best of our knowledge, only one study to date has evaluated microvascular responsiveness as an index of endothelial function after recreational football training. Schmidt et al.[23] used RH-PAT to measure peripheral microvascular function and found no changes in microvascular indices after 4 or 12 months.

Given the conflicting findings and the scarce published data on recreational football, we assessed microvascular responsiveness after 12 weeks of recreational football to determine whether a dose-response relationship exists. For this purpose, we used NIRS during VOT to monitor and assess microvascular responsiveness as a marker of endothelial health.

7.1.2 Cardiac function

Regular endurance training leads to improvements in aerobic fitness, which is one of the most important factors for the prevention of cardiovascular diseases (See Chapter 1). The physiological adaptations that result in improved aerobic fitness are both peripheral (vascular system) and central[24], with the latter related to cardiac remodelling induced by regular training. Maximal cardiac output is the central factor that plays a determinant role in the calculation of maximal oxygen consumption (VO2max), the most important index of aerobic fitness. Cardiac output (Q) is the product of heart rate (beats • min^{-1}) and stroke volume (mL • min^{-1}). After endurance training, it can improve from 5 L • min^{-1} to 15 L • min^{-1} at rest in young women [25] and from 35 L • min^{-1} to 40 L • min^{-1} in trained athletes during maximal exercise, which involves many large muscle groups.[26] Since maximal heart rate is not affected by training, the increase in maximal cardiac output (Qmax) is the result of a higher maximal stroke volume. It has been demonstrated that improvements in sub-maximal and maximal stroke volume, and cardiac output as a consequence, are associated with functional and structural changes in the heart. Henschen, in his study conducted more than a century ago, described both dilation and hypertrophy of the left and right side of the heart in well-trained athletes.[27]

More recently, advanced technologies are used to assess changes in the heart chambers. In their review, Hellsten and Nyberg [24] summarised several studies that showed increments in left ventricular (LV) and right ventricular (RV) end-diastolic diameter, LV and RV wall thickness, and LV and RV mass. From a functional point of view, endurance training leads to an increase in early diastolic filling and a quicker filling of the LV during high-intensity exercise. Similar results were found in sedentary people engaged in endurance training for 12 months: LV and RV mass and LV and RV end-diastolic volume were greater after training. Furthermore,

modifications in ventricular mass were observed at 3 months from baseline, without any changes in end-diastolic volume, which increased after 3 additional months.[28] Suboc et al.[29] found changes in LV end-diastolic volume and end-systolic volume in older adults after 12 weeks of light-to-moderate exercise. They also found a decrease in septal wall thickness.

Few studies to date have investigated cardiac adaptations following recreational football in women and men, and only one involved healthy men. Schmidt et al.[23] studied untrained elderly men (age range 65 to 75 years) who played recreational football for 12 months. Echocardiographic assessment showed changes in cardiac structure and function after just 4 months of training. Krustrup et al.[30] studied the effect of recreational football on 7 young healthy women over a period of 16 months and compared them with a running and a control group. Some cardiac functional indices, such as the pulsed-wave tissue Doppler-derived S', the average peak systolic velocity, and the LV systolic longitudinal displacement improved in the football group after just 4 months of training, whereas structural changes appeared only at 16 months. A very similar study published by Andersen et al.[31] confirmed many of the previous results: large cardiac dimensions and increments in ventricular function after 16 months of recreational football in young healthy women. Because of the long time period between the evaluations in these studies, we cannot know when the structural changes actually occurred.

To the best of our knowledge, only two studies have focused on the cardiac adaptations induced by recreational football in sedentary men. Twenty hypertensive men, who played recreational football for 6 months, showed greater LV volume as well as improved systolic and diastolic function after just 3 months, with no other changes in most of the indices after 6 months of training.[32]

In all these studies, the training frequency was 2 or 3 times a week and none involved healthy and sedentary middle-aged men. With the present study, we assessed cardiac adaptations in sedentary men engaged in recreational football at low or moderate frequency (once or twice a week).

7.2 Methods

Subjects arrived at the laboratory at about the same time for each session and were asked to refrain from vigorous physical activity during the 24 hours before, as well as from alcohol and caffeine consumption during the 12 hours before testing. The microvascular responsiveness test and echocardiography were performed during the third assessment session of the study.

7.2.1 Near-infrared Spectroscopy (NIRS)

Near-infrared spectroscopy (NIRS) of muscle relies on the relative transparency of the body tissue at infrared light wavelengths (650-1000 nm) as well as on the oxygenation-dependent absorption characteristics of oxyhemoglobin and oxymyoglobin (HbO2, MbO2), deoxyhemoglobin and deoxymyoglobin（Hb, Mb). Changes in tissue oxygenation are instantly measured by changing the emitted wavelength.[20] The device used in this study (NIRS, Nimo-Nirox, Brescia Italy) works at three different light wavelengths (685, 830, and 980 nm) emitted by three low-power diodes (< 10 mW) and a receiver mounted on an optic probe. Each diode is turned on for maximum of 500 µs at a fixed sequence; the emitted light (frequency 40 Hz) penetrates the tissues under the probe, and its intensity is attenuated by absorption and scattering (Figure 7.3).

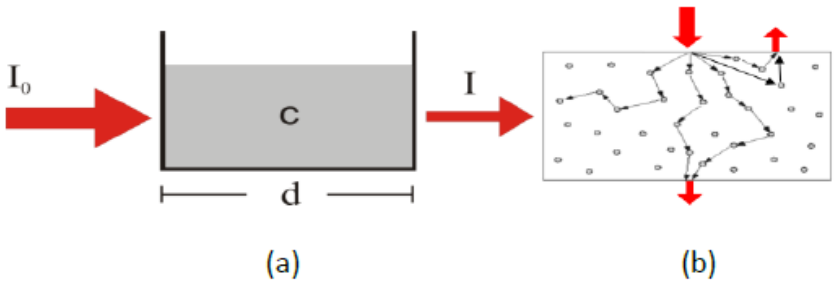

Figure 7.3 - Absorption (a) and Scattering (b) - From NIMO manual

Absorption is the weakening of the optical signal caused by the tissues and is proportional to molecule concentration (chromophores). Scattering is the deviation of the photon route due to the difference in the refractive index of the different kinds of tissues. Because of these signal deviations, the photon path through the tissues resembles a banana (called banana-shape), and allows to measure the refracted signal on the same side as the emission (Figure 7.4).

The processed signal obtains a quantitative measure of HbO_2 and Hb for calculating

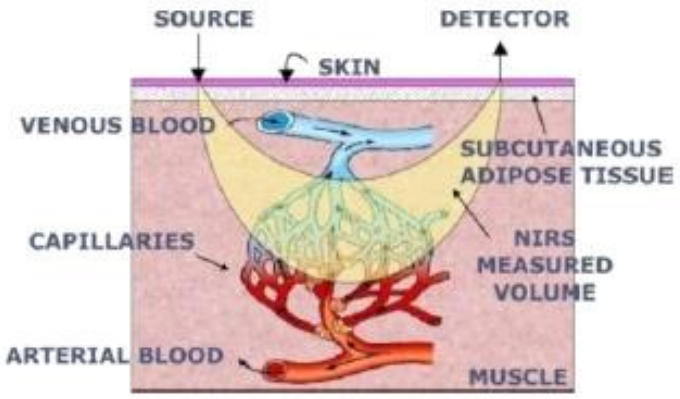

Figure 7.4 - Optical signal route - From NIMO manual

the oxygenation muscle index (TOI %) according to the formula:

$$\frac{[HbO_2]}{[Hb] + [HbO_2]} \times 100\%$$

A 20-min warm up period preceded the first test of the session. The equipment was calibrated before each test using a calibration block (phantom) with known absorption and scattering indices and following the manufacturer's instructions. The site of measurement was the belly of the tibialis anterior muscle; the probe was placed about 15 cm distal from the centre of the patella, with the long side along the tibial crest. Anatomical reference points were marked, and measures taken so that the probe was placed exactly in the same position in each assessment session. The skin was shaved and cleaned before placing the probe. Skinfold thickness at the tibialis anterior was measured using a high-quality metal caliper (precision of

0.2 mm) and the value of the subcutaneous adipose tissue was entered into the Nimo-Nirox software. The probe was firmly attached to the skin with bioadhesive tape and an elastic bandage. An optically dense black vinyl sheet covered the probe to minimise intrusion of external light.[22]

7.2.2 Vascular occlusion test (VOT)

The vascular occlusion test (VOT) creates a hyperaemic response after prolonged ischemia. A pneumatic cuff was placed just below the knee, avoiding interference with the NIRS probe. The test entailed three different phases (Figure 7.5): 1) baseline measurement for 2 min, 2) arterial occlusion for 5 min, and 3) post-release for 8 min. After baseline measurement, the cuff was inflated to an occlusion pressure of 250 mm Hg and kept constant for 5 min. The subject lay reclined and was asked not to move his legs during the test.

The desaturation curve during ischemia (Slope 1) and the saturation curve (Slope

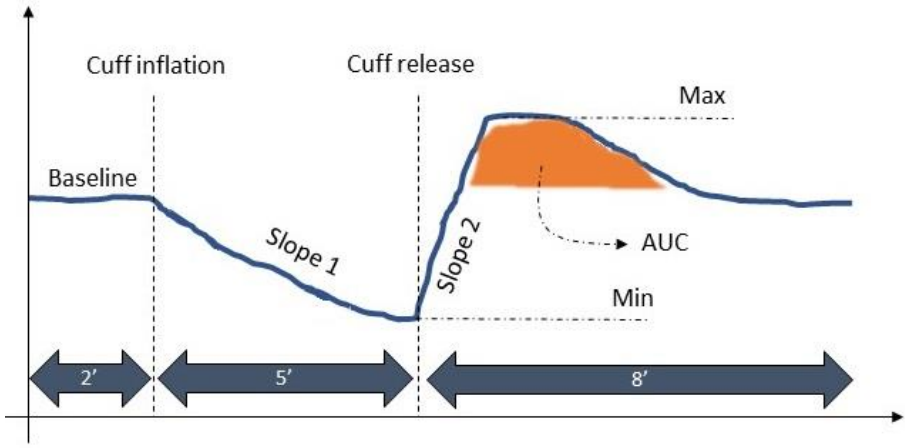

Figure 7.5 - Vascular occlusion test procedure

2) were calculated, as was the baseline value (average of 1 min before occlusion). The difference between maximum and minimum yields the hyperaemia reserve which, with the area under the hyperaemia curve (AUC), represents two important parameters related to training status, age, and health. We also calculated the magnitude of saturation at the end of the ischemic period, which is basically the difference between baseline and minimum values (negative amplitude).

7.2.3 Echocardiographic assessment

Cine-loops of the parasternal long-axis and apical four-chamber, two-chamber, and long-axis views were recorded. LV and RV dimensions were calculated from the parasternal long-axis two-dimensional (2D) recordings at the mid-ventricular level. The proximal RV outflow tract (RVOT) dimension was measured from the RVOT anteriorly to the aortic root posteriorly in the parasternal long-axis view at end-diastole. LV volumes and ejection fraction (EF) were calculated using Simpson's biplane method from apical two- and four-chamber views.[38] Due to a technical issue during the final (third) session, we were unable to record the cine-loops for calculating the EF. The LV mass was calculated according to the American Society of Echocardiography guidelines using the formula 0.832 [1.05 [(LVID + IVST + PWT)3] – (LVID)3] and indexed according to body weight (BW). Pulsed Doppler measurements of mitral inflow were obtained from the apical four-chamber view, with the Doppler beam aligned perpendicular to the plane of the mitral annulus and the sample volume (2 mm) placed between the tips of the mitral leaflets. Measurements included peak transmitral flow in early diastole (E), peak transmitral flow velocity in late diastole during atrial contraction (A), and their ratio (E/A).

Pulsed-wave tissue Doppler imaging (TDI) was performed in four-chamber apical projections with a 5-mm pulsed tissue Doppler volume placed at the level of the mitral annulus in the septum and the lateral wall. The myocardial velocity curves were recorded, and the peak systolic velocity (S′), early diastolic velocity (E′), and late diastolic velocity (A′) were measured and are presented as the average of each measurement. The ratio of E/E′ was calculated as an estimate of LV filling pressure[39]. RV systolic function was measured as peak systolic velocity (S′-RV) in four-chamber apical projections with a 5-mm pulsed tissue Doppler volume placed at the level of the tricuspid annulus in the lateral wall. Two-dimensional colour TDI was recorded with a minimum frame rate of 130/s.

7.3 Results

Statistical analysis was performed on comparison of the three groups (CG vs LFG vs MFG). The two training groups were merged (FG) then compared as CG vs. FG. The results are presented as mean and mean differences with 95% confidence interval. Tables 7.1 to 7.4 refer to the VOT, while Tables 7.5 to 7.8 refer to echocardiograph assessment.

7.3.1 Vascular occlusion test (VOT)

When the training groups were compared separately (LFG vs CG, MFG vs CG and MFG vs LFG), no differences were found for most of the parameters measured. There were no differences among groups for baseline ($p = 1$, $p = 1$, and $p = 1$), Slope 1 ($p = 0.7$, $p = 0.311$, and $p = 1$), Slope 2 ($p = 1$, $p = 1$, and $p = 1$), and negative amplitude ($p = 1$, $p = 0.558$, and $p = 1$) after 12 weeks (Figures 7.6, 7.7, 7.8, and 7.9, respectively). The hyperaemia reserve was higher in the MFG than in the CG ($p = 0.047$); although the comparison between the LFG and the CG did not reach statistical significance ($p = 0.068$), also the LFG appeared to follow a similar trend (Figure 7.10). No differences in hyperaemia reserve were observed between the MFG and the LFG ($p = 1$), while the AUC (Fig 7.11) was higher in the LFG than in the CG ($p = 0.040$) but it did not differ between the MFG and the CG ($p = 1$) or between the MFG and the LFG ($p = 0.3$).

When we merged the two training groups in one (FG) to analyse the effect of training irrespective of frequency and volume, the trend was similar to the first analysis: no differences were found between the groups after 12 weeks from baseline ($p = 1$, $p = 1$, and $p = 1$), Slope 1 ($p = 0.7$, $p = 0.311$, and $p = 1$), Slope 2 ($p = 1$, $p = 1$, and $p = 1$), and negative amplitude ($p = 1$, $p = 0.558$, and $p = 1$) (Figures 7.12, 7.13, 7.14, and 7.15). The hyperaemia reserve ($p = 0.013$) and the AUC ($p = 0.045$) were higher in the FG than in the CG at 12 weeks (Figures 7.16 and 7.17).

7.3.2 Echocardiographic assessment

7.3.2.1 Cardiac dimensions

Left ventricular end-diastolic diameter (LVEDd) was increased after 12 weeks in the LFG vs. the CG ($p = 0.076$) and in the MFG vs. the CG ($p = 0.005$), while no difference was found between the MFG and the LFG ($p = 1$) (Figure 7.18). Left ventricular mass (LV mass) (Figure 7.19) and LV mass normalised for body weight (LV mass/BW) (Figure 7.20) were higher in the LFG ($p = 0.036$ and $p = 0.021$) and the MFG ($p = 0.001$ and $p < 0.001$) as compared with the CG. No differences were found between the MFG and the LFG for LV mass ($p = 1$) or LV mass/BW ($p = 1$). RVOT was increased only in the MFG as compared with CG ($p = 0.003$), whereas the comparisons of LFG vs. CG and MFG vs. LFG showed no changes ($p = 0.203$ and $p = 0.905$) at 12 weeks (Figure 7.21).

Comparison of the FG and CG after 12 weeks showed differences in structural variables: LV end diastolic diameter ($p = 0.001$), LV mass ($p = 0.027$), LV mass/BW ($p = 0.016$), and RVOT ($p = 0.001$) were all higher in the FG than the CG (Figures 7.26, 7.27, 7.28 and 7.29 respectively).

7.3.2.2 Cardiac function

After 12 weeks of training, no differences between LFG vs. CG, MFG vs. CG, and MFG vs. LFG were found in E/A ($p = 1$, $p = 1$, and $p = 0.569$) (Figure 7.23) and E/E' ratio ($p = 1$, $p = 1$, and $p = 1$) (7.24). E' was increased in the MFG as compared with the CG ($p = 0.027$), whereas no differences were noted between LFG vs. CG ($p = 0.107$) and MGF vs. LFG ($p = 1$) (Figure 22). The S'-RV was unchanged at 12 weeks ($p = 0.068$, $p = 0.386$, and $p = 1$) (7.25).

When the merged training group (FG) was compared with the CG, both E/A (Figure 7.31) and E/E' (Figure 7.32) showed no differences ($p = 0.848$ and $p = 0.586$), whereas both E' (Figure 7.30) and S'-RV (Figure 7.33) were higher in the FG than the CG ($p = 0.005$ and $p = 0.019$).

7.4 Discussion

7.4.1 Vascular occlusion test (VOT)

One of the main aims of this study was to determine whether microvascular parameters, as measured by NIRS during a VOT, were changed after 12 weeks of recreational football played once or twice a week. To the best of our knowledge, this is the first study to assess the impact of recreational football training by means of microvascular responsiveness using NIRS. Previous studies using NIRS to assess microvascular responsiveness as a marker of endothelial function showed differences between young and elderly, trained and untrained, healthy and unhealthy adults[-42], documenting the ability of NIRS to discriminate different levels of endothelial function. Rosenberry et al. found that Slope 1, Slope 2, hyperaemic reserve, and AUC values are higher in young adults (< 35 years) than the elderly. Lower values in older adults suggest impairment in vasodilatory stimulus and metabolic rate, along with impaired microvascular responsiveness. The authors suggested that metabolic function, as well as microvascular responsiveness, plays a crucial role in predicting endothelial dysfunction. Better muscle function and a higher metabolic rate will result in a steeper desaturation slope (Slope 1) and greater values of tissue ischemia than a case of impaired muscle function and metabolic rate. For this reason, the authors pondered what would happen before cuff release when the hyperaemia response has been analysed.

A recent study[23] used peripheral arterial tonometry to assess microvascular endothelial function and arterial stiffness at 4 and 12 months of recreational football in elderly adults. No differences were found. The authors attributed the lack of improvement to the poor influence of this kind of physical activity on microvascular endothelial health and arterial stiffness. Partially in line with these results, we found no differences among the groups for the kinetics (Slope 1 and Slope 2) and the ischemic variation (negative amplitude). However, differences were found in the AUC between the LFG and the CG and in the hyperaemic reserve between the MFG and the CG. Although the differences were not statistically significant, the latter index showed an increasing trend in the LFG vs CG comparison as well. Similar

results were found when we compared the FG and the CG, with both the hyperaemic reserve and the AUC increased in the FG.

Our results may be explained from different points of view: 1) the lack of improvements in kinetics and desaturation parameters is shared by Schmidt et al. Since the two techniques used to assess the microvascular endothelial function were not the same, their results are not directly comparable to the those we found in our study. In addition, McLay et al. observed that changes in microvascular responsiveness after short-term training might be better detected using a VOT with shorter occlusion. It seems that the shorter the occlusion, the more sensible the measure; 2) according to Rosenberry et al., the hyperaemic reserve and the AUC could describe not only microvascular endothelial function but metabolic rate as well. The changes in these two parameters we found in our study suggest that 12 weeks of recreational football produced some beneficial adaptations; 3) although the participants were sedentary, apparently none had endothelial dysfunction. Adults with normal endothelial function may need a higher volume/frequency and a longer training period to further improve it.

7.4.2 Echocardiographic assessment

According to the literature, the practice of regular, moderate-to-intense physical exercise induces cardiac remodelling, including physiological hypertrophy of heart muscle and increased dimension of cardiac cavities.[24] The athlete's heart has been defined and is now accepted as increased in end-diastolic dimensions of both RV and LV, LV mass, and left atrial volume. These changes were primarily shown in elite athletes performing sports involving high workloads with a discrete intensity variability, i.e., rowers, cyclists, cross-country skiers.[33,34] Although the published data are more controversial for untrained men and women, several studies have demonstrated positive adaptations after a training period, usually more than 3 months, of different kinds of sports, including recreational football. [28,31,32,35]

One of the aims of this study was to determine whether a dose-response relationship exists in cardiac adaptations induced by recreational football, i.e., if low frequency (once a week) of this kind of activity can lead to any improvements.

In line with other similar studies involving both healthy and diseased populations, we found changes that indicate training-induced cardiac remodelling. LVEDd and LV mass were increased after 12 weeks in both the LFG (12% and 4%, respectively) and the MFG (13% and 3%, respectively). RVOT was increased by about 2.5% only in the MFG. These changes are consistent with the concentric vs. eccentric cardiac hypertrophy theory proposed several decades ago that suggests an influence of the nature of physical activity on cardiac adaptations.[36] Basically, football, also at the recreational level, involves different kinds of dynamic and static activities (walking, jogging, acceleration and deceleration, shooting, etc.) at highly variable intensity throughout the game, and this can be a powerful stimulus to induce cardiac hypertrophy. Comparison between the merged training groups and the CG confirmed the changes in cardiac structure, consistent with the aforementioned literature.

Analysis of diastolic function produced controversial findings. No differences were found for the E/A and E/E' ratios, which are classically used for the assessment of diastolic function. However, debate surrounds the validity of these indices of early diastolic filling. The E/A ratio seems to be load dependent and influenced by heart rate and other factors, while the E/E' ratio may not be accurate in normal subjects, as was the case in our population. Furthermore, E' is considered to be more load independent than E/A and can be used to correct for the effect of LV relaxation on mitral E velocity.

We found an increase in E' in the MFG as compared with the CG and in the FG as compared with the CG, which could suggest better LV diastolic function after 12 weeks of training. Although we cannot state that diastolic function improved, our data do suggest that a favourable change occurred in the MFG and when the combined groups were compared with the CG. Moreover, almost all subjects had normal diastolic function at baseline, and this condition could be difficult to further improve with a relatively low volume of physical activity. There was no difference

in RV systolic function (S'RV) among the groups at 12 weeks, although the effect size between LFG vs. CG and MFG vs. CG was large. When we compared the merged football group (FG) with the CG, we found a clear increase in S'RV for the FG, suggesting an improvement in RV systolic function, as previously documented. This finding assumes an important value, considering that S'RV is related to RV ejection fraction.[37]

7.5 Conclusion

Summarising, we found several interesting and novel findings in this study. Echocardiographic assessment showed ventricular remodelling, as demonstrated by increased LV and RV diameters and LV mass after 12 weeks of recreational football played once or twice a week. It seems that low-frequency recreational football is not enough to improve an already normal diastolic function in healthy middle-aged men. Previous studies involving either hypertensive or elderly adults have shown that low-frequency recreational football could be effective in improving impaired diastolic function, but this finding requires further investigations.

Microvascular endothelial function evaluation detected changes in two important parameters - hyperaemic reserve and AUC - suggesting that a small positive effect after 12 weeks of training may be present. Nevertheless, further studies are needed. A future area of focus is training studies designed to better describe the endothelial function – exercise dose-response relationship.

7.6 Tables

	SatO2 - Negative Amplitude [%]	SatO2 - Slope 1 [%•sec^{-1}]	SatO2 - Slope 2 [%•sec^{-1}]
	Mean [95% CL]		
GC	31.20 [27.64 - 34.74]	-0.123 [-0.141 - -0.104]	1.75 [1.45 - 2.05]
LFG	30.22 [27.12 - 33.32]	-0.119 [-0.135 - -0.103]	1.70 [1.43 - 1.96]
MFG	31.58 [27.90 - 35.27]	-0.124 [-0.143 - -0.105]	1.77 [1.46 - 2.09]
Covariate	*27.99*	*-0.11*	*1.76*
	Change in the mean [95% CL]		
LFG - CG	-0.97 [-6.79 - 4.85] *p = 1* *ES 0.05 - trivial*	0.004 [-0.027 - 0.034] *p = 0.700* *ES 0.03 - trivial*	-0.05 [-0.55 - 0.44] *p = 1* *ES 0.09 - trivial*
MFG -CG	0.39 [-5.94 - 6.71] *p = 0.558* *ES 0.03 - trivial*	-0.001 [-0.034 - 0.032] *p = 0.311* *ES 0.03 - trivial*	0.03 [-0.51 - 0.56] *p = 1* *ES 0.04 - trivial*
MFG - LFG	1.36 [-4.59 - 7.31] *p = 1* *ES 0.23 - small*	-0.005 [-0.036 - 0.026] *p = 1* *ES 0.12 - trivial*	0.08 [-0.43 - 0.59] *p = 1* *ES 0.12 - trivial*

Table 7.1 – Negative amplitude, desaturation slope (Slope 1) and saturation slope (Slope2). Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

	$SatO_2$ - Baseline [%]	Hyperaemic reserve [%]	SatO2 - AUC [%•sec]
	Mean [95% CL]		
GC	58.51 [55.97 - 61.05]	9.55 [8.38 - 10.71]	1059.08 [847.36 - 1270.80]
LFG	58.14 [55.78 - 60.49]	11.44 [10.37 - 12.51]	1425.12 [1233.14 - 1617.10]
MFG	57.92 [55.11 - 60.73]	11.14 [9.93 - 12.35]	1187.14 [975.73 - 1398.55]
Covariate	*59.95*	*9.73*	*1155.59*
	Change in the mean [95% CL]		
LFG - CG	-0.37 [-4.66 - 3.91] *p = 1* *ES 0.07 - trivial*	1.89 [-0.06 - 3.85] *p = 0.068* *ES 0.78- moderate*	366.04 [12.56 - 719.52] *p = 0.040* *ES 0.91 - moderate*
MFG -CG	-0.59 [-5.27 - 4.09] *p = 1* *ES 0.07 - trivial*	1.60 [-0.48 - 3.68] *p = 0.047* *ES 0.66 - moderate*	128.06 [-241.99 - 498.11] *p = 1* *ES 0.32 - small*
MFG - LFG	-0.22 [-4.75 - 4.31] *p = 1* *ES 0.05 - trivial*	-0.30 [-2.29 - 1.70] *p = 1* *ES 0.14 - trivial*	-237.98 [-591.18 - 115.21] *p = 0.300* *ES 0.58 - small*

Table 7.2 – Baseline, Hyperaemic reserve and AUC. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

	$SatO_2$ - Baseline [%]	Hyperaemic reserve [%]	SatO2 - AUC [%•sec]
	Mean [95% CL]		
GC	58.51 [56.01 - 61.01]	9.55 [8.34 - 10.75]	1059.08 [840.50 - 1277.67]
FG	58.27 [56.59 - 59.95]	11.27 [10.45 - 12.09]	1315.70 [1168.99 - 1462.41]
Covariate	*59.95*	*9.73*	*1155.59*
	Change in the mean [95% CL]		
FG - CG	-0.23 [-3.25 - 2.78] *p = 0.906*	1.73 [0.27 - 3.18] *p = 0.013*	256.62 [-6.63 - 519.87] *p = 0.045*
	ES 0.05 - trivial	*ES 0.72- moderate*	*ES 0.64- moderate*

Table 7.3 - Baseline, Hyperaemic reserve and AUC. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

	SatO2 - Negative Amplitude [%}	SatO2 - Slope 1 [%•sec^{-1}]	SatO2 - Slope 2 [%•sec^{-1}]
	Mean [95% CL]		
GC	31.20 [27.72 - 34.67]	-0.12 [-0.14 - -0.10]	1.75 [1.45 - 2.05]
FG	30.79 [28.49 - 33.10]	-0.12 [-0.13 - -0.11]	1.72 [1.52 - 1.92]
Covariate	*27.99*	*48.31*	*1.76*
	Change in the mean [95% CL]		
FG - CG	-0.40 [-4.58 - 3.77] *p = 0.274*	0.00 [-0.02 - 0.02] *p = 0.848*	-0.03 [-0.39 - 0.33] *p = 1*
	ES 0.05 - trivial	*ES 0.04 - trivial*	*ES 0.04 - trivial*

Table 7.4 - Negative amplitude, desaturation slope (Slope 1) and saturation slope (Slope2). Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

	LVEDd [mm]	LV mass [gr]	LV mass/BW	RVOT [mm]
	Mean [95% CL]			
GC	47.83 [46.85 - 48.81]	141.20 [22.81 - 259.60]	69.52 [65.79 - 73.25]	34.36 [23.78 - 24.94]
LFG	49.64 [48.38 - 50.91]	155.06 [11.91 - 298.21]	77.06 [72.34 - 81.79]	25.30 [24.42 - 26.17]
MFG	50.14 [49.16 - 51.12]	158.75 [62.86 - 254.63]	79.55 [75.67 - 83.44]	25.83 [25.22 - 26.43]
Covariate	*48.15*	*139.14*	*68.76*	*25.20*
	Change in the mean [95% CL]			
LFG - CG	1.81 [-0.17 - 3.80] *p = 0.076* *ES 1.32 - large*	13.85 [-0.39 - 28.10] *p = 0.036* *ES 1.28 - large*	7.54 [0.08 - 15.00] *p = 0.021* *ES 1.41 - large*	0.94 [-0.36 - 2.24] *p = 0.203* *ES 0.90 - moderate*
MFG -CG	2.31 [0.59 - 4.03] *p = 0.005* *ES 1.63 - large*	17.55 [4.80 - 30.29] *p = 0.001* *ES 1.53 - large*	10.00 [3.35 - 16.71] *p < 0.001* *ES 1.78 - large*	1.47 [0.43 - 2.50] *p = 0.003* *ES 1.56 - large*
MFG - LFG	0.50 [-1.48 - 2.48] *p = 1* *ES 0.26 - small*	3.69 [-10.78 - 18.16] *p = 1* *ES 0.24 - small*	2.49 [-5.09 - 10.07] *p = 1* *ES 0.30 - small*	0.53 [-0.79 - 1.85] *p = 0.905* *ES 0.35 - small*

Table 7.5 – Heart dimension parameters. LVEDd = Left ventricular end-diastolic diameter, LV mass = Left ventricular mass, LV mass / BW = Left ventricular mass indexed to body weight, and RVOT = Right ventricular outflow track. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

	E' [cm•sec^{-1}]	E/A	E/E'	S'RV [cm•sec^{-1}]
	Mean [95% CL]			
GC	11.82 [10.95 - 12.68]	1.533 [-0.974 - 4.040]	5.51 [4.98 - 6.03]	13.231 [3.849 - 22.613]
LFG	13.25 [12.32 - 14.17]	1.478 [-1.259 - 4.215]	5.88 [5.28 - 6.48]	15.157 [2.798 - 27.516]
MFG	13.63 [12.72 - 14.54]	1.647 [-0.783 - 4.078]	5.62 [5.05 - 6.20]	14.657 [-1.062 - 30.376]
Covariate	*12.46*	*1.46*	*5.35*	*13.69*
	Change in the mean [95% CL]			
LFG - CG	1.43 [-0.14 - 3.01] *p = 0.107* *ES 1.33 - large*	-0.055 [-0.444 - 0.335] *p = 1* *ES 0.19 - trivial*	0.38 [-0.62 - 1.37] *p = 1* *ES 0.56 - small*	1.927 [-0.199 - 4.052] *p = 0.068* *ES 1.46 - large*
MFG -CG	1.82 [0.25 - 3.38] *p = 0.027* *ES 1.59 - large*	0.115 [-0.248 - 0.477] *p = 1* *ES 0.23 - small*	0.12 [-0.85 - 1.08] *p = 1* *ES 0.19 - trivial*	1.426 [-0.667 - 3.519] *p = 0.386* *ES 1.45 - large*
MFG - LFG	0.38 [-1.24 - 2.00] *p = 1* *ES 0.19 - trivial*	0.169 [-0.224 - 0.562] *p = 0.596* *ES 0.45 - small*	-0.26 [-1.30 - 0.77] *p = 1* *ES 0.28 - small*	-0.500 [-2.737 - 1.736] *p = 1* *ES 0.21 - small*

Table 7.6 – Cardiac function parameters. E' = Peak early diastolic velocity, E/A = Peak transmitral flow in early diastole (E) / peak transmitral flow velocity in late diastole during atrial contraction (A) ratio, E/E' = Peak transmitral flow in early diastole (E) / Peak early diastolic velocity (E') ratio and S'RV = Right ventricular peak systolic velocity. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

	LVEDd [mm]	LV mass [gr]	LV mass/BW	RVOT [mm]
	Mean [95% CL]			
GC	47.83 [46.87 - 48.79]	141.20 [63.01 - 219.40]	69.52 [65.73 - 73.31]	24.36 [23.78 - 24.93]
FG	49.90 [49.15 - 50.64]	156.51 [78.43 - 234.59]	78.10 [75.11 - 81.10]	25.69 [25.22 - 26.16]
Covariate	*48.15*	*139.14*	*68.75*	*25.20*
	Change in the mean [95% CL]			
FG - CG	2.07 [0.85 - 3.28] *p = 0.001*	15.31 [6.20 - 24.42] *p = 0.027*	8.58 [3.76 - 13.41] *p = 0.016*	1.33 [0.59 - 2.07] *p = 0.001*
	ES 1.51 - large	*ES 1.36 - large*	*ES 1.55 - large*	*ES 1.46 - large*

Table 7.7 - Hearth dimension parameters. LVEDd = Left ventricular end diastolic diameter, LV mass = Left ventricular mass, LV mass / BW = Left ventricular mass indexed to body weight and RVOT = Right ventricular outflow track. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

	E' [cm•sec^{-1}]	E/A	E/E'	S'RV [cm•sec^{-1}]
	Mean [95% CL]			
GC	11.82 [5.20 - 18.43]	1.53 [-0.44 - 3.72]	5.51 [-3.67 - 14.68]	13.23 [12.14 - 14.32]
FG	13.44 [7.65 - 19.22]	1.57 [-0.57 - 3.72]	5.75 [1.95 - 9.55]	14.93 [14.11 - 15.76]
Covariate	*12.46*	*1.46*	*5.35*	*13.69*
	Change in the mean [95% CL]			
FG - CG	1.62 [0.58 - 2.67] *p = 0.005*	0.12 [-0.21 - 0.29] *p = 0.848*	0.25 [-0.40 - 0.89] *p = 0.586*	0.67 [0.33 - 3.07] *p = 0.019*
	ES 1.44 - large	*ES 0.18 -*	*ES 0.21 - small*	*ES 1.69 - large*

Table 7.8 - Cardiac function parameters. E' = Peak early diastolic velocity, E/A = Peak transmitral flow in early diastole (E) / peak transmitral flow velocity in late diastole during atrial contraction (A) ratio, E/E' = Peak transmitral flow in early diastole (E) / Peak early diastolic velocity (E') ratio and S'RV = Right ventricular peak systolic velocity. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

7.7 Charts

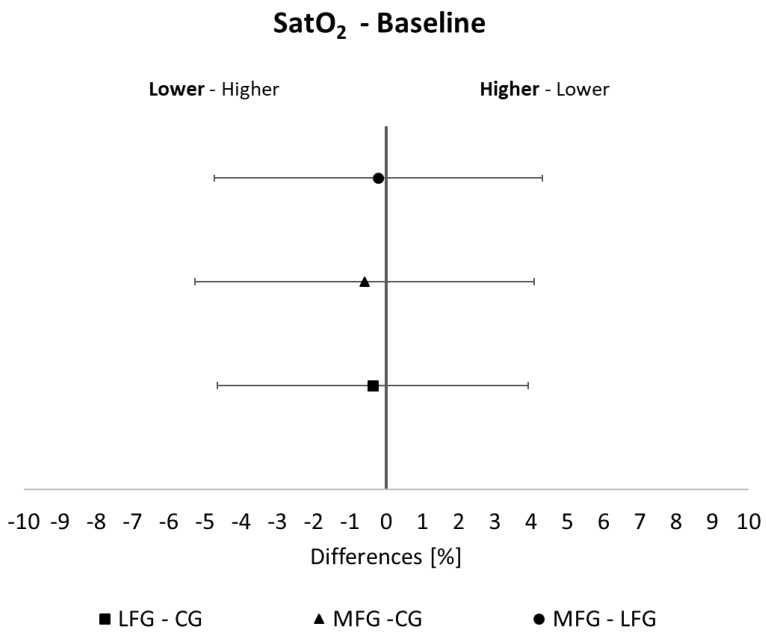

Figure 7.6 – Baseline saturation at the beginning of VOT. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

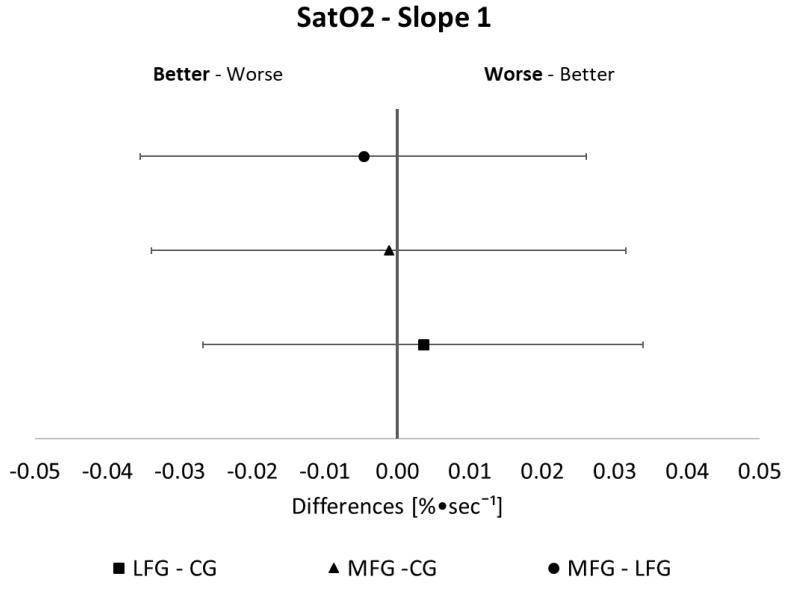

Figure 7.7 – Desaturation Slope during ischemia period (between 30th – 150th s.). Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

Figure 7.8 – Saturation slope during the 10 seconds after cuff release. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

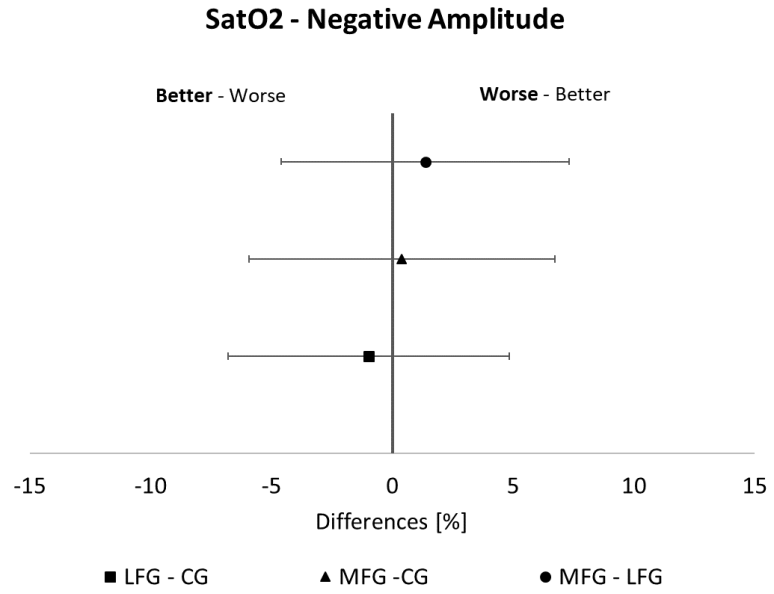

Figure 7.9 – Negative amplitude is the difference between the baseline value and the minimal value during the ischemia. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

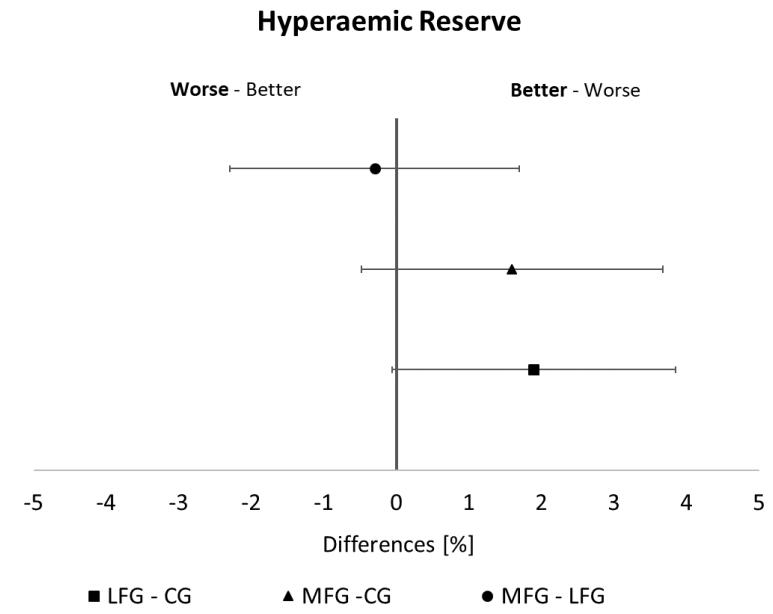

Figure 7.10 – Hyperaemic reserve is the difference between the maximal value reached during hyperaemic response and the minimal value during ischemia. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

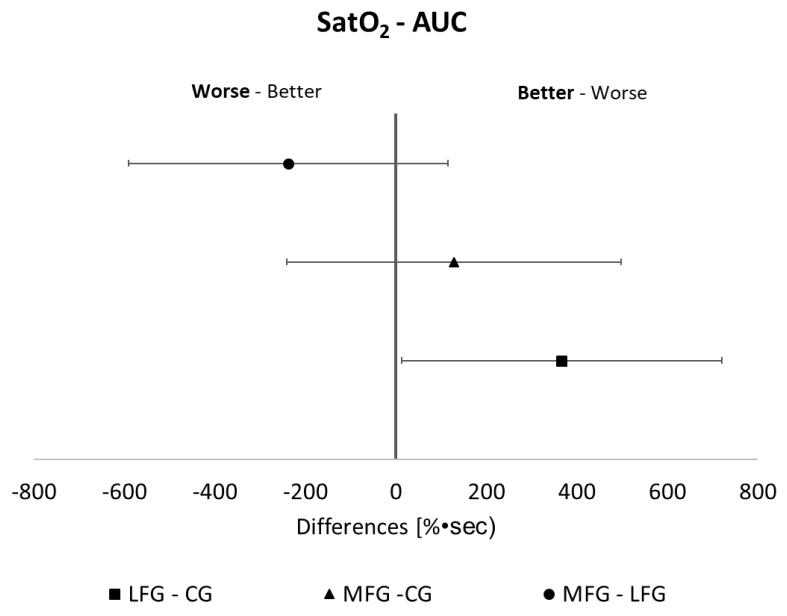

Figure 7.11 – AUC is the area under the hypereamic curve, considering only the above baseline values. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI.

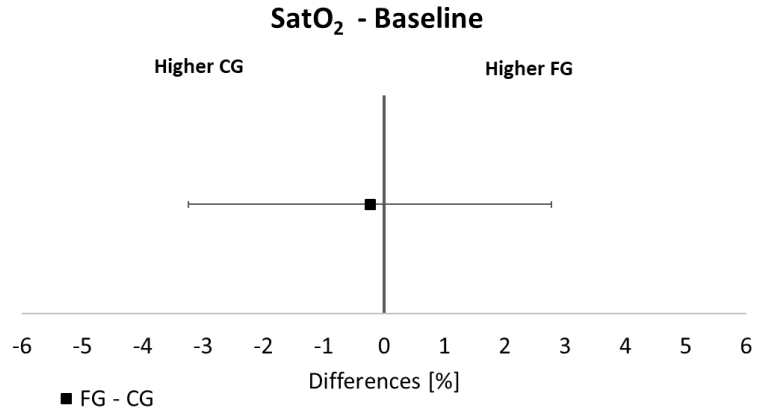

Figure 7.12 - Baseline saturation at the beginning of VOT. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

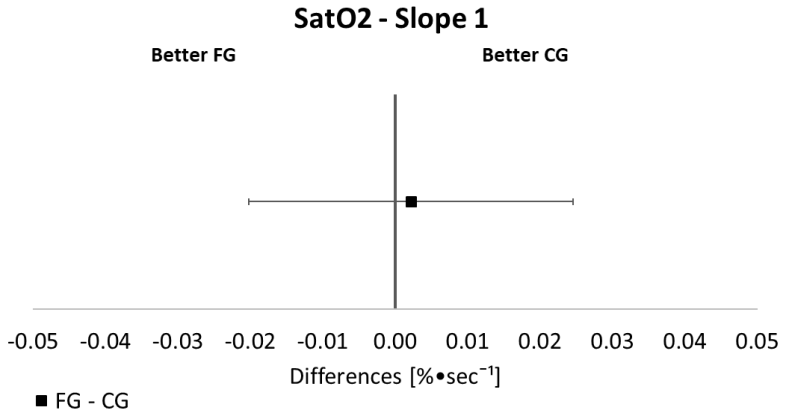

Figure 7.13 - Desaturation Slope during ischemia (30^{th} – 150^{th} s). Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

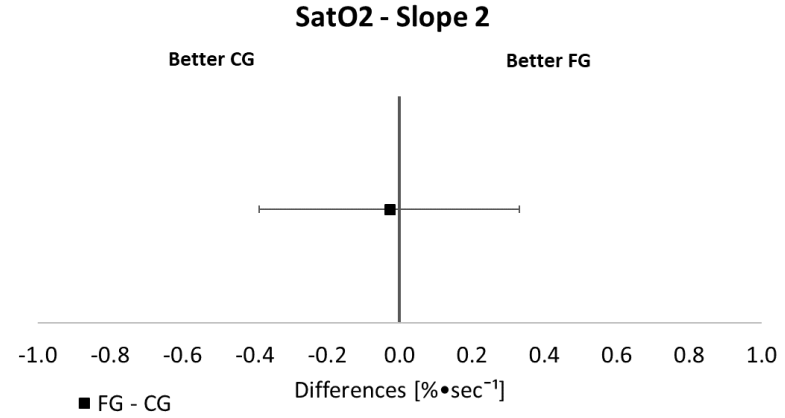

Figure 7.14 - Saturation slope during the 10 seconds after cuff release. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

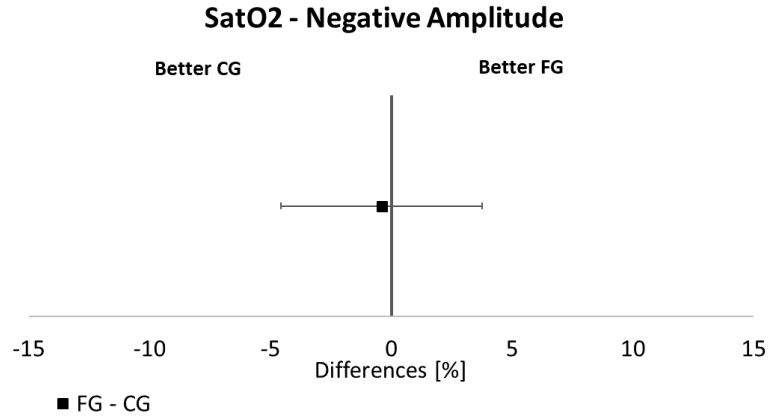

Figure 7.15 - Negative amplitude is the difference between the baseline value and the minimal value during ischemia. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

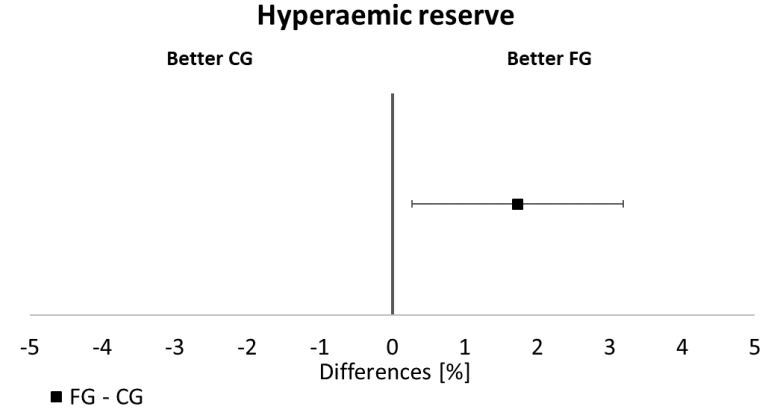

Figure 7.16 - Hyperaemic reserve is the difference between the maximal value during hyperaemic response and the minimal value during ischemia. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

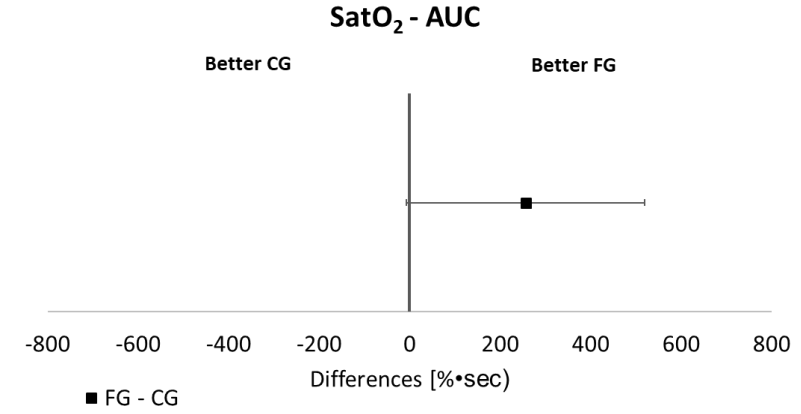

Figure 7.17 - AUC is the area under the hypereamic curve, considering only the above baseline values. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

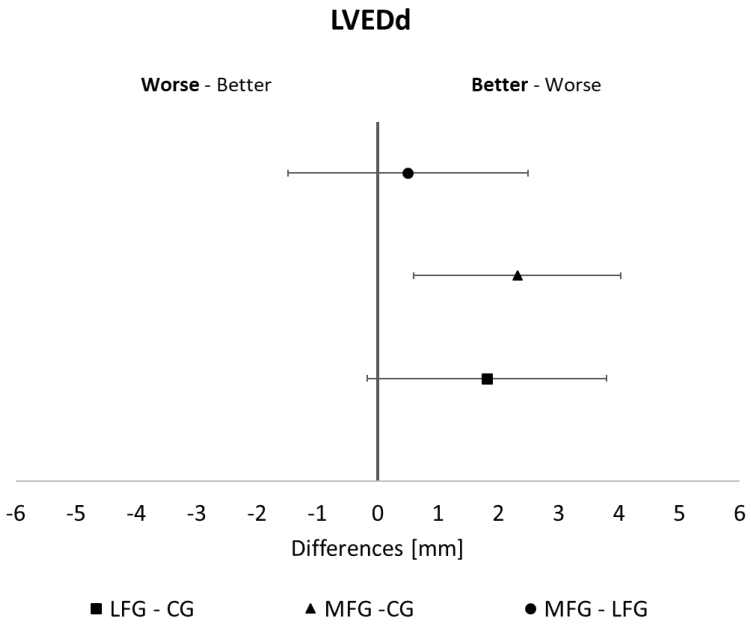

Figure 7.18 – Left ventricular end diastolic diameter. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

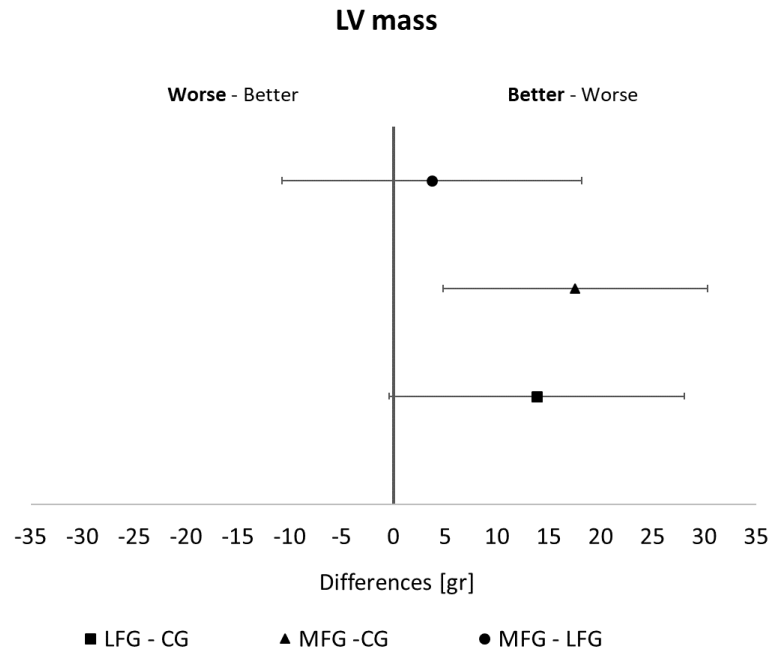

Figure 7.19 - Left ventricular mass. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

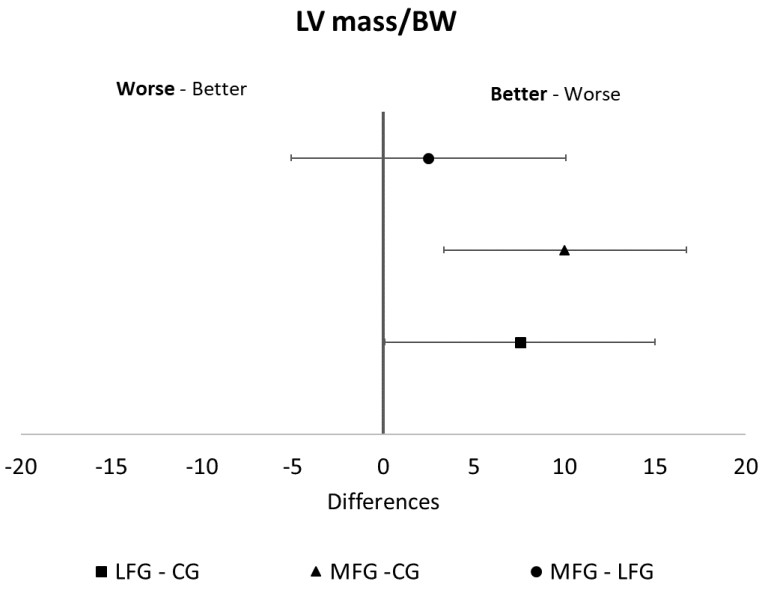

Figure 7.20 - Left ventricular mass indexed to body weight. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

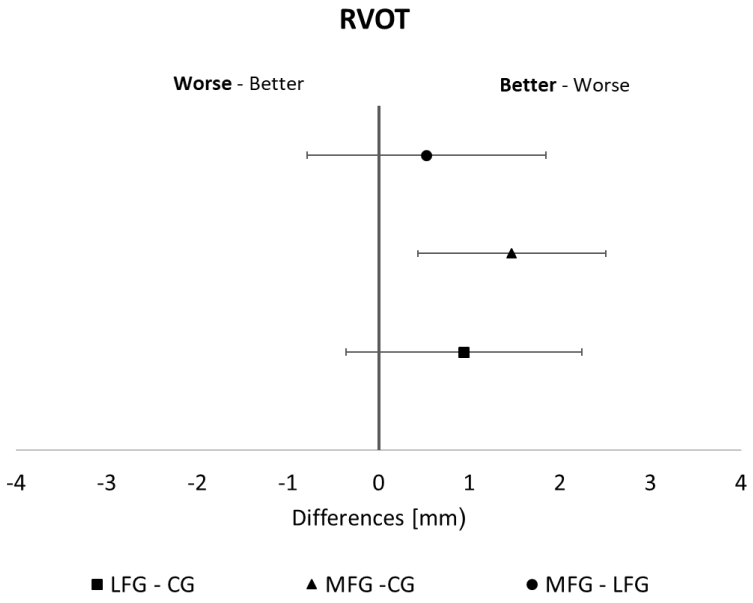

Figure 7.21 - Right ventricular outflow track. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

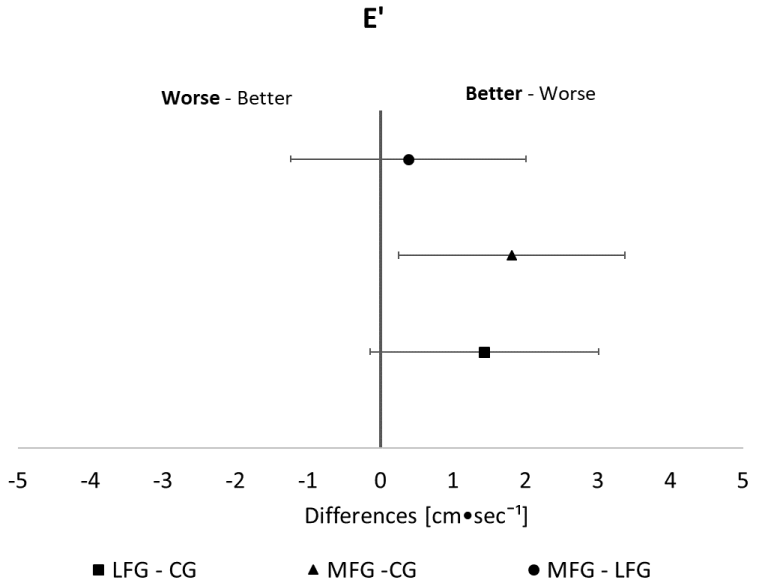

Figure 7.22 – Peak early diastolic velocity. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

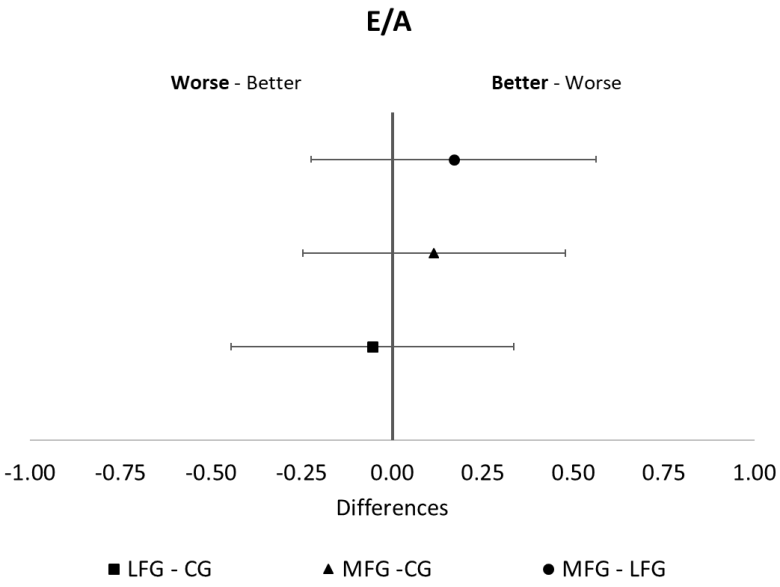

Figure 7.23 - Peak transmitral flow in early diastole (E) / peak transmitral flow velocity in late diastole during atrial contraction (A) ratio. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

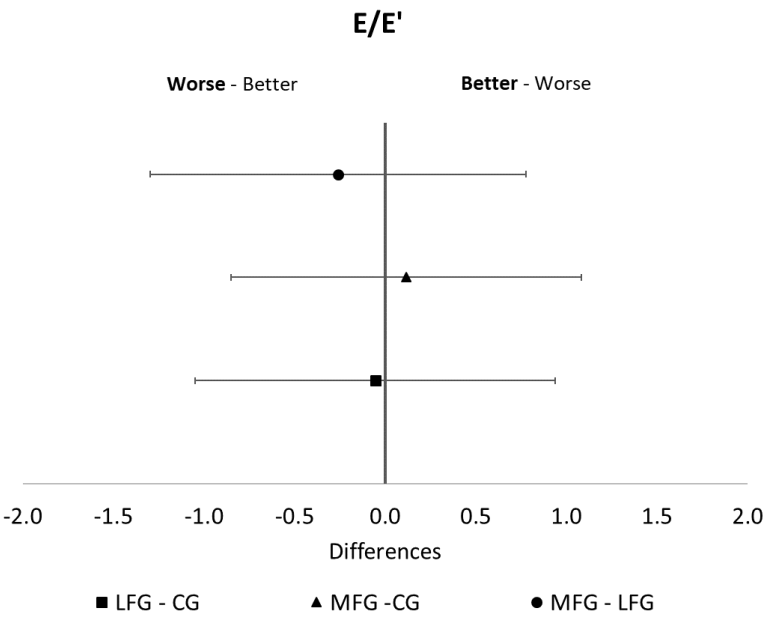

Figure 7.24 – Peak transmitral flow in early diastole (E) / Peak early diastolic velocity (E') ratio. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

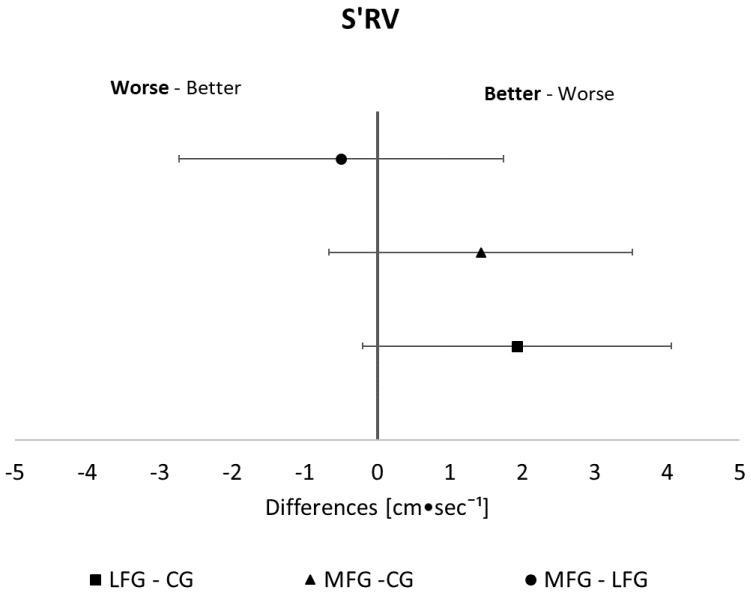

Figure 7.25 - Right ventricular peak systolic velocity. Pairwise comparison between LFG = Low frequency group, MFG = Moderate frequency group and CG = Control group. Change in the mean and 95% CI

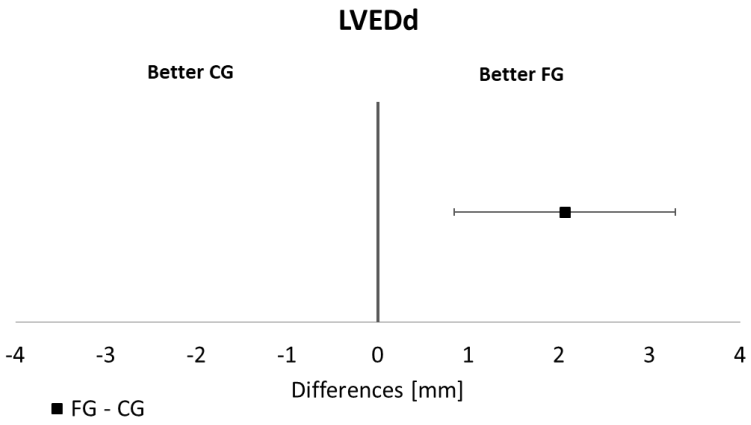

Figure 7.26 - Left ventricular end diastolic diameter. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

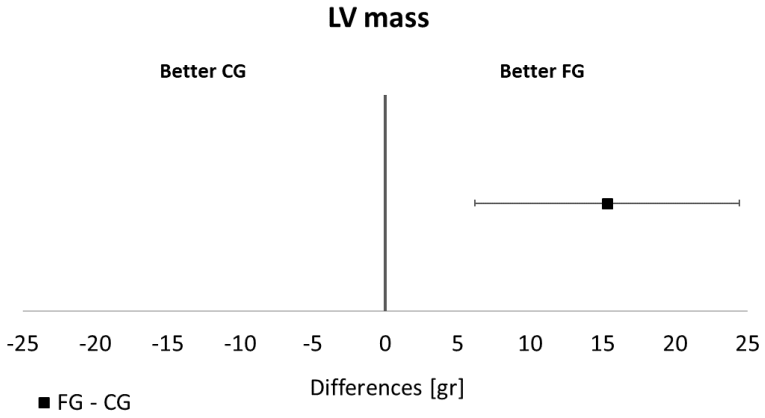

Figure 7.27 – Left ventricular mass. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

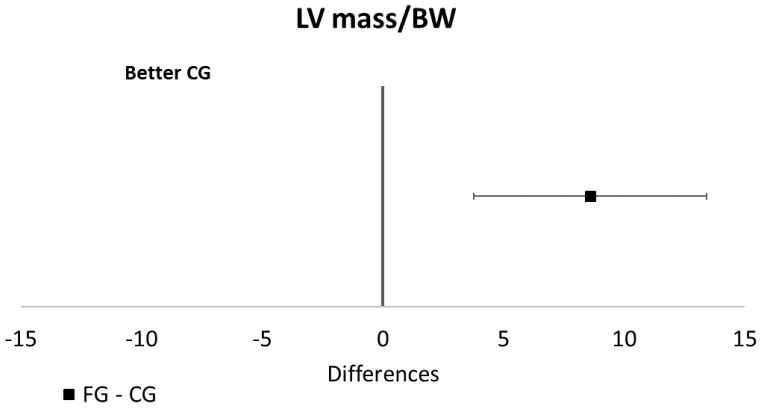

Figure 7.28 - Left ventricular mass indexed to body weight. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

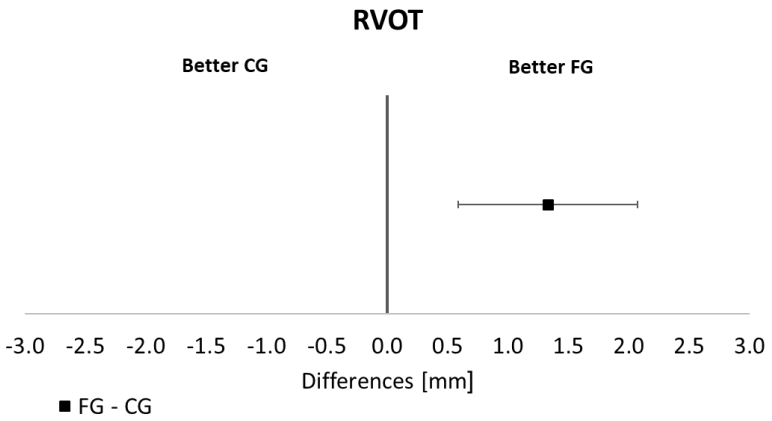

Figure 7.29 – Right ventricular outflow track. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

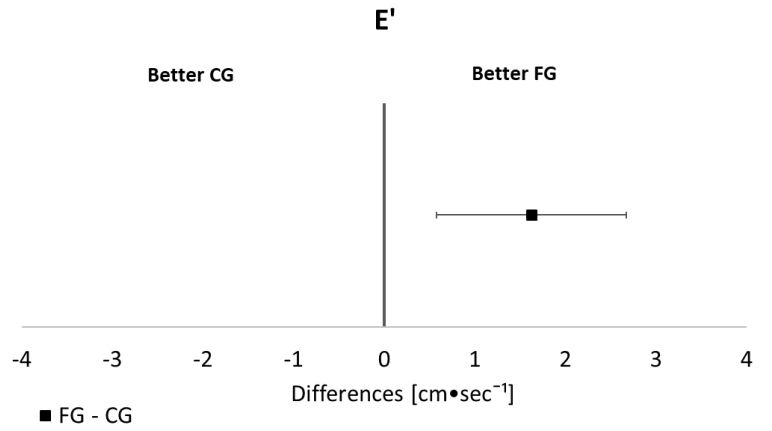

Figure 7.30 - Peak early diastolic velocity. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

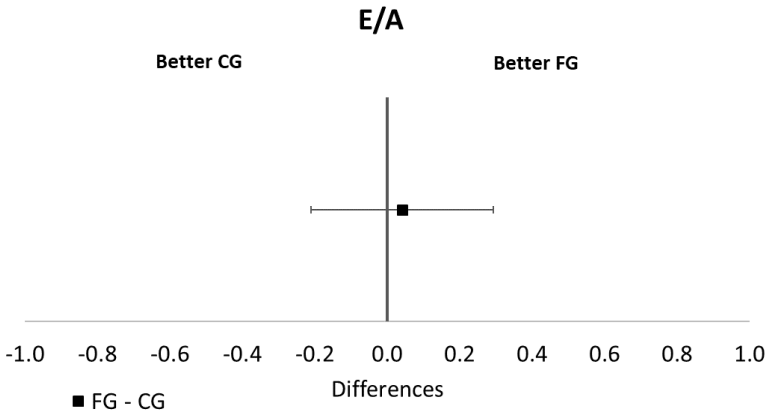

Figure 7.31 – Peak transmitral flow in early diastole (E) / peak transmitral flow velocity in late diastole during atrial contraction (A) ratio. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

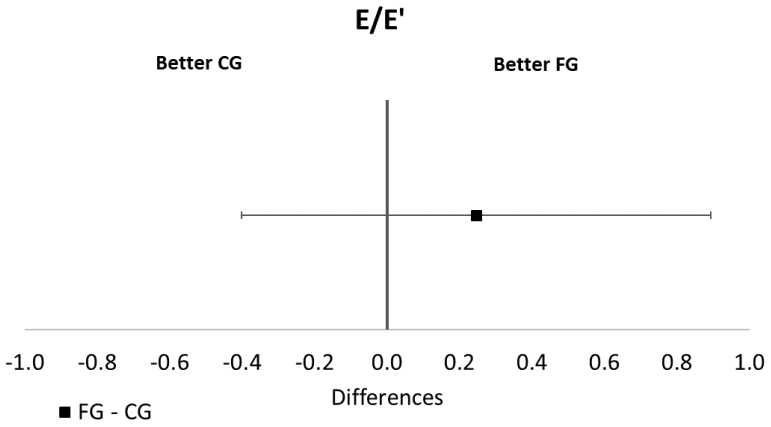

Figure 7.32 - Peak transmitral flow in early diastole (E) / Peak early diastolic velocity (E') ratio. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

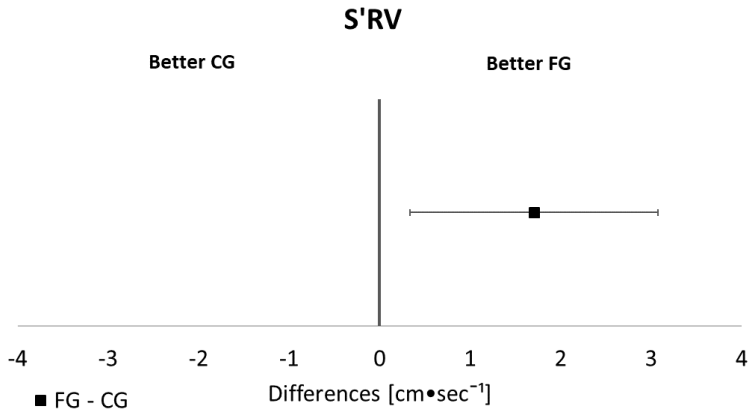

Figure 7.33 – Right ventricular peak systolic velocity. Pairwise comparison between FG = Football group, and CG = Control group. Change in the mean and 95% CI

7.8 References

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8 Limitations

The main aim of these studies was to compare two different doses of recreational football, in order to evaluate even if a low frequency (once a week) can lead to beneficial effects on cardiovascular health. Since the most people play recreational football either once or twice a week, assessing the effect of a low dose of this kind of activity has an important ecological meaning that may represent a weak methodological point too. Some participants with a target frequency of one hour a week, cause of personal commitments, light injuries or illness carried out the experimental period with a lower frequency that could have underestimate the training effect.

Some cardiovascular risk factors included in our studies, such as lipid profile and body composition, are usually influenced by nutritional behavior. We did not prescribe any diet interventions or restrictions to participants. Although we know that the individual nutritional habits might affect some adaptations, it is particularly complicated asking 40 healthy people to modify their diet for 12 weeks.

In addition, related to body composition assessment, we used a skinfolds equation to estimate the fat mass percentage. Other evaluation methods (i.e. dual-energy x-ray absorptiometry, DEXA) allow more accurate and reliable measures, assessing bone density as well. Unfortunately, the planned use of DEXA in our study it has not been possible cause of a technical issue that affected the DEXA machine.

9 Future perspectives

The novel findings of these studies, the efficacy of a low frequency of recreational football to improve some cardiovascular risk factors, filled a gap in the consistent body of literature about recreational football and cardiovascular health in both healthy and unhealthy people throughout lifespan. A 3-month period of low frequency football training led several positive adaptations, but we do not know if a longer period might result in greater improvements in these parameters and changes in those that were unchanged after 3 months.

Moreover, as highlighted by Castagna et al.[1] in a recent editorial, few studies focused on the effects of other type of team sport for improving health. On the one hand different kind of recreational team sports potentially appeal to more people with different skills and interests, on the other hand, team sport involving arms (e.g. basketball, handball, etc.) are expected to lead beneficial effect at upper limb in terms of strength and bone health. More studies involving different recreational team sports and different cohorts of people should be carried out.

9.1 References

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10 Acknowledgements

At the end of this important experience, that lasted three years, I'd like to thank some people who have been very important. Firstly, I want to start by thanking my supervisor Prof. Federico Schena and co-supervisor Prof. Franco M. Impellizzeri who have been fundamental, helping me to plan and develop the research project and this manuscript. Actually, I think the beginning of the journey started on March 2011 when I started to work at CeRiSM (Research Center of Sport Mountain and Health) and I became part of a great group of people and researchers who I can call "mates". Some of them, Alessandro and Spyros, spent a lot of time helping me, during the numerous testing sessions, with our "football players". But, everyone encouraged me with their presence or some advice, especially about either statistics or Matlab programming: Aldo, Alexa, Alfredo, Barbara, Chiara, Federica, Federico, Laura and Lorenzo. I've met other researchers and sport scientists over these last few years, but with three of them there is a special relationship; Maurizio, Alan and Samuele are colleagues and mates who I could collaborate and discuss with, learning a lot.

I have chosen to leave these acknowledgements to the end but, actually, they are the most important people, my family. During these three years I have stolen time from my daughters, Caterina and Margherita and from my wife, Veronica, but they never stopped to encouraging me. I love you, a lot! The family is represented also by your roots, my mum Rosanna, my dad Mariano and my brother Daniele. I have to thank my parents for everything that they did in my life, they supported me in every decision that I made, teaching to accept both the advantages and the consequences of every choice. For these reasons, I miss my dad a lot, he was always a reference point for me and when he died last year, a new, more difficult life started. I would like to dedicate this thesis to my dad Mariano and my grandmother Pia, you can't see this work finished but I know that you are here with us.

Ringraziamenti

Al termine di questa importante esperienza, durata tre anni, voglio ringraziare alcune persone che sono state molto importanti. Innanzitutto, il mio supervisor Prof. Federico Schena e il co-supervisor Prof. Franco M. Impellizzeri, il loro aiuto è stato

fondamentale per la pianificazione e lo sviluppo del progetto di ricerca e di questa tesi. In realtà, penso che l'inizio di questo viaggio sia avvenuto a marzo del 2011 quando ho iniziato la mia collaborazione al CeRiSM (Centro di Ricerca Sport Montagna e Salute) e sono entrato a far parte di un fantastico gruppo di persone e ricercatori che posso chiamare amici. Alcuni di loro, specialmente Alessandro e Spyros, hanno passato un bel po' di tempo in laboratorio aiutandomi nelle sessioni di test. Ognuno comunque, mi ha sostenuto e aiutato con informazioni e suggerimenti, specialmente con la statistica o la programmazione in Matlab: Aldo, Alexa, Alfredo, Barbara, Chiara, Federica, Federico, Laura e Lorenzo. Anche al di fuori del CeRiSM ho avuto modo in questi anni di conoscere molti altri ricercatori, e con tre di loro si è creato un rapporto profondo: Maurizio, Alan e Samuele sono colleghi e amici con cui ho potuto collaborare e confrontarmi, imparando un sacco.

Ho scelto di lasciare loro per ultimi, ma in realtà sono le persone più importanti nella mia vita, la mia famiglia. Durante questi tre anni ho tolto tempo ed energie da dedicare alle mie figlie, Caterina e Margherita e a mia moglie Veronica, ma nemmeno per un momento loro hanno mancato di incoraggiarmi e sostenermi. Vi amo!

La famiglia è anche rappresentata dalle proprie radici e quindi la mia mamma Rosanna, il mio papà Mariano e mio fratello Daniele. Devo molto ai miei genitori per tutto quello che hanno fatto per me, supportandomi in ogni decisione presa, insegnandomi ad assumere la responsabilità ed accettare le conseguenze di ogni scelta. Per queste e altre ragioni, papà, mi manchi tanto, sei sempre stato un punto di riferimento e quando te ne sei andato, lo scorso anno, una nuova e più difficile vita è iniziata.

Voglio dedicare questa tesi a mio padre Mariano e mia nonna Pia, non avete potuto vedere questo lavoro finito ma so che siete qui con noi.