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Physical activity and cardiovascular risk factors

Studies in adults without established cardiovascular disease in primary health care context

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To Carolina and Camilo

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Resumo

A presente tese tem dois objetivos principais. O primeiro, consiste em determinar as possíveis associações entre a atividade física diária e o tempo sedentário com a rigidez arterial e com a função autonómica cardíaca em adultos sem diagnóstico de doença cardiovascular. O segundo, consiste na avaliação do efeito de um programa de intervenção de educação e aconselhamento para a saúde, na atividade física diária, na rigidez arterial e na função autonómica cardíaca.

Para cumprir o primeiro objetivo foi realizado um estudo transversal, envolvendo cento e noventa e sete utentes (47 ± 13 anos) recrutados de forma aleatória em uma unidade de cuidados primários de saúde. Procedeu-se à avaliação da atividade física, do tempo sedentário, da rigidez arterial [velocidade da onda de pulso carótida femoral (VOPcf)] e da função autonómica cardíaca [variabilidade da frequência cardíaca (VFC)]. Os resultados revelaram que o tempo sedentário está associado à VOPcf independentemente da idade, tensão da arterial sistólica e dos níveis de glicose em jejum. Adicionalmente, observou-se que indivíduos com agregação de fatores de risco (síndrome metabólica) e uma maior quantidade de tempo sedentário exibem uma VOPcf significativamente superior do que a registada em indivíduos com síndrome metabólica e menor quantidade de tempo sedentário. Os resultados do estudo para determinação da associação entre atividade física e do tempo sedentário com a VFC, mostraram que a atividade física de intensidade moderada a vigorosa se associava negativamente com o índice do domínio da frequência, razão entre a potência de baixa frequência e a potência de alta frequência, independentemente do sexo, perímetro de cintura e idade.

Para dar resposta ao segundo objetivo, um outro estudo, com desenho longitudinal, incluiu amostra de conveniência de sujeitos com risco cardiovascular moderado a elevado (n = 164), posteriormente alocados a um grupo de intervenção (IG; n = 87) ou a um grupo de controlo (CG; n = 77). O IG foi submetido a um programa de educação e aconselhamento para a saúde, com duração de quatro meses, mais os cuidados médicos habituais, tendo como alvo principal o incremento da atividade física de intensidades ligeira e moderada a vigorosa e a redução do tempo sedentário. O programa de intervenção consistiu em três sessões de formação e aconselhamento em grupo, antecedidas de caminhada. Posteriormente às sessões de formação, os participantes do IG receberam mensagens escritas nos telemóveis, para reforço e encorajamento da prática de atividade física. Os participantes do CG receberam cuidados médicos habituais. As avaliações da atividade física, VOPcf e VFC foram realizadas no início do estudo, imediatamente após o final do programa e oito meses após o seu término. Os resultados do estudo revelaram que o programa de educação e aconselhamento para a saúde não se mostrou eficaz para alterar a atividade física, para reduzir o tempo sedentário e modificar a VOPcf e VFC.

A educação para a saúde e a promoção da atividade física para a prevenção das doenças cardiovasculares, particularmente em indivíduos com risco cardiovascular moderado a elevado, devem incidir na redução de comportamentos sedentários, substituindo-os por atividade física de qualquer outra intensidade acima de 1,5 equivalentes metabólicos, mas idealmente por atividade física de intensidade moderada a vigorosa (acima de 3,9 equivalentes metabólicos).

Palavras-chave: atividade física, rigidez arterial, função autonómica cardíaca, cuidados primários de saúde, educação e aconselhamento em saúde

Abstract

The present thesis has two main aims. The first consists in determining possible associations between daily physical activity and sedentary time with arterial stiffness, and cardiac autonomic function in adults without diagnosis of cardiovascular disease. The second consists in the assessment of the effect of a health education and counseling program on physical activity, arterial stiffness and cardiac autonomic function.

To accomplish the first objective, a cross-sectional study was carried out, involving a total of one hundred and ninety-seven users (47 ± 13 years) randomly recruited from a primary health care unit. Daily physical activity, sedentary time, arterial stiffness (carotid femoral pulse wave velocity, cfPWV), and cardiac autonomic function (heart rate variability, HRV) were evaluated. Results showed that sedentary time is associated with cfPWV independently of age, systolic blood pressure and fasting plasma glucose. Additionally, it was observed that, in individuals with a cluster of risk factors (metabolic syndrome) and superior amounts of sedentary time, the cfPWV is higher compared to individuals with metabolic syndrome and inferior sedentary time. Results of the study, which determine the association between physical activity and sedentary time with HRV, showed that moderate to vigorous intensity physical activity was negatively associated with the index ratio between low frequency power and high frequency power of the frequency domain, independently of sex, waist circumference and age.

To accomplish the second aim, another study was carried out with a longitudinal design with a convenience sample of participants classified as having moderate to high cardiovascular risk (n = 164) that were allocated or into an intervention group (IG; n = 87) or into a control group (CG; n = 77). The IG underwent a four-month health education and counseling program that pretended to improve light and moderate to vigorous physical activity intensities, and reduce sedentary time. Additionally, the IG participants had habitual medical care. The intervention program consisted of three counseling group sessions and walks. Following the sessions, IG participants received text messages on mobile phones to reinforce and encourage the practice of physical activity. Participants from the CG received habitual medical care. Assessments on physical activity, cfPWV, and HRV were performed at baseline, immediately after the intervention, and after eight-month follow-up. Study results showed that the health educational and counseling program was not efficient to modify physical activity, to reduce sedentary time and consequently, to change cfPWV and HRV.

Health education and physical activity promotion for cardiovascular disease prevention, particularly in individuals with moderate to high cardiovascular risk, should target the reduction of sedentary behaviors and their replacement by any physical activity intensity above 1,5 multiples of the basal metabolic rate, but ideally by moderate to vigorous intensity of physical activity (above 3,9 multiples of the basal metabolic rate).

Keywords: physical activity, arterial stiffness, cardiac autonomic function, primary health care, health education and counseling

List of Abbreviations

ANS: autonomic nervous system

a.u.: arbitrary units

BMI: body mass index

c_o: wave speed

CG: control group

cfPWV: carotid femoral pulse wave velocity

CVD: cardiovascular disease

DBP: diastolic blood pressure

DFA1: short term fractal scaling exponent

E: Young's modulus

ECG: electrocardiogram

h: wall thickness

HbA1C: glycated haemoglobin

HDL: high density lipoprotein

hs-CRP: high sensitivity C-reactive protein

HVR: heart rate variability

HF: high frequency power

IG: intervention group

LDL: low density lipoprotein

LF: low frequency power

Ln: natural logarithm

LPA: light physical activity

LF/HF: ratio between low frequency power and high frequency power

IL: interleukin

MET: multiples of the basal metabolic rate

MVPA: moderate to vigorous physical activity

OECD: Organization for Economic Cooperation and Development

ρ: density

PA: physical activity

PNS: parasympathetic nervous system

rMSSD: square root of the mean of the squared differences between successive NN intervals

SBP: systolic blood pressure

SCORE: systematic coronary risk evaluation

SD1: short diameter ellipse

SD2: long diameter ellipse

SD12: Poincaré ratio

SDNN: standard deviation of the normal-to-normal R-R intervals

SNS: sympathetic nervous system

Sqrt: squared root

VFC: variabilidade da frequência cardíaca

VLF: very low frequency power

VOPcf: velocidade de onda de pulso carótida-femoral

TNF-α: tumor necrosis factor- α

CHAPTER I

GENERAL INTRODUCTION

Epidemiology of Cardiovascular Diseases

Over the past decades, age-adjusted cardiovascular mortality has been decreasing in developed countries, although it still remains the leading cause of death (WHO, 2012a). From the 54.7 million deaths that occurred worldwide in 2012, 17.5 million (31%) were due to cardiovascular diseases (CVD) (WHO, 2012a). In Portugal, the mortality rate from CVD dropped from 44.4 in 1988 to 29.5% in 2015 (Direção Geral de Saúde, 2016). Between 2009 and 2013, in Portugal, the potential years of life lost as a consequence of CVD decreased 8.8% (Direção Geral de Saúde, 2016). Possible reasons that might explain the reduction on cardiovascular mortality are related to improvements in diagnostic and therapeutic interventions (J. Liao & Farmer, 2014), and with the development of strategies aiming to promote the adoption of healthy lifestyles (Perk et al., 2012; WHO, 2012b).

Between European Union members, cerebrovascular and ischemic heart diseases are the sixth and fourth cause of death, respectively (Direção Geral de Saúde, 2016). Portugal has a standardized mortality rate from cerebrovascular disease for all ages of 61.9%, which is higher than the standardized rate in European Union (48.1%). For coronary artery disease, the Portuguese's standardized mortality rate for all ages is 34.7%, which is lower than the 75.4% reported for European Union (Direção Geral de Saúde, 2016).

In 2013, in Portugal continental, the standardized mortality rate per 100 000 inhabitants for diseases of the circulatory system was 23.5 and 1125.5 for individuals below and above the age of 65 years, respectively (Direção Geral de Saúde, 2016). According to sex, the standardized mortality rate from diseases of the circulatory system for all ages was 182.1 for males and 128.6 for females per 100 000 inhabitants.

Based on the above-mentioned epidemiological data, the National Plan for Health ("*Plano Nacional de Saúde*") highlights the importance of primary prevention in the Portuguese context (Direção Geral de Saúde, 2013).

Arterial Stiffness

Definition and Measurement

Arterial stiffness is a general term that refers to the loss of elasticity of the large central arteries as a consequence of structural changes in the arterial wall (Laurent & Boutouyrie, 2015; Laurent et al., 2006; Nilsson, 2014).

The arterial tree has two functions. First, to conduit the blood from ventricles to tissues according their needs. Second, to cushion the pulsations generated by the heart, safeguarding a continuous blood flow (Berne & Levy, 2008; O'Rourke & Hashimoto, 2007; Safar, Levy, & Struijker-Boudier, 2003).

In normal conditions, during the left ventricular systole, part of the blood is forwarded directly into the peripheral tissues and part is momentarily stored in the aorta and central arteries, stretching its walls and raising local blood pressure (Laurent & Boutouyrie, 2015, Berne & Levy, 2008; Safar et al., 2003). During the diastole, the elastic recoil of the arterial wall of central arteries will move the accumulated blood to the peripheral tissues, ensuring a continuous flow (Berne & Levy, 2008; Laurent & Boutouyrie, 2015; Safar et al., 2003).

The stretching of the arterial wall generates a forward wave that travels downstream along the arterial tree. A second backward wave is produced at sites of impedance discontinuity in the vascular system that travels back to the heart (Laurent et al., 2006; O'Rourke, Pauca, & Jiang, 2001). The cushioning function of normal elastic central arteries guarantees slowly forward and backward waves, with the backward wave approaching the left ventricle during its diastole, with no further increment on systolic pressure (Laurent et al., 2006; O'Rourke et al., 2001). Conversely, the loss of arterial elasticity is responsible for faster forward and backward waves that are related with central and peripheral consequences. Centrally, an early backward wave reaches the ventricle during its systole, leading to a further increment in systolic pressure (e.g. augmentation pressure) (O'Rourke et al., 2001) and consequently to a reduction of diastolic pressure, which compromises coronary perfusion (Berne & Levy, 2008; Nilsson, Khalili, & Franklin, 2014; O'Rourke & Hashimoto, 2007;

Safar et al., 2003). In the periphery, the increased stiffness of central arteries will induce an elevation in the speed of the forward wave, therefore changing the moving pattern of the blood to the peripheral tissues to a more pulsatile-like pattern and at higher pressures during ventricular systole (Berne & Levy, 2008; Laurent et al., 2006). This will lead to disturbances of metabolic exchanges at the level of the capillaries and increases the risk of damaging target organs such as kidneys, brain and heart (Laurent & Boutouyrie, 2015).

Arterial stiffness can be measured non-invasively at various sites along the arterial tree. Local arterial stiffness is related with changes in vessel size (diameter or area) according to distending pressure, and it is often measured on superficial arteries through echotracking techniques (Laurent et al., 2006; Sakuragi & Abhayaratna, 2010). The local stiffness of the carotid artery is of major clinical importance because of its association with the atherosclerotic process. Distensibility and compliance of deep arteries might be assessed by magnetic resonance imaging (Laurent et al., 2006; Sakuragi & Abhayaratna, 2010). The high costs, high technical expertise, and high time-consuming process required to measure local arterial stiffness has limited its utilization in epidemiological studies (Laurent et al., 2006).

Central wave-reflection analysis is measured non-invasively with applanation tonometry (Sakuragi & Abhayaratna, 2010; Laurent et al., 2006). Indexes of the wave-reflection (e.g. augmentation pressure, augmentation index and central pressures) are generated from the recording of brachial pulse waves using a transfer function (Gallagher, Adji, & O'Rourke, 2004; Laurent et al., 2006) (Figure 1). The augmentation pressure is the amount of pressure that the backward wave adds to the forward wave [i.e. difference between the second (P2) and the first (P1) systolic peak] (Figure 1). The augmentation index is the quotient of augmentation pressure on pulse pressure expressed as a percentage (Figure 1) (Laurent et al., 2006).
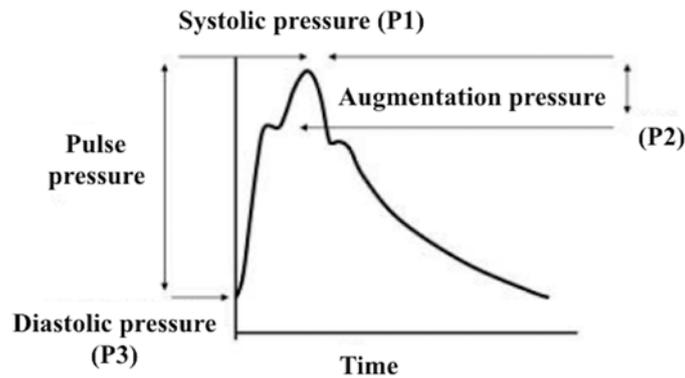
Figure 1. Carotid pressure waveform

Central systolic pressure is also estimated by this approach. Its importance derives from the amplification phenomenon, which is related with the increment of pulse pressure along the arterial tree (Sakuragi & Abhayaratna, 2010). In healthy young subjects, the larger elasticity of the central arteries determine a much lower central than peripheral systolic pressure; whereas diastolic blood pressure is stable along the arterial tree (Latham et al., 1985). Consequently, in healthy young subjects, the amplification phenomenon is large. In elderlies, the pulse pressure amplification phenomenon is attenuated by the stiffening of the arteries (Latham et al., 1985). Therefore, central blood pressure might not often be represented by brachial blood pressure (Latham et al., 1985; Sakuragi & Abhayaratna, 2010).

The utility of pulse wave analysis indices to predict future cardiovascular events and all-cause mortality has already been confirmed (Vlachopoulos, Aznaouridis, O'Rourke, et al., 2010). However, it remains to be demonstrated whether its predictive power for risk stratification is greater or not than peripheral pressure indices (Mitchell, 2015).

Regional arterial stiffness is assessed through of pulse wave velocity (PWV), where a propagative model is applied to the circulatory system. The theoretical basis of PWV is described by the Moens-Korteweg equation:

$$c_o{}^2 : E*h / 2r\rho,$$

Where, "c_o" is the wave speed; "E" is the slope of the stress–strain relationship for a given vessel (Young's modulus); "h" is the wall thickness; "r" the radius, and "ρ" is the density of the fluid.

In practical terms, PWV refers to the time that the forward wave takes to travel a path between two distant points (Laurent et al., 2006). The waves from these two points can be simultaneously read as in Complior systems (Alam Medical, Vincennes, France); or recorded separately as in the Sphygmocor system (AtCor, Sydney, Australia). When assessed separately, in addition to waves it is necessary to use a reference point, which is normally the R wave obtained from an electrocardiogram (ECG) performed simultaneously.

The assessment of PWV over carotid and femoral pulses is the most trustful approach, and it is known as carotid-femoral PWV (cfPWV), or aortic PWV (Weber, Wassertheurer, Hametner, Parragh, & Eber, 2015). According to the literature, cfPWV is the best index of regional stiffness (Boutouyrie & Vermeersch, 2010; Laurent et al., 2006; Mancia et al., 2014; Van Bortel et al., 2012) due to is simplicity (Laurent et al., 2006), reliability (Mancia et al., 2007), and, fundamentally, due to its strong association with CVD incidence, independently of traditional risk factors (Ben-Shlomo et al., 2014a; Laurent et al., 2001; Vlachopoulos, Aznaouridis, & Stefanadis, 2010). Indeed, cfPWV is referred and accepted as a functional marker for CVD (Van Bortel et al., 2012) (Laurent et al., 2001; Mitchell et al., 2010). Conversely, PWV measured outside the aortic track, such as femoro-tibial PWV does not confer prognostic information (Laurent et al., 2006; Pannier, Guerin, Marchais, Safar, & London, 2005). In practice, PWV depends on the time [in seconds (s)] that the wave

needs to cover a certain distance [in meters, (m)], (Laurent et al., 2006; Van Bortel et al., 2012; Boutouyrie & Vermeersch, 2010; Salvi et al., 2008).

There are different ways to determine "time" and "distance" (Boutouyrie & Vermeersch, 2010; Laurent et al., 2006; Vermeersch et al., 2009).

Time depends on how waves are recorded (simultaneously or subsequently), and on which algorithm is used to identify the "foot of the wave". In the Sphygmocor system waves are recorded subsequently (AtCor, Sydney, Australia), and the intersecting-tangent algorithm is used to point out the "foot of the wave", which is the precise intersection point between a line tangent to the initial systolic upstroke of the waveform and a horizontal line through the minimum point (ShygmoCor Software Operator´s Guide, 2008). After determining the foot of wave, the "transit time" is obtained by the delay between the ECG R-wave and the foot of the wave of the femoral pulse subtracted from the delay between the ECG R-wave and the foot of the wave of carotid pulse (Salvi et al., 2008).

Distance depends on the precise points in where pulse waves are registered and the "path" chosen to connect the two points (Boutouyrie & Vermeersch, 2010; Vermeersch et al., 2009). To assess cfPWV, the path between carotid and femoral pulses must be measured. Distance between the two pulse points must be measured precisely once small inaccuracies may influence the absolute value of cfPWV (Boutouyrie & Vermeersch, 2010; Laurent et al., 2006; Vermeersch et al., 2009).

Different distances have been proposed (i.e. direct distance between carotid and femoral pulses; direct distance minus the distance between carotid location and sternal notch; subtracted distance "carotid location to the sternal notch" from "femoral location to the sternal notch"), but the direct distance between carotid and femoral pulses at the body surface seems to be the best predictor (Boutouyrie & Vermeersch, 2010; Van Bortel et al., 2012; Weber et al., 2015). Because the direct distance overestimates cfPWV (when compared with magnetic resonance imaging and invasive measurements), a scaling factor of 0.8 suggested by Sugawara el al. (2008) and Weber et al. (2009) must be

applied to correct the carotid-femoral distance. The cut-off point of 10 m/s is defined as the upper limit from which the arteries are characterized as stiff (Boutouyrie & Vermeersch, 2010). Values higher than that cut-off indicates the presence of a cardiovascular risk factor, linked with target organ damage (Mancia et al., 2014).

Arterial Stiffness: Risk factors

Arterial stiffness is primarily determined by age (Boutouyrie & Vermeersch, 2010; McEniery et al., 2010) and blood pressure (Boutouyrie & Vermeersch, 2010; Laurent & Boutouyrie, 2015; McEniery et al., 2010) that together may account up to 70% of arterial stiffness variance (McEniery et al., 2010). However, risk factors such obesity (Gottsater et al., 2015; Scuteri et al., 2014), hyperglycemia (Gottsater et al., 2015; Schram et al., 2004), dyslipidemia (Gottsater et al., 2015; Laurent et al., 2006), metabolic syndrome (Schram et al., 2004; Scuteri et al., 2014), diabetes (Cameron & Cruickshank, 2007; George, Bantwal, Ayyar, & Mathew, 2015), chronic low-grade inflammation (Jain, Khera, Corrales-Medina, Townsend, & Chirinos, 2014; Korkmaz, Sahin, Ipekci, Temel, & Kebapcilar, 2014), and behavioral risk factors, such as the lack of physical activity (PA) (Ferreira, Boreham, & Stehouwer, 2006; Gomez-Marcos et al., 2014) might also impair arterial compliance. These risk factors are those underlying atherosclerosis and, therefore, atherosclerosis can be viewed as a pathology that amplifies the stiffening of arteries.

Arterial Stiffness and Age

Younger individuals have more distensible arteries, while elders have stiffer ones (Nilsson et al., 2014; O'Rourke & Hashimoto, 2007). As mentioned earlier, stiffer arteries determine a high-speed forward wave, which is responsible for an early wave reflection that reaches the left ventricle during its late systole, adding more pressure to systolic pressure (Laurent et al., 2006; Nilsson et al., 2014; Safar et al., 2003). Age-related degenerative changes occur principally on

central elastic arteries (Benetos et al., 2002; Nilsson, 2014), especially on the aorta artery, which is the major vessel of interest (Laurent et al., 2006). As a natural consequence, vascular aging is associated to the burden of CVD (Avolio, 2013).

Arterial Stiffness and Hypertension

Hypertension is a major risk factor underlying arterial stiffness (AlGhatrif et al., 2013; Benetos et al., 2002; Boutouyrie & Vermeersch, 2010; Laurent & Boutouyrie, 2015; Laurent et al., 2001; Mancia et al., 2014).

In a general sample population followed during 6 years, the increment in arterial stiffness was higher in hypertensive than in normotensives (Benetos et al., 2002), with the highest rate of increment observed in the older (> 50 year) hypertensive group (0.2 m/s/year) (Benetos et al., 2002). In the Baltimore Longitudinal Study on Aging serial measurements of arterial stiffness were collected in adults from 21 to 94 years old and free from clinically significant CVD (AlGhatrif et al., 2013). Results showed that men had a higher rate of increment on arterial stiffness compared to women, especially after the age of 50. Moreover, there was a continuous deleterious effect on arterial stiffness caused by systolic blood pressure (SBP) above 120 mmHg, with a marked acceleration in pressures higher than 140 mmHg. This result suggests that pre-hypertension already exerts a negative impact on arteries.

Arterial Stiffness and Coexistence of Multiple Risk Factors

The coexistence of multiple risk factors further potentiates arterial stiffness (Scuteri et al., 2004). Metabolic syndrome involves a cluster of interrelated risk factors, including raised blood pressure, diminished high-density lipoprotein cholesterol, raised triglycerides, raised blood glucose, and central obesity (Alberti et al., 2009). Metabolic syndrome is established when at least three risk factors coexist simultaneously, and its presence doubles the risk of cardiovascular events in 5 to 10 years-time, and increases 5-fold the risk of type

2 diabetes (Alberti et al., 2009). Metabolic syndrome is associated with impaired arterial functionality and augmented arterial stiffness (Schram et al., 2004; Scuteri et al., 2014; Scuteri et al., 2004). Indeed, the association between metabolic syndrome and increased arterial stiffness was previously reported (Schram et al., 2004; Scuteri et al., 2014; Scuteri et al., 2004). Moreover, waist circumference, a marker of central obesity (Alberti et al., 2009), is associated with insulin resistance and low-grade inflammation (Jia et al., 2015; Safar, Czernichow, & Blacher, 2006), which are correlates or predictors of vascular remodeling and arterial stiffness (Safar et al., 2006; Strasser et al., 2015). A recent longitudinal study, conducted in the middle-age population followed for 17 years, indicated that central obesity, hyperglycemia and atherogenic dyslipidemia are non-hemodynamic predictors of arterial stiffness (Gottsater et al., 2015).

Arterial Stiffness and Atherosclerosis

Atherosclerosis is a focal and potentially occlusive process that is initiated by insults to the endothelium, leading to endothelial dysfunction, enhanced permeability, and inflammation (Libby, Ridker, & Maseri, 2002; Nilsson, 2014). Clinical studies have demonstrated an association between arterial stiffness and atherosclerotic burden (Oberoi et al., 2013; van Popele et al., 2001). A recent study by Selwaness et al. (2014) with 6527 participants from the Rotterdam Study found that increased aortic stiffness is linked to a higher prevalence of carotid atherosclerosis and also to increased intraplaque hemorrhage, which is as a marker of plaque instability. Despite this association, it remains controversial if arterial stiffness is a cause or a consequence of atherosclerosis (Hansen & Taylor, 2016).

Arterial Stiffness, Physical Activity and Sedentariness

Physical activity confers a protective effect on cardiovascular system (Haskell et al., 2007; Nocon et al., 2008), but biological mechanisms underlying cardiovascular protection involve different pathways that are not totally

unraveled (Mora, Cook, Buring, Ridker, & Lee, 2007; Seals, 2014). Most of the research on physiological mechanisms of PA and cardiovascular health are based on systematic and oriented exercises approaches (e.g. structured training) that are transposed to non-structured regular PA.

Physiological benefits from PA on cardiovascular system can be direct or indirect. Indirect benefits might occur through modification on traditional risk factors (e.g., blood pressure, dyslipidemia, glucose control, body fatness) (Nocon et al., 2008; Seals, 2014), and account to about one-half of the CVD risk-reduction from regular PA (Mora et al., 2007). Further benefits from PA are attributed to its influences on vascular hemodynamics, endothelial function (Ribeiro, Alves, Duarte, & Oliveira, 2010; Seals, 2014), inflammation (Ribeiro et al., 2010; Seals, 2014), arterial remodeling and arterial compliance (Seals, Jablonski, & Donato, 2011). It is important to highlight that the development of chronic adaptations (e.g., increased elastin content and inhibition of collagen activity within the arterial wall) require long-term exposure to regular PA (Ferreira et al., 2006).

Until now, only a few studies investigated the possible association between daily PA and arterial stiffness, and they provide conflicting results. For instance, it was shown that ≥ 150 min/week of moderate to vigorous PA (MVPA) (Andersson et al., 2015) and vigorous lifetime PA intensity (van de Laar et al., 2010) is associated with lower arterial stiffness. In addition, it was also observed that greater levels of sedentary time were associated with increased arterial stiffness (Gomez-Marcos et al., 2014; Horta et al., 2015). On its turn, data from the third generation cohort of the Framingham Heart Study based on 2376 apparently healthy individuals showed that higher values of MVPA (per 10 min increment) were associated with lower cfPWV estimate (0.53 m/s, P = 0.006), but the association between light PA intensity (LPA) or sedentary time with arterial stiffness was not observed (Andersson et al., 2015). The Amsterdam Growth and Health Longitudinal Study showed those adults characterized by stiffer carotid arteries at the age of 36 also spent significantly less time in vigorous PA between the ages of 13 and 36 years (-26.5 min/day; CI = -45.9 to -7.1), but no association was observed in relation to previous light-to-moderate PA intensity (-11.2

min/day; CI = -53.5 to 31.1) (van de Laar et al., 2010). Finally, the EVIDENT group showed that total daily PA and moderate PA were negatively associated with augmentation index (β = -0-007, p < 0.01; and β = -0.015, p = 0.04) and positively associated with sedentary time (β = 0.015; p = 0.04) (Gomez-Marcos et al., 2014).

Despite the above results, it has been questioned if the protection against risk factors and clinical manifestations of CVD remains in those persons who, although accomplishing the PA recommendations, also accumulate large amounts of sedentary time (Chau et al., 2013; Thyfault, Du, Kraus, Levine, & Booth, 2015). Indeed, sedentariness is a risk factor that affects cardiovascular heath independently of PA (Thorp, Owen, Neuhaus, & Dunstan, 2011), but the evidences concerning how sedentariness and MVPA relates with arterial stiffness still are scarce. In a retrospective cohort study enrolling 373 apparently healthy adults, it was noted that the time spent watching television over a period of four years was positively associated with arterial stiffness indexes, independently of the amount of vigorous PA and other lifestyle risk factors (van de Laar et al., 2014). In addition, in a cross sectional study with 1241 Brazilian young adults, sedentary time was positively associated with cfPWV after adjustments for MVPA (Horta et al., 2015). Despite these results, the body of evidence remains weak and further longitudinal and cross sectional studies on different populations is needed to close these gaps.

Cardiac Autonomic Function

Definition and Measurement

The autonomic nervous system (ANS) is the part of the nervous system responsible for an adequate interaction between internal and external environment (Koeppen & Stanton, 2008). The ANS is divided into two branches: sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). The ANS regulates smooth muscles, myocardium, and glands in an involuntary and automatic fashion (Koeppen & Stanton, 2008). Despite

frequently described that ANS branches works in opposite ways, a more appropriate description of branches' actions is a coordinated modus aiming the maintenance of the internal body homeostasis (Koeppen & Stanton, 2008).

To guarantee body homeostasis, PNS has a vegetative and restorative function, while SNS is linked with energy mobilization (Thayer, Yamamoto, & Brosschot, 2010). Table 1 describes actions of some effector organs mediated by ANS impulses.

Table 1. Responses of some effector organs to Autonomic Nerve Impulses (Used with permission, this table was adapted from: Berne & Levy Physiology sixth edition, Mosby Elsevier 2008)

Effector Organs	Adrenergic impulse (SNS)	Cholinergic impulse (PNS)
Heart		
- Sinoatrial node	Increase in heart rate	Decrease in heart rate
- Atria	Increase contractility and conduction velocity	Decrease in contractility
- Atrioventricular node	Increase in automaticity and conduction velocity	Decrease in conduction velocity, atrioventricular block
- His-Purkinje system	Increase in automaticity and conduction velocity	Little effects
- Ventricles	Increase contractility, conduction velocity, automaticity, and rate of pacemakers	Slight decrease in contractility
Arterioles		
- Coronary	Constriction ($\alpha 1$); dilation (β_2)	Dilation
- Skin and mucosa	Constriction	Dilation
- Skeletal muscle	Constriction ($\alpha 1$); dilation (β_2)	Dilation
- Cerebral	Constriction (slight)	Dilation
- Pulmonary	Constriction ($\alpha 1$); dilation (β_2)	Dilation
- Abdominal viscera, renal	Constriction ($\alpha 1$); dilation (β_2)	-
- Salivary glands	Constriction	Dilation
Veins (systemic)	Constriction ($\alpha 1$); dilation (β_2)	
Lung		
- Bronchial muscle	Relaxation	Contraction
- Bronchial glands	Inhibition (?)	Stimulation
Kidney	Renin secretion	-

Effector Organs	Adrenergic impulse (SNS)	Cholinergic impulse (PNS)
Adrenal medulla	Epinephrine and norepinephrine secretion	-
Liver	Glycogenolysis, Gluconeogenesis	Glycogen synthesis
Pancreas		
- Acini	Decreased secretion	Secretion
- Islets (beta cells)	Decreased secretion	-
Fat cells	Lipolysis	-

There are two major intrinsic cardiac pacemakers, the sinoatrial and atrioventricular nodes, capable of spontaneously initiating heartbeats (Shaffer, McCraty, & Zerr, 2014). In healthy individuals, because the sinoatrial node has the higher depolarization frequency (60 to 100), it sets the basic rhythm of the heartbeat. The depolarization propagates through the atria and approaches the atrioventricular node, inducing it to fire. The signal fast spreads down leading to the completed ventricular depolarization and synchronized contraction (Koeppen & Stanton, 2008). Despite the innate capacity of intrinsic heart pacemakers to generate a normal cardiac rhythm, cardiac heartbeat rhythm and myocardial contraction force are strongly influenced by ANS, circulating hormones, and ions (Koeppen & Stanton, 2008). This allows the heart to continuously adjust its performance in order to adequately respond to a myriad of challenges and stimuli (Koeppen & Stanton, 2008). The cardiovascular control center, which is located in the brainstem, is responsible for integrating sensory information [from proprioceptors (limb position), chemoreceptors (blood chemistry), and baroreceptors (blood pressure)], and information from the cerebral cortex and limbic system. After integrating information, the cardiovascular center responds by adjusting heart rate via shifts between sympathetic and parasympathetic outflow according to the needs (Shaffer et al., 2014).

Parasympathetic (vagus) nerves decelerate heart rate, while sympathetic nerves accelerate (Table 1) (Koeppen & Stanton, 2008). The time required for parasympathetic activity to slowdown the heartbeat is faster (< 1 s) than the time required for the sympathetic nerves to increase it (> 5 s) (Nunan,

Sandercock, & Brodie, 2010). Vagus nerve innervates sinoatrial and atrioventricular nodes, and atrial cardiac muscle. Additionally, vagus nerve has a scarce innervation in ventricles, reason why it marginally affects ventricular contractility (Table 1) (Shaffer et al., 2014). Parasympathetic neurotransmitters have a short latency and a high turnover rate (cholinesterase is more slowly hydrolyzed than acetylcholine) (Koeppen & Stanton, 2008), which allows the vagal nerve to exert its influence on a beat-by-beat basis (Koeppen & Stanton, 2008; Shaffer et al., 2014). Regarding sympathetic branches, they innervate intrinsic cardiac nodes, atria and ventricles (Table 1). Augmenting sympathetic outflow to the heart is the principal way to increase heart rate above the intrinsic level generated by the sinoatrial node (Koeppen & Stanton, 2008). Contrary to the immediate response in heart rate reduction from vagal modulation, sympathetic branch prompts heart rate increment with a small delay (5 - 10 seconds) after outflow (Koeppen & Stanton, 2008; Shaffer et al., 2014). Thus, it is the dynamic balance between the sympathetic and parasympathetic influences on sinoatrial node activity that primarily determines the actual heart rate of a given physiological state.

The resting heart's sinus rhythm of healthy individuals is regular, with vagal modulation predominance (Koeppen & Stanton, 2008; Shaffer et al., 2014). An over activation of sympathetic outflow and /or a diminished vagal activity on cardiac structures are synonyms of a poor cardiovascular health, and are related with cardiac and non-cardiac disorders (Hillebrand et al., 2013; Kleiger, Miller, Bigger, & Moss, 1987; Thayer et al., 2010; Tsuji et al., 1996). Indeed, an imbalanced ANS is associated with the pathogenesis of malignant arrhythmias (e.g. supraventricular tachycardia, atrioventricular block, and ventricular arrhythmias), myocardial ischemia and sudden death (Johnson, Gray, Lauenstein, Newton, & Massari, 2004; Lahiri, Kannankeril, & Goldberger, 2008). Contrarily, an augmented vagal tonus might have a cardio protective effect linked with a better cardiac electrical stability (Billman, 2002; Curtis & O'Keefe, 2002). Consequently, the clinical assessment of cardiac ANS has gained a notorious importance but it remains a challenge due to its extreme complexity (Lahiri et al., 2008). Most often measurements are non-invasive and encompass

resting heart rate, heart rate recovery, heart rate variability (HRV), and baroreflex sensitivity. Autonomic nervous function might also be assessed invasively (e.g. muscle sympathetic nerve activity), but this approach gives information only about the sympathetic branch.

Heart rate is an index of cardiac ANS that tends to reproduce the overall combination of sympathetic and vagal inputs to sinoatrial node. Nevertheless, heart rate is constantly being adjusted, therefore limiting its use as an index of cardiac ANS. Despite that, there are evidences showing that higher resting heart rate is associated with higher all-cause mortality and cardiovascular mortality, particularly sudden cardiac death (Shaper, Wannamethee, Macfarlane, & Walker, 1993). During exercise, the SNS is increased and vagal outflow is weakened, leading to a necessary and expected increase in heart rate. Heart rate recovery to pre-exercise levels encompasses further modifications of cardiac ANS (e.g. vagal reactivation and sympathetic withdraw) (Cole, Foody, Blackstone, & Lauer, 2000). Evidences suggest that the fast heart rate recovery (during the first and second minutes after exercise cessation) is principally due to parasympathetic reactivation. Diminished sympathetic activity and non-autonomic components such as temperature changes and atrial stretch are less important (Kannankeril, Le, Kadish, & Goldberger, 2004; Lahiri et al., 2008). Furthermore, heart rate recovery seems to have a prognostic significance to predict cardiovascular mortality (Cole et al., 2000).

Heart rate variability is the quantification of fluctuations in the interval between normal heartbeats (Stein & Kleiger, 1999; Task Force, 1996), and results from complex and non-linear interactions between autonomic neural activity and respiratory control (McCraty & Shaffer, 2015). A healthy system changes constantly and dynamically, reason why an optimal level of HRV reflects a healthy function with a self-regulatory capacity, adaptability, or resilience (McCraty & Shaffer, 2015). Impaired HRV characterizes a system unable to adapt to different situations and is associated with numerous clinical conditions (Stein & Kleiger, 1999; Task Force, 1996). Excessive instability (e.g. arrhythmias) is harmful and is related with increased mortality, and excessive stability is linked with ageing, pathology and potentially with an unsuitable

functioning of self-regulatory systems (Stein & Kleiger, 1999; Task Force, 1996).

Heart rate variability data is acquired from short-term (e.g. 5 to 20 minutes) or long-term (e.g. 24-hour) ECG recordings (R-R intervals). Heart rate variability might be assessed with various analytical approaches, and the most commonly used are time-domain, frequency-domain (or power spectral density analysis), and non-linear methods (Task Force, 1996). Long-term recordings allow to track circadian differences in HRV, and daytime and nighttime vagal respiratory variation. Short-term recordings are performed under laboratory environments with standardized conditions, with individuals generally in supine position and under respiratory control. Short time recordings provide information about resting parasympathetic variation in heart rate, but do not give information on circadian rhythm or sleep-related variations (Task Force, 1996). The time intervals between each successive normal QRS complex are recorded and subsequently the abnormal beats that are not generated by the depolarization of the sinus node are eliminated from recordings.

Heart rate variability: time domain analysis

Time domain method of HRV is the simplest to calculate and is based on statistical operations on R-R intervals. Commonly used measures include standard deviation of normal R-R intervals (SDNN) that summarizes all variation in R-R intervals (Task Force, 1996). Moreover, it is possible to calculate the root mean square of successive R-R interval differences (rMSSD) and the number of interval differences between successive intervals greater than 50 ms (pNN50) (Task Force, 1996). In short-term recordings, indexes inform about cardiac parasympathetic outflow (Lahiri et al., 2008). In long-term recordings, indexes are predominantly related with the circadian rhythm (Bigger, Fleiss, Rolnitzky, & Steinman, 1993).

Heart rate variability: frequency-domain analysis

Frequency-domain methods are based on estimates of power spectrum density, which is calculated from R-R interval series with the fast Fourier transformation or autoregressive modeling (Task Force, 1996). Spectral analysis of the R-R interval is used to show the power's distribution (the variance and amplitude of a given rhythm) according to frequency (the time period of a given rhythm) (Lahiri et al., 2008). Values from power density analysis are expressed as the area under the curve (peak) in a given segment of the spectrum (Shaffer et al., 2014).

Three spectral bands are originated from short-term recordings of R-R intervals: very low frequency (VLF; < 0.04 Hz), low frequency (LF; 0.04 – 0.15 Hz), and high frequency (HF; 0.15 – 0.40 Hz) (Task Force, 1996).

In short-term recordings, the physiological meaning of VLF is not established, and there is no agreement about the meaning of the LF band (Kleiger et al., 1987; Shaffer et al., 2014). Possibly, LF results from both branches of ANS (O'Rourke & Hashimoto, 2007; Shaffer et al., 2014). More precise is the physiological meaning of HF band, which may reflect the vagal activity from the respiratory sinus arrhythmia phenomena. Heart rate accelerates during inspiration and slows during expiration. The cardiovascular center inhibits vagal outflow during inspiration leading to heart rate acceleration; during exhalation, the cardiovascular center restores vagal outflow, and heart rate decelerates (Shaffer et al., 2014).

Low frequency and HF are expressed in absolute values (ms^2), and in normalized units (n. u.) (Task Force, 1996), minimizing the effect of the total power on their values. An additional index is the ratio between LF and HF (LF/HF). The physiological meaning might reflect sympathovagal balance, but there is no agreement in the literature due to the dubious origin of the LF band (Billman, 2013; Task Force, 1996).

Heart-rate variability: non-linear analysis

Non-linear methods aims to calculate the structure and complexity of R-R intervals because it is assumed that mechanisms involved in the origin of HRV are non-linear (Task Force, 1996). Examples of non-linear methods are detrended fluctuation analysis and Poicaré plot (Kleiger, Stein, & Bigger, 2005).

Detrended fluctuation analysis reflects randomness and correlativeness of R-R intervals patterns. A detrended fluctuation analysis with a value of 0.05 is associated with a totally random R-R interval pattern, while a value of 1.5 reflects a totally correlated pattern (Kleiger et al., 2005).

Poincaré plot is a graphical representation of the R-R intervals patterns. It results from the ratio between two axes (e.g. a short diameter of an ellipse-SD1; and a long diameter of an ellipse- SD2) that reflect the deviation of instantaneous beat-to-beat R-R intervals variability. The ratio between SD1 and SD2 provides information on the organization of heart rate patterns. Abnormal Poincare's plots are characteristic of low HRV (Kleiger et al., 2005).

Autonomic Function: Risk factors

Traditional risk factors such as age (De Meersman & Stein, 2007; Voss, Schroeder, Heitmann, Peters, & Perz, 2015), sex (Voss et al., 2015), obesity (Canale et al., 2013), hypertension (Mancia & Grassi, 2014) and metabolic syndrome risk factors (Canale et al., 2013; Vinik, Maser, & Ziegler, 2011) might influence the ANS function.

The deleterious effect of ageing is transversal to many physiological systems, affecting also ANS. Indeed, age-related changes in HRV are characterized by a diminished parasympathetic activation and an incremented sympathetic activation (De Meersman & Stein, 2007; Umetani, Singer, McCraty, & Atkinson, 1998; Voss et al., 2015). Age-related reductions in HRV may reflect cardiovascular modification such the loss of sinoatrial cells and reduced arterial distensibility (Voss et al., 2015).

There is a lack of agreement regarding the sex influence on ANS. Women compared to men showed lower cardiac vagal modulation (e.g. time domain

indexes) until the decade of 30 years. After this age, differences between sexes disappear (Umetani et al., 1998). In another study, it was shown that for time-domain indexes both sexes showed similar results, but females compared to males had a higher vagal modulation (e.g. HF) between the age-range of 35 and 54 years (Voss et al., 2015). Despite the dubious results between sexes, differences on cardiac autonomic function disappear with aging, presumably by the hormonal reorganization particularly triggered by the menopause process (Umetani et al., 1998; Voss et al., 2015).

Autonomic impairment is present in hypertension (Palatini & Julius, 2009), even in its early phases (Mancia & Grassi, 2014). Unbalanced ANS is a potentially causative mechanism of higher blood pressure (Palatini & Julius, 2009). Indeed, both sympathetic and parasympathetic divisions are altered in individuals who are at risk of developing hypertension, but do not have the clinical manifestation of high blood pressure (e.g. normotensives with family history of hypertension) (Mancia & Grassi, 2014; Palatini & Julius, 2009). In established hypertension, sympathetic overactivity is a generalized phenomenon, linked probably with other conditions such as obesity, metabolic syndrome and diabetes mellitus (Mancia & Grassi, 2014). Mechanisms responsible for the occurrence of sympathetic activation in hypertension are not fully clarified and are multifactorial (Grassi, 1998; Mancia & Grassi, 2014). One possibility is that the adrenergic overactivity is secondary to activation of the renin-angiotensin-aldosterone system, given that angiotensin II has a stimulatory effect on sympathetic outflow and an inhibitory effect on vagal activity (Grassi, 1998; Mancia & Grassi, 2014). Another possibility is linked with insulin-resistance, a condition frequently correlated with hypertension (Grassi, 1998; Mancia & Grassi, 2014). Insulin stimulates the SNS to release epinephrine and norepinephrine to heart and vessels, augmenting even more cardiac output and blood pressure (Grassi, 1998; Mancia & Grassi, 2014). Excessive environmental stressors might also trigger and overactivation of sympathetic branches (Grassi, 1998; Mancia & Grassi, 2014). Finally, baroreflex impairment might also increase sympathetic activity (Mattace-Raso et al., 2007). In normal conditions, arterial baroreceptors provide continuous information of blood

pressure firing afferent stimulus to ANS that respond with appropriate discharge or to vagal or to sympathetic neurons, adjusting blood pressure and arterial tonus (Mattace-Raso et al., 2007). In chronic adrenergic conditions such as hypertension, baroreflex sensor areas might suffer reduced vascular compliance, and, consequently, the baroreflex sensitivity get impaired, reducing their afferent firing to augment vagal tonus (Mattace-Raso et al., 2007) and compromising its role on blood pressure control. Physiological theory supporting long-term regulation of blood pressure by baroreflex sensitivity is linked to arterial stiffness (Mattace-Raso et al., 2007). In animal models, the chronic exposure to elevated levels of epinephrine has induced to replication of vascular smooth muscle cells (Mancia & Grassi, 2014). Vascular smooth muscle cells proliferation is linked both with arterial stiffness and atherosclerotic plaque formation (Mancia & Grassi, 2014).

Obesity and sympathetic overactivation are strongly associated (Canale et al., 2013; Malpas, 2010). In obese individuals, sympathetic modulation is enlarged to kidney, skeletal muscle and peripheral vessels to elicit hypertension (Moreira et al., 2015). More important than the amount of fat, the impact of adipose tissue on ANS is more influenced by its localization /distribution in the body (Grassi et al., 2004). While subcutaneous fat is mainly a fat depot (Canale et al., 2013), visceral fat is a metabolically active organ that leads to a bigger activation of sympathetic branches (Alvarez, Beske, Ballard, & Davy, 2002; Canale et al., 2013). Visceral fat, comprised by insulin-resistant cells and inflammatory cells (Canale et al., 2013), produces and releases adipokines with a pro-inflammatory and pro-atherogenic action (Canale et al., 2013). There are many mechanisms that justify the association between obesity and sympathetic overactivation, and probably most of them coexist. Examples are obesity-related insulin and leptin resistance as both can modulate SNS activity (Canale et al., 2013).

There is an inverse association between cardiac ANS and metabolism (Curtis & O'Keefe, 2002; D. Liao et al., 1998; Vinik et al., 2011). The overactivation of sympathetic nerve is associated with specific metabolic syndrome components, but especially with obesity and hypertension (Grassi, 2006; Moreira et al.,

2015). As above-mentioned, an increased sympathetic nerve activity is detectable in obese patients, but when obesity is presented together with hypertension, the degree of sympathetic activation is greater (Grassi, 2006). The pathophysiological mechanisms linking sympathetic overactivity and metabolic syndrome are complex and still need to be fully elucidated, but insulin resistance was suggested to be the main one (Moreira et al., 2015).

Additionally, the negative effect on ANS from adipokine, baroreflex impairment, oxidative stress and low-grade inflammation might not be neglected (Canale et al., 2013; Moreira et al., 2015). The augmented sympathetic nervous activity strengthens the hemodynamic stress and reduces shear stress-induced dilation of the vessels, potentially impairing endothelial function and triggering a consequent inflammatory response (Haensel, Mills, Nelesen, Ziegler, & Dimsdale, 2008; Sajadieh et al., 2004). An augmented inflammatory response leads to an overproduction of reactive oxygen species, that in its turn, reduces nitric oxide bioavailability and endothelial-mediated vasodilatation (Chistiakov, Ashwell, Orekhov, & Bobryshev, 2015; Touyz & Schiffrin, 2004). Nitric oxide modulates cardiac vagal activity because reductions in its synthesis or increases in its degradation reduce the vagal tone (Patel, Li, & Hirooka, 2001).

Sympathetic activation exerts a causative role on arterial vascular remodeling, but there is a possible bidirectional pathway between arterial stiffness and ANS activity: sympathetic activity modulates arterial structure and function, but carotid artery wall structure and function may alter sympathetic neural tone (Dinenno, Jones, Seals, & Tanaka, 2000). Indeed, there are some evidences showing the associations between ANS and arterial stiffness (Dinenno et al., 2000; Mattace-Raso et al., 2007).

Lastly, an increased cardiac sympathetic modulation and reduced cardiac vagal modulation leads to an electrical instability of the heart, with a consequent risk of ventricular arrhythmia, mainly on conditions of myocardial ischemia and infarction (Johnson et al., 2004; Koeppen & Stanton, 2008).

Cardiac Autonomic Function, Physical Activity and Sedentariness

There is an abundance of literature on PA and its effects cardiac autonomic function measured by HRV (Buchheit et al., 2004; Dietrich et al., 2008; Soares-Miranda et al., 2014; Stein, Ehsani, Domitrovich, Kleiger, & Rottman, 1999). Moderate to vigorous PA intensity in regular basis might positively modulate cardiac autonomic function, by promoting an antiarrhythmic effect (Billman, 2002), once MVPA promotes vagal outflow and reduces sympathetic activity (Billman, 2009; Dietrich et al., 2008; Soares-Miranda et al., 2012). The mechanisms by which MVPA modulates cardiac ANS are not well established (Billman, 2002), but are potentially linked with (i) reduction of the sympathetic neural outflow to the sinoatrial node leading to an attenuation of heart rate in response to myocardial stretch (Smith, Hudson, Graitzer, & Raven, 1989), (ii) reduction of GABAAergic neurotransmissions that are involved in heart rate control, and therefore promoting an augmented vagal influence on cardiac sinoatrial node activity (Mueller & Hasser, 2006); and (iii) improving cardiomyoctyes contractility, enhancing cardiac electrical stability (Billman, 2009). Additionally, there are some evidences showing that MVPA is associated with increased vascular compliance and arterial restructuring (Andersson et al., 2015; Krieger, Da Silva, & Negrao, 2001). Like the arteries, baroreceptors might also suffer remodeling, and as a result, there is an improved afferent activity, leading to an augmented outflow of the parasympathetic branch (Green, O'Driscoll, Joyner, & Cable, 2008). Moderate to vigorous PA is also associated with an enhanced production of nitric oxide and consequently the better endothelial function (Seals et al., 2011).

The positive association between MVPA and parasympathetic outflow measured by HRV were mainly constructed by comparing very active or even athletes individuals against sedentary controls (Melanson, 2000; Stein et al., 1999). However, studies including general population have provided divergent results, some found positive associations with PA (Buchheit et al., 2004; Dietrich et al., 2008; Garet et al., 2005; Kiviniemi et al., 2016; Sandercock, Hardy-Shepherd, Nunan, & Brodie, 2008; Soares-Miranda et al., 2009), while others did not find any association (Fagard, Pardaens, & Staessen, 1999;

Greiser et al., 2009; Kluttig et al., 2010). Methodological constraints might contribute for divergent results. Most studies assessed PA through self-reporting questionnaires (Dietrich et al., 2008; Garet et al., 2005; Kiviniemi et al., 2016; Sandercock et al., 2008), which tend overestimate total PA and time spent at different PA intensities (Dyrstad, Hansen, Holme, & Anderssen, 2014). Second, studies were mainly performed with a restricted age range [adults (Sandercock et al., 2008; Soares-Miranda et al., 2009), middle-aged adults (Buchheit et al., 2005, 2006) or elderly (Buchheit et al., 2004; Soares-Miranda et al., 2014) and with homogeneous health conditions, which might limit the clinical utility of results for the general population as users of primary health care centers.

Another focus of discussion is the impact of different intensities of PA on HRV. There is almost no information on the association between sedentary time and LPA with HRV. Recently, sitting time at work was negatively associated with time and frequency HRV indices in blue-collar workers, regardless of MVPA (Hallman et al., 2015). The valid number of days for PA measurement was 1.9, which is far below the recommendations for assessment of daily PA levels (Troiano et al., 2008). Future studies are needed to ascertain the possible relationships between HRV and sedentary time.

Cardiovascular Risk Estimation

Cardiovascular diseases are associated with prolonged exposure to modifiable and non-modifiable CVD risk factors that impair the normal function of the heart and vessels, culminating in cardiovascular events (Mancia et al., 2014; Cooney, Dudina, & Graham, 2009). A single risk factor increases the chances of a CVD event, but the clustering of multiple risk factors cause it to rise exponentially (D'Agostino et al., 2008; D'Agostino, Pencina, Massaro, & Coady, 2013; Mancia et al., 2014). It is possible to quantify CVD risk through estimation systems that encompass risk factors with stronger predictive capacity (e.g. age, sex, smoking, lipid status and blood pressure) (Conroy et al., 2003; Cooney et al., 2009; D'Agostino et al., 2013). The purpose of risk estimation systems is to

identify individuals at greater risk in order to define strategies to prevent or delay the onset of a cardiovascular event (Cooney et al., 2009; Mancia et al., 2014). After risk determination, risk factors might be corrected through behavioral education and counseling and, when this is ineffective, patients will start doing medication (D'Agostino et al., 2013; Grundy, Pasternak, Greenland, Smith, & Fuster, 1999).

The best-known risk estimation system, and probably the most widely used, is The Framingham Risk Score (D'Agostino et al., 2013). This calculator was developed based on intermediate-sized samples (n = 5 209 individuals aged between 30 and 59 years old) representative of the general population (D'Agostino et al., 2013). The Framingham Risk Score estimates the probability of an individual to develop CVD (including coronary heart disease, cerebrovascular disease, intermittent claudication, and congestive heart failure) within a ten-year time frame. It calculates the risk using sex, age, systolic blood pressure (SBP), total cholesterol, high-density lipoprotein cholesterol, smoking behavior, and diabetes (D'Agostino et al., 2008; D'Agostino et al., 2013). In Europe, the calculator Systematic Coronary Risk Evaluation (SCORE) was developed based on a substantially larger dataset (> 205 000) from 12 European countries mainly based on general population (Conroy et al., 2003). The SCORE system estimates the risk of a fatal cardiovascular event within 10 years. The SCORE is based on age, sex, SBP, total cholesterol, and smoking behavior (Conroy et al., 2003). The greatest advantage of this system is that each European country has an adjusted risk estimation calibrated according national mortality statistics (Conroy et al., 2003).

Independently of the calculator adopted, it is easy to determine the risk categories "high" or "very high" in individuals with already established CVD or diabetes (Boutouyrie & Vermeersch, 2010; Grundy et al., 1999; Mancia et al., 2007; Mancia et al., 2014). However, in individuals without any evident risk factor, total risk determination is not easily performed (Grundy et al., 1999; Mancia et al., 2014), because both calculators depend fundamentally on age (Mancia et al., 2007; Mancia et al., 2014). Consequently, for young adults (specially women), the risk level might be low, even in the presence of

hypertension associated with other cardiovascular risk factor (Mancia et al., 2014). The main consequence is an inadequate risk stratification in young individuals that are excluded from strategies designed to control risk factors and, consequently, stay exposed to risk factors during a long time, which might lead to a high and potentially irreversible risk at middle and advanced ages (Mancia et al., 2014).

Based on the aforementioned, refining cardiovascular risk prediction to better target preventative therapy among individuals considered to be at low or moderate risk has been subject of study. Indeed, the 2007 (Mancia et al., 2007) and 2013 (Mancia et al., 2014) European Guidelines for the management of hypertension and guidelines for CVD prevention in clinical practice has suggested an alternative risk quantification score based in two main doorways: i) blood pressure, and ii) the presence of cardiovascular risk factors, asymptomatic organ damage or diabetes mellitus, chronic kidney disease (starting in stage 3), and symptomatic chronic disease.

Long-term exposure to hypertension might lead to asymptomatic changes in organs such as kidney, brain and heart, indicating progression of disorders or pathologies, underlying CVD (Mancia et al., 2007; Mancia et al., 2014). Arterial stiffness, micro albuminuria, increased left ventricular hypertrophy and carotid plaques are markers of organ damage, enabling to predict mortality due to CVD (Sehestedt et al., 2012), and are related with at least, a moderate risk (Mancia et al., 2007; Mancia et al., 2014).

Carotid-femoral PWV has emerged as a potential candidate to improve risk stratification (Laurent et al., 2001) and its utility was already demonstrated in longitudinal studies (Boutouyrie et al., 2002; Mitchell et al., 2010), and meta-analysis (Ben-Shlomo et al., 2014b). Therefore, the ability of arterial stiffness to predict coronary heart disease (beyond the Framingham Risk Score) was tested in 1 045 participants with essential hypertension (Boutouyrie et al., 2002). After adjusted for Framingham Risk Score, the increment of 1 standard deviation (SD) in cfPWV was associated with a 34% increase in risk for coronary artery disease (95% CI = 1.01 to 1.79; P = 0.039) (Boutouyrie et al., 2002). In another

study, enrolling 2 232 individuals from The Framingham Heart Study, with 7.8 years follow up, a higher cfPWV was associated with a 48% increased CVD risk (95% CI = 1.16 to 1.91, P = 0.002) after adjusting for age, sex, SBP, lowering blood pressure medication, high density lipoprotein cholesterol, smoking and diabetes (Mitchell et al., 2010). In the same study, the addition of cfPWV to the standard risk factor model, result in a risk discrimination improvement of 0.7% (95% CI = 0.05 to 1.3%, P < 0.05), suggesting that cfPWV might be a useful biomarker of CVD risk (Mitchell et al., 2010). Finally, a meta-analysis based on 16 studies with a minimum of 1-year follow-up, and encompassing a total number of 17 635 individuals, revealed that cfPWV, after adjusted for conventional risk factors, remained a strong predictor of coronary heart disease (HR = 1.23 [95% CI = 1.11 to 1.35]; P < 0.001), stroke (HR = 1.28 [95% CI = 1.16 to 1.42]; P < 0.001), and CVD events (HR = 1.30 [95% CI = 1.18 to 1.43]; P < 0.001) (Ben-Shlomo et al., 2014b). Furthermore, the addition of cfPWV to Framingham risk score improved the overall 10-year risk classification by 13%, which was particularly important for the reclassification of younger individuals at intermediate (Ben-Shlomo et al., 2014b).

As previously mentioned, impaired ANS and inadequate autonomic responsiveness to stimulus are markers of an unhealthy cardiovascular system and a powerful risk factor for adverse cardiovascular events, including malignant arrhythmias (Andresen & Bruggemann, 1998; Johnson et al., 2004), morbidity and mortality after myocardial infarction (Bigger et al., 1993) and mortality (Curtis & O'Keefe, 2002; Vinik et al., 2011). After the first cardiac event, individuals with lower HRV have an increased risk of cardiovascular morbidity and mortality compared with those with high HRV (Andresen & Bruggemann, 1998; Dutsch, Burger, Dorfler, Schwab, & Hilz, 2007), which indicates that a less adaptive ANS is a marker of cardiovascular risk. Consequently, after the first cardiac event, the ANS becomes less sensitive for minor hemodynamic changes, which can cause of a secondary cardiovascular event (Dutsch et al., 2007).

Although reduced HRV is associated with cardiovascular events in individuals with known CVD, the evidence on the association between HRV and the risk of

a first cardiovascular event in a population without known CVD, remain inconclusive. The ability of cardiac autonomic function to predict the risk of adverse cardiovascular events was tested in 2 501 participants free of clinically apparent heart disease from the Framingham Heart Study (Tsuji et al., 1996). After adjustments for cardiovascular risk factors, time and frequency domain indexes of HRV, except the ratio between LF and HF, were significantly related with adverse cardiac events. A 1SD reduction in the SDNN was associated with a hazard ratio of 1.47 for new cardiac event (95% CI = 1.16 to 1.86) (Tsuji et al., 1996). Recently, a meta-analysis from prospective cohort studies with 21 988 participants (follow-up raging between 3.5 to 15 years), pointed out that individuals with low HRV have an additional risk of 32-45% of fatal and non-fatal cardiovascular event compared to individuals with high HRV (Hillebrand et al., 2013). The HRV indexes with significant risk were SDNN (RR = 1.35; 95% CI = 1.10 to 1.67), LF (RR = 1.45; 95% CI = 1.12 to 1.87) and HF (1.32; 95% CI = 0.96 to 1.81). Despite the above results, methodological issues limit between-studies comparisons. Additionally, the capacity of cardiac autonomic function in improving risk determination further than Framingham score is questionable for apparently healthy populations.

Physical activity is defined as any movement produced by skeletal muscles that requires energy expenditure (Caspersen, Powell, & Christenson, 1985), meaning that the spectrum of PA ranges from low energy expenditure behaviors to those related with vigorous and very vigorous occupational and leisure time activities. Joint guidelines from the American College of Sports Medicine and the American College of Sports Medicine and the American Heart Association recommend that healthy persons aged 18 to 65 years should engage in moderate-intensity PA for at least 30 minutes 5 days a week (150 minutes per week) or vigorous-intensity activity for at least 20 minutes at least 3 times per week (60 minutes per week) to promote and maintain health (Haskell et al., 2007). Moderate intensity is related with activities between 3.0 to 5.9 multiples of the basal metabolic rate (METs), and vigorous those activities that occur over 6.0 METs (Ainsworth et al., 1993).

The non-commitment with these international guidelines defines physical inactivity (WHO, 2012a), which has been identified as a leading risk factor for several conditions (Kohl et al., 2012; WHO, 2010, 2012a). Indeed, strong evidence shows that physical inactivity is associated with an increase rate of all-cause mortality, coronary heart disease, hypertension, stroke, metabolic syndrome, type II diabetes, and breast and colon cancers (Lee et al., 2012; Roger et al., 2011; Shaw, Gennat, O'Rourke, & Del Mar, 2006).

Almost one-third of world´s population is insufficiently active (Lee et al., 2012). Economic consequences from physical inactivity are significant, with costs being estimated in 1.5% to 3% of total direct healthcare expenditure in developed countries (Oldridge, 2008). The majority of studies relating CVD and PA have focused on MVPA and effectively, showed that MVPA confers a cardiovascular protective effect (Erlichman, Kerbey, & James, 2002; Paffenbarger et al., 1993; Shiroma & Lee, 2010). Indeed, PA practiced in regular bases is associated with a 35% reduction from CVD (Nocon et al., 2008).

Time spent in sedentary behaviors has emerged as another important CVD risk factor, which is distinct from physical inactivity (Owen, Healy, Matthews, & Dunstan, 2010; Pate, O'Neill, & Lobelo, 2008; Thorp et al., 2011). Sedentary behaviors are related with activities at an absolute intensity from 1.0 to 1.5 METs (Ainsworth et al., 1993) that normally occurred in sitting position (e.g. screen-time behaviors, workplace sitting, and time spent in automobiles). Studies have shown that time spent in sedentary behavior is positively associated premature mortality specifically all-cause and cardiovascular-related mortality (Katzmarzyk, Church, Craig, & Bouchard, 2009; Thorp et al., 2011). Furthermore, the deleterious impact of sedentary behaviors was shown to be independent of concomitant sufficient MVPA (Koolhaas et al., 2017; Thorp et al., 2011). Matthews at el (2012) shows that individuals with 7 hours per day or more of TV viewing (a marker of sedentary behavior) had an increased risk of all-cause mortality and mortality from CVD compared to those individuals who had less than 1 hour per day of TV viewing; and this was independent of accomplishing the MVPA recommendations. In the INTERHEART study (Held

et al., 2012), similar results were reported based on 24 260 participants from low, middle and high-income countries. Assessments were done on leisure and labor PA behavior, and the ownership of car and television. Compared to participants with high amounts of sedentary time, participants with LPA during leisure time and moderate (but not hard) PA in occupation had a reduced risk of myocardial infarction. In addition, having a car and a TV were related with sedentary behavior and to an increased risk of myocardial infarction (Held et al., 2012). Recently, data from The Rotterdam study shows that adults and elders with 11 or more hours per day in sedentary time, had a superior mortality risk (HR = 1.80, 95% CI = 1.14 to 2.84), compared to those who had a maximum of 8 hours /day in sedentary time. After adjusting daily PA the association was attenuated (HR = 1.50, 95% CI = 0.93 to 2.41), but still significant (Koolhaas et al., 2017).

In summary, it can be concluded that physical inactivity and mortality show a dose-response relationship, and meeting the recommendations for the dose of MVPA might not be enough to attenuate the independent effect of sedentary activities on increased risk for CVD (Biswas et al., 2015; Matthews et al., 2012).

Lifestyle and Health Education Programs to Control Cardiovascular Risk

An inappropriate lifestyle is associated with the onset of CVD (WHO, 2011a). According to the WHO, over three-quarters of cardiovascular mortality could be prevented with adequate changes in lifestyle (WHO, 2011b). This means that prevention is an enormous opportunity for general population, politicians and health caregivers (WHO, 2011a). Therefore, the aim of cardiovascular prevention programs in primary health care should be to develop an integrational strategy to prevent CVD by managing and controlling risk factors (Perk et al., 2012). According to the WHO, health education means the improvement of health knowledge, attitudes, skills and behaviors, to empower individuals (WHO, 2012b). Primary health care centers seems to be an

adequate setting in where lifestyle and health education programs can be potentially successful to prevent of CVD. Indeed, based on Organization for Economic Cooperation and Development (OECD) statistics, almost 80% of adults in developed countries visit their general practitioner at least once a year (van Doorslaer, Masseria, Koolman, & Group, 2006). In 2013, the OECD average was 6.5 appointments per year, despite the high variability between countries. In Portugal, citizens are encouraged to visit the general practitioner once a year, and at each new episode of illness, if indicated, general practitioner will refer citizen to a specialist (OECD, 2015). On average, the number of appointments with a general practitioner in the Portuguese public healthcare system is 4.1 (OECD, 2015). Taken together, the combination of these data suggests that in Portugal, the ongoing public health care encompass a large segment of the population. Given that health care professionals are considered one of the primary sources of information about health (Abramson, Stein, Schaufele, Frates, & Rogan, 2000; Loprinzi & Beets, 2014), they hold the chance to influence and empower patients to gain healthier behaviors (Weintraub et al., 2011). Behavioral risk factors that should be included in health education programs include unhealthy diet, alcohol consumption, smoking, physical inactivity and sedentariness.

Studies aiming to improve PA in primary care are normally based on parallel group design, and produced divergent results, suggesting the needed of further research (Armit et al., 2009; Elley, Kerse, Arroll, & Robinson, 2003; S. Hardcastle, Taylor, Bailey, & Castle, 2008; Koelewijn-van Loon et al., 2010). For example, the Hoorn Prevention Study (Lakerveld et al., 2013) and the IMPALA study (Koelewijn-van Loon et al., 2010) aimed to promote lifestyle modifications through health education and counseling programs, including daily PA levels, but both studies reported no changes in PA. Conversely, Armit et al. (2009) showed an increase in PA at weeks 12 and 24 with no significant difference between intervention and control groups; nevertheless, at week 24, the intervention group was more likely to report meeting PA guidelines than the control group. Recently, it was published a review about the success of PA implementation in primary health care context based on reviews published

between 2002 and 2012 (Sanchez, Bully, Martinez, & Grandes, 2014). This study showed that results are influenced by sample characteristics, with better results in studies with samples of insufficiently active or sedentary participants (Sanchez et al., 2014).

Comparison between studies is difficult due to a number of reasons: the differences in the structure of the health education and counseling programs; differences in the strategies of communication to deliver the interventions; length of intervention and duration of follow-up periods; the skills of caregivers or lifestyle facilitators who deliver the program (physicians, nurses, physiotherapists, or PA specialists); and the participant characteristics in intervention and control groups (Orrow, Kinmonth, Sanderson, & Sutton, 2012). Furthermore, an important limitation in almost all studies is the PA assessment through questionnaires. We find only one study that measured PA with accelerometry (Griffin et al., 2014), and results pointed out that PA, after intervention, has increased in both control and intervention groups, with non-significant differences between groups after 1 year of follow-up. Self-reported PA measures have low sensitivity, high variance, are less accurate, and frequently overestimate the PA levels (Dyrstad et al., 2014).

Further than PA, health education interventions studies also observe cardio metabolic risk factors as primary or secondary outcomes. Harris et al (2012), designed a parallel-group study in high-risk patients to evaluate the impact of a lifestyle intervention on self-report PA, weight, body mass index (BMI), waist circumference, blood lipid, fasting blood glucose, and blood pressure. After 6 and 12 months, the only outcome that has significantly changed was the self-report PA. Cochrane el al. (2012), in a randomized trial with participants with Framingham score higher than 20%, have observed the effect of an intervention program to support lifestyle change based on motivational interview /counseling over 1-year period compared to the support delivered by the NHS Health Check service. Main outcome was Framingham risk score and secondary outcomes were high blood, total cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose, smoking, body weight, waist circumference, BMI, diet, and PA. Results pointed out that both groups decreased in the same magnitude the

Framingham risk score. Similarly, the prevalence of high blood pressure, high cholesterol and smoking were reduced significantly in both groups. Self-report PA increased in both groups, and the prevalence of central obesity was favorable for the intervention group.

Davies et al. (2008) evaluated the effectiveness of education program to newly diagnosed type 2 diabetes and found that the intervention was effective to improve weight loss after one year. Triglycerides were reduced at 8 months from intervention, but after 4 months, differences between groups were not different. Hardcastle et al. (2013) looked at the effectiveness of an intervention based on motivational interviewing on weight loss, PA and cardiovascular risk factors. After 6 months of intervention, participants of the intervention group had better blood pressure, weight, and BMI, but these positive changes were not maintained after the 12 months follow-up. However, the intervention brought long-term changes in health-related outcomes such as walking and cholesterol levels.

The modification on daily PA as a consequence of primary care educational and counseling interventions might bring modifications on CVD risk factors (Armit et al., 2009; Elley et al., 2003; S. Hardcastle et al., 2008), however the evidence is not yet conclusive. To be best of our knowledge, arterial stiffness and cardiac autonomic function were never included as secondary outcomes in this kind of research, which might be interesting once both risk factors are related with cardiovascular risk.

Primary prevention strategies aim to identify individuals at moderate to high risk of CVD in order to delay the onset of cardiovascular events. Since daily PA is a lifestyle-related behavior and its lack is also a risk factor for cardiovascular health, interventions to promote PA modifications may have side effects of modulation on other risk factors (e.g. arterial stiffness and cardiac autonomic function). Interventions on daily PA may potentially help to control risk factors and overall cardiovascular risk. Despite of this, in Portugal, lifestyle interventions to promote better daily PA in primary health contexts are almost inexistent, reason why this thesis is pioneering in our country.

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CHAPTER II

STRUCTURE and AIMS

Structure

The present thesis is organized into five chapters. The general introduction to the literature supporting the research problems of the present thesis was provided in chapter I. The main aims of the thesis and the purposes of each study are outlined in chapter II. The fieldwork is presented into three original studies, and is presented in chapter III. The general discussion, encompassing both the methodological issues and the overall results is presented in chapter IV. The main conclusions of the present thesis are outlined on chapter V.

Aims

The present thesis has two main aims. The first consisted in the determination of possible associations between daily PA and sedentary time with arterial stiffness and cardiac autonomic function in adults without established CVD. The second, consisted in the evaluation of the effects of a 4-month health education and counseling intervention in primary health care, and 8-month follow-up period, on daily PA (primary outcome), and on arterial stiffness and cardiac autonomic function (secondary outcomes), in adults with moderate to high cardiovascular risk.

To accomplish the above-mentioned aims, three original studies are presented.

Title, authors, aims and status of each study

Original study I

Title: Sedentary behavior and arterial stiffness in adults with and without metabolic syndrome

Authors: Lucimére Bohn, Ana Ramoa Castro, Gustavo Silva, Nuno Silva, Sandra Abreu, Fernando Ribeiro, Pierre Boutouyrie, Stéphane Laurent, José Oliveira

Aim: To investigate whether sedentary time and PA are associated with arterial stiffness in individuals with and without metabolic syndrome.

Status: Published ahead of print in International Journal of Sports Medicine.

Original study II

Title: Objective measures of moderate to vigorous physical activity are associated with the frequency domain index LF/HF of heart rate variability independently of age, sex, heart rate and waist circumference in adults

Authors: Lucimére Bohn, Fernando Ribeiro, Nuno Silva, Ana Ramoa Castro, José Oliveira

Aim: To evaluate the association of daily PA with HRV in adults free from established CVD.

Status: under review

Original study III

Title: Effects of a health education intervention on physical activity, arterial stiffness and cardiac autonomic function in individuals with moderate-to-high cardiovascular risk

Authors: Lucimére Bohn, Pedro Sá-Couto, Ana Ramoa Castro, Fernando Ribeiro, José Oliveira

Aim: To evaluated the effects of a health education and counseling intervention program, in a primary health care setting, on daily PA, arterial stiffness, and cardiac autonomic function in individuals with moderate-to-high risk of CVD.

Status: under review

CHAPTER III

ORIGINAL STUDIES

Original Study I

Sedentary behavior and arterial stiffness in adults with and without metabolic syndrome

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Abstract

This study aimed to investigate whether sedentary time and physical activity (PA) are associated with arterial stiffness in individuals with and without metabolic syndrome. This cross-sectional study comprised 197 individuals (47 ± 13 years; 58 % female) from a primary health care center. Arterial stiffness was assessed using carotid-femoral pulse wave velocity (cfPWV). Metabolic syndrome was determined as clustering of at least 3 out of 5 risk factors (central obesity, hypertension, impaired glucose, triglycerides and high-density lipoprotein cholesterol). Daily PA was objectively assessed and classified in sedentary time, light and moderate to vigorous PA. Physical activity was used as a continuous variable for multiple regression analysis. For mean comparisons of cfPWV between subjects with and without metabolic syndrome, a binary split at the median of sedentary time and PA was used. Sedentary time was associated with cfPWV (β = 0.11; p = 0.01) explaining 1.3 % of its variance; independently of age (β = 0.49; p < 0.001), systolic blood pressure (β = 0.27; p < 0.001) and fasting glucose (β = 0.19; p < 0.001). Participants with metabolic syndrome and more sedentary time had higher cfPWV than those with metabolic syndrome and less sedentary time (9.9 ± 1.0 vs. 8.9 ± 1.0 m/s; p < 0.05). Sedentary time is associated with cfPWV independently of age and metabolic risk factors. A higher sedentary time in metabolic syndrome individuals lead to a worse arterial stiffness profile.

1. Introduction

Metabolic syndrome involves a cluster of interrelated risk factors, including raised blood pressure, dyslipidemia, raised glucose and central obesity (Alberti, et al., 2009), which double the risk of cardiovascular events (Alberti, et al., 2009; Grundy, 2008). Given its growing prevalence worldwide, coupled with an obesity pandemic and increase in sedentary lifestyles, metabolic syndrome has become a major public health problem (Alberti, et al., 2009).

Evidence has shown that the odds of developing metabolic syndrome increase with sedentary behavior (Edwardson, et al., 2012; Ekblom, et al., 2015), independently of individuals meeting international guidelines for moderate to vigorous PA (MVPA) (Bankoski, 2011). Improved MVPA concomitant with less sedentary time is an important goal for the primary prevention of metabolic syndrome (Alberti, et al., 2009).

Metabolic syndrome is also associated with a chronic inflammatory and prothrombotic state (Alberti, et al., 2009), which prompts vascular arterial wall remodeling characterized by incremental arterial stiffness (Scuteri, et al., 2014; Scuteri, et al., 2004). By extension, given its association with fatal and nonfatal cardiovascular events, arterial stiffness is an important risk factor for cardiovascular disease (CVD) (Vlachopoulos, Aznaouridis, & Stefanadis, 2010).

Although arterial stiffness is largely determined by age and metabolic risk factors, the impact of lifestyle upon the condition is not negligible (Laurent, et al., 2006). However, to the best of our knowledge, the independent association between PA and arterial stiffness is poorly established, as is whether higher levels of MVPA and reduced sedentary time mitigate the deleterious effects of age and metabolic syndrome upon arterial stiffness.

In response, in this study we investigated the relationship among arterial stiffness, objective measures of daily PA intensity and sedentary time in individuals either with or without metabolic syndrome.

2. Materials and Methods

Study design

This cross-sectional study was conducted in a primary health care center (Porto, Portugal). Inclusion criterion was age ≥ 18 and ≤ 65 years old. Exclusion criteria were established CVD or cognitive disorders, neurological and orthopaedic impairments, arrhythmias, severe hypertension [systolic blood pressure (SBP) > 180 mmHg or diastolic blood pressure (DBP) > 100 mmHg], acute coronary syndromes and peripheral arterial disease, thyroid disorders, severe pulmonary and renal disorders, or infectious and chronic immunological diseases.

Participants

Participants were recruited from database including 8000 registered individuals. An age filter was applied to the database, leaving 4600 potential participants. From those, 1200 were random sampled in 6 unique sets of 200 numbers. This study enrolled the general population, because participants were randomly selected from the registries of the primary health care center. In Portugal access to National Health Service is universal, and everyone is registered, even those who do not access healthcare services (Carreira, Pereira, Azevedo, & Lunet, 2012). The study was approved by the Ethics Committee of the North Regional Health Authority (I.P. 25/2010) and met the ethical standards of the International Journal of Sports Medicine (Harriss, & Atkinson, 2015).

Data collection

Participants were invited through phone calls and those who accepted went twice to the health care center. Participants were instructed to refrain from strenuous exercise and to avoid consuming caffeine-containing products or alcohol for at least 24 h before evaluation. During the first appointment, eligibility criteria, data on sociodemographics, pre-existing clinical conditions and medications were verified. In this appointment, anthropometrics, blood

pressure and arterial stiffness were taken and each participant was given an accelerometer. After 7 days, participants returned for the second appointment to return the accelerometers and to collect blood samples.

Anthropometry and clinical variables

Height was assessed with a standard wall-mounted stadiometer and weight using a scale (Tanita, Inner Scan BC-522, Japan). Body mass index (BMI) was calculated as the ratio of weight to squared height. Waist circumference was measured at the midpoint between the lowest rib and iliac crest. Metabolic syndrome was defined as the clustering of at least 3 out of 5 conditions: central obesity (waist circumference ≥ 102 cm in men and ≥ 88 cm in women); triglycerides ≥ 150 mg/dL or drug treatment for elevated triglycerides; high density lipoprotein (HDL) cholesterol < 40 mg/dL in men and < 50 mg/dL in women or drug treatment for reduced HDL-cholesterol; SBP ≥ 130 and/or DBP ≥ 85 mmHg or presence of antihypertensive medication; and, elevated fasting glucose (≥ 100 mg/ dL) or drug treatment for elevated glucose (Alberti, et al., 2009)].

Blood pressure and arterial stiffness measurements

A single trained researcher performed blood pressure and arterial stiffness measurements after 20 min resting in supine position. Blood pressure was assessed (Colin, BP 8 800; Critikron, Inc., USA) in the left arm and SBP and DBP were computed as the average of 3 readings. Additional readings were performed when differences between readings exceeded 5 mmHg. SBP and DBP were used to calculate pulse pressure and mean blood pressure (Boutouyrie & Vermeersch, 2010). Arterial stiffness was measured as carotid-femoral pulse wave velocity (cfPWV) using the SphygmoCor device (AtCor Medical, Australia) according to international guidelines (Boutouyrie & Vermeersch, 2010). Two valid measures were performed and the average was used for analysis. Arterial stiffness assessment was made at rest in a quiet, semi-dark room with an average temperature of 21 °C.

Physical activity

Daily PA was assessed using accelerometers (Actigraph GT1M, Actigraph LLC, Pensacola, FL) over the right hip, for 7 consecutive days, during the waking hours, except while bathing and water-based activities (Dyrstad, Hansen, Holme, & Anderssen, 2014). ActiLife software (Actigraph, Florida, USA, version 6.9) was used to reduce raw activity data into daily PA. The accelerometer measures the intensity of movement, which was averaged for 1-min sampling intervals (counts/min). For data analysis, non-wear periods were defined as ≥ 10 consecutive 1-min sampling intervals with "zero" counts. To be considered as valid data, individuals must have had a minimum of 4 days recorded with at least 8 wear-time hours per day. The average minutes/day spent at different categories of PA intensity was determined according to cut points that relate PA to counts/min: sedentary time (≤ 99 counts/min), light PA (LPA) (100 – 2 019 counts/min) and MVPA (≥ 2 020 counts/min) (Troiano, et al., 2008).

Blood sampling

Twelve hours fasting blood samples were collected by venipuncture of the antecubital vein into serum separator and EDTA coated tubes. The following parameters were assessed: glycated hemoglobin (HbA1c), serum glucose, total cholesterol, HDL-cholesterol, triglycerides, high-sensitivity C-reactive protein (hs- CRP) and plasma insulin. Low-density lipoprotein (LDL) was calculated using the Friedewald equation. A high sensitive Milliplex map kit (Millipore, Germany), with the Luminex 200™ analyzer (Luminex Corporation, USA) were used to assess the plasma Interleukin-6 (IL-6), Tumor Necrosis Factor alpha (TNF-a) and leptin. Adiponectin plasma levels were determined using a commercial enzyme-linked immunosorbent assay (Mercodia AB, Sweden). The ratio leptin-to-adiponectin was calculated dividing leptin by adiponectin (Al-Hamodi, Al-Habori, Al-Meeri, & Saif-Ali, 2014). Assays were assessed in duplicated.

Statistical methods

Not normally distributed variables were transformed into a natural logarithm (cfPWV) or ranked (LPA and MVPA) for analysis and then transformed back to the original scale for the purpose of clarity. Data are expressed as mean ± standard deviation. Pearson's correlation was used to analyze the relationship between cfPWV and PA for the total sample. Multivariate linear regression with stepwise selection of variables was performed to determine the relationship between cfPWV, risk factors and PA. Variables were organized in clusters in order to sort out the contribution of redundant variables (e.g., morphometric, lipid, inflammatory, diabetes and hypertension) (Briet, el al, 2006). Within each cluster, variables were included in a competitive manner in multivariate models. The variable with the highest univariate significant level with cfPWV was kept. Variables retained from clusters were tested with age. Those that sustained the significant level were included in the first model. The second model, encompassed variables retained from the first model plus PA variables with bivariate significant association with cfPWV. The sample was classified according to the presence or absence of metabolic syndrome and then metabolic syndrome and non-metabolic syndrome groups were divided by the median of PA levels and sedentary time. Comparisons between groups were performed using independent t-test, chi-square and ANCOVA, with Bonferroni post hoc test. For these analyses, mean and standard error were adjusted for age. Statistical analysis was performed using the IBM SPSS 20 software (SPSS, USA). Power analysis was calculated post hoc and it was higher than 0.8. P-values < 0.05 were considered significant.

3. Results

From the 1200 individuals randomly sampled, 318 did not answer, 244 declined to participate, 348 had exclusion criteria and 33 missed the first appointment. A total of 257 individuals attended to the study and 197 had valid data for arterial stiffness and PA, being considered the final sample size. Sample characteristics are presented in Table 1.

Table 1. Sample characteristics and between groups comparison

	Overall (n = 197)	No Metabolic Syndrome (n = 116)	With Metabolic Syndrome (n = 81)
Age, years	47 ± 13	42 ± 12	54 ± 10 **
Female, n (%)	114 (58)	71 (61)	43 (53)
BMI, kg/m^2	26.8 ± 4.3	25.2 ± 3.9	29.0 ± 3.9 **
Waist circumference, cm	92 ± 12	88 ± 11	99 ± 10 **
Risk factors and medications			
Hypercholesterolemia, %	71	60	88 **
Hypertension, %	43	16	82 **
Type II Diabetes, %	9	0	22 **
Obesity, %	20	9	37 **
Current smoker, %	29	35	22 *
Antihypertensive, %	33	14	61 **
Lipid-lowering, %	22	1	53 **
Oral anti-diabetic, %	8	0	20 **
Blood pressure, heart rate and arterial stiffness			
Brachial SBP, mm Hg	127 ± 16	120 ± 13	137 ± 15 **
Brachial DBP, mm Hg	75 ± 11	71 ± 10	80 ± 10 **
MBP, mm Hg	94 ± 13	89 ± 12	102 ± 12 **
Brachial PP, mm Hg	52 ± 10	49 ± 8	57 ± 11 **
Aortic PP, mm Hg	41 ± 10	38 ± 9	47 ± 10 **
Resting heart rate, bpm	64 ± 9	63 ± 10	64 ± 9
cfPWV, m/s	9.1 ± 1.9	8.3 ± 1.5	10.2 ± 1.9 **

	Overall (n = 197)	No Metabolic Syndrome (n = 116)	With Metabolic Syndrome (n = 81)
Physical activity			
Mean wear time, min/day	798 ± 90	792 ± 96	804 ± 90
Sedentary time, min/day	460 ± 93	455 ± 93	469 ± 92
LPA, min/day	301 ± 99	299 ± 100	305 ± 99
MVPA, min/day	36 ± 26	39 ± 27	32 ± 24*

Note. Hypercholesterolemia= total cholesterol > 190 mg/dL; and/or any low-density lipoprotein > 115 mg/dL; and/or, high-density lipoprotein: men < 40 mg/dL, women < 46 mg/dL; and/or presence of lipid lowering medication. Obesity= body mass index ≥ 30 kg/m^2. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; PP: pulse pressure; cfPWV: carotid-femoral pulse wave velocity; LPA: light physical activity; MVPA: moderate to vigorous physical activity. *$p < 0.05$; **$p < 0.001$

Regarding PA, mean wear time was 13.3 ± 1.5 h/day, ranging from 8.7 to 17.5 h/day. Comparisons between participants with and without metabolic syndrome showed that those with metabolic syndrome tended to be older, exhibit significantly worse indexes for metabolic syndrome risk factors, had greater inflammatory biomarkers and cfPWV (Table 1, 2).

Table 2. Overall and between groups comparisons for lipid, metabolic, inflammatory and hormonal characteristics

	Overall (n = 197)	No Metabolic Syndrome (n = 116)	With Metabolic Syndrome (n = 81)
Lipid profile			
Triglycerides, mg/dL	111.4 ± 55.8	93.2 ± 37.6	138.7 ± 66.9 ***
HDL- Cholesterol, mg/dL	56.9 ± 15.2	59.4 ± 15.3	50.5 ± 13.4 ***
LDL-Cholesterol, mg/dL	118.9 ± 35.8	115.7 ± 35.1	123.9 ± 36.6
Total Cholesterol, mg/dL	197.1 ± 38.6	193.6 ± 37.1	202.7 ± 40.4
Metabolic profile			
Fasting glucose, mg/dL	96.1 ± 29.5	86.1 ± 10.4	111.2 ± 40.6 ***
Insulin, µIU/mL	10.3 ± 7.0	8.5 ± 5.3	13.0 ± 8.4 ***
Glycated hemoglobin, %	5.5 ± 0.7	5.2 ± 0.2	5.8 ± 0.9 ***
Inflammatory biomarkers			
hs C-reactive protein, mg/dL	0.3 ± 0.6	0.2 ± 0.4	0.4 ± 0.8 *
Interleukin-6, pg/mL	1.8 ± 1.5	1.6 ± 1.4	2.2 ± 1.6 **
TNF α, pg/mL	3.3 ± 1.4	3.0 ± 1.2	3.7 ± 1.6 **
Adipocyte-specific proteins			
Leptin, mg/mL	13.6 ± 12.0	13.2 ± 13.1	14.0 ± 10.4
Adiponectin, mg/mL	10.7 ± 5.2	11.3 ± 5.5	10.0 ± 4.6 *
Leptin-to-Adiponectin ratio	1.3 ± 1.3	1.2 ± 1.1	1.6 ± 1.6 **

Note. HDL: High-density lipoprotein cholesterol; LDL: low-density lipoprotein; TNFα: tumor necrosis factor alpha. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Considering the total sample, cfPWV was positively associated with sedentary time (r = 0.14; p = 0.03), and negatively associated with MVPA (r = − 0.20; p = 0.005). The correlation between cfPWV and LPA was not significant (r = − 0.05, p = 0.48).

Table 3 shows two multivariate models for the natural logarithm of cfPWV. Since not all clusters were significant predictors for cfPWV, some clusters have no variables in the first model. In the second model, sedentary time became an independent predictor of cfPWV (β = 0.11; p = 0.01) that explained 1.3 % of its variance. However, MVPA was not an independent predictor of cfPWV (p > 0.05).

Table 3. Multivariate relationships between natural logarithm of carotid-femoral pulse wave velocity and independent variables

Parameters	R^2 increment %	Beta	*p*
Model 1 (R^2: 0.56)			
Age, years	44.6	0.49	< 0.001
Systolic blood pressure, mm Hg	8.2	0.27	< 0.001
Fasting glucose, mg/dL	3.7	0.20	< 0.001
Model 2 (R^2: 0.58)			
Age, years	45.5	0.49	< 0.001
Systolic blood pressure, mm Hg	7.7	0.27	< 0.001
Fasting glucose, mg/dL	3.5	0.19	< 0.001
Sedentary time, min/day	1.3	0.11	0.01

Note. The dependent variable (cfPWV) is in natural logarithm. Beta: standardized coefficients.

Figure 1 presents the results for ANCOVA. Participants with metabolic syndrome who spent more sedentary time showed significantly greater cfPWV than not only those with metabolic syndrome and less sedentary time, but also all individuals in both non-metabolic syndrome groups regardless of sedentary time (Figure 1A). However, that pattern did not emerge in the distinction between LPA (Figure 1B) and MVPA (Figure. 1C), since individuals with metabolic syndrome who engaged greater PA had the same mean cfPWV value as those who engaged less PA.
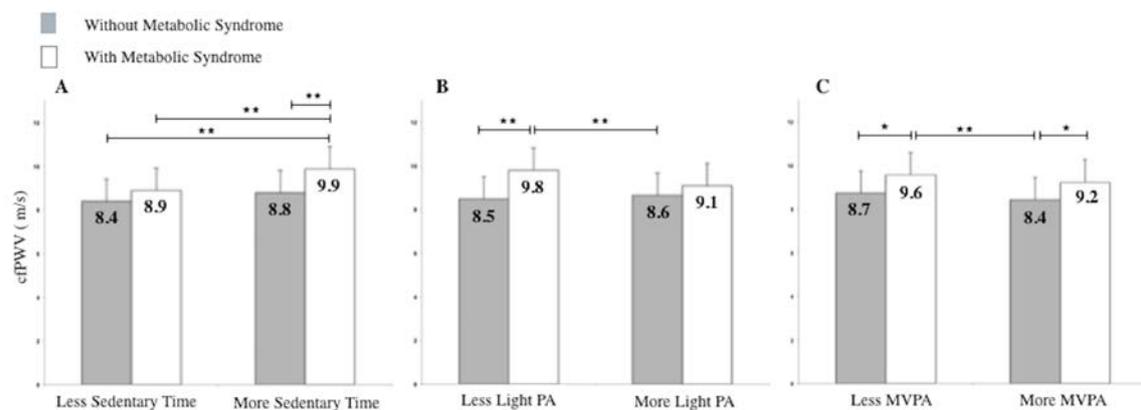
Figure 1. Carotid-femoral pulse wave velocity according to metabolic syndrome and sedentary behavior and physical activity intensities

Carotid-femoral pulse wave velocity means (label inside bars) adjusted for age, according to the presence of metabolic syndrome and to the median of time spent in sedentary behavior (A), light physical activity (B), and moderate to vigorous physical activity (C). White and grey bars are participants with and without metabolic syndrome, respectively. Physical activities intensities are divided by the median (median of sedentary behavior: 467 min/day; median of light physical activity: 284 min/day, and median of moderate to vigorous physical activity: 30 min/day). * $p < 0.05$; ** $p < 0.001$

4. Discussion

The findings of the study are twofold: sedentary time is independently associated with cfPWV in participants with or without metabolic syndrome, and individuals with metabolic syndrome and more sedentary time display a significantly greater cfPWV than those with metabolic syndrome and less sedentary time. To the best of our knowledge, our study is the first to investigate

the interaction of daily PA and metabolic syndrome on cfPWV at the same time. Our multivariate analysis explained nearly half (R^2 = 58%) of all variation in cfPWV, 1.3% of which was explained by sedentary time. As clinical values indicate, for individuals of the same age with similar SBP and fasting glucose, sedentary time augments cfPWV. Despite the abovementioned, the combination of metabolic syndrome with more sedentary time precipitated greater cfPWV close to 10 m/s, which indicates a risk of a cardiovascular event (Mancia, et al., 2014). In our analysis, we removed the effect of age and controlled for it with MVPA. Since both metabolic syndrome and non-metabolic syndrome groups demonstrated sufficient MVPA (e.g. > 30 min/day) the overall results suggest that sufficient MVPA does not negate the effects of sedentary time. Indeed, earlier studies examined the association between arterial stiffness and sedentary time in apparently healthy populations (Horta, et al., 2015; van de Laar, et al., 2014), and despite the studies' different methodological approaches, they generally reported the deleterious effect of sedentary time on arterial stiffness (Horta, et al., 2015; van de Laar, et al., 2014).

Contrary to our expectations, MVPA was not an independent predictor of cfPWV. By contrast, an association between meeting international guidelines of PA and lower cfPWV in adults free of established CVD study was demonstrated (Andersson, et al., 2015). However, others have reported results similar to those observed in our study (Gomez-Marcos, et al., 2014). Future research should seek to clarify the association between MVPA and arterial stiffness.

Metabolic syndrome entails a cluster of critical metabolic, inflammatory, and hemostatic conditions (Alberti, et al., 2009) that affect large arteries at all ages (Scuteri, et al., 2014; Scuteri, et al., 2015; Scuteri, et al., 2004). Our data reinforce that description since participants with metabolic syndrome exhibited worse metabolic, lipid-related, proinflammatory, and arterial stiffness profiles. However, our data showed that participants with metabolic syndrome with higher PA and lower sedentary time have less cfPWV than those with worse PA profiles. As such, PA arguably plays a protective role in humoral and inflammatory secondary effects in arterial stiffness. Physiological mechanisms linking sedentary time (Alibegovic, 2009; Pavy-Le Traon, Heer, Narici,

Rittweger, & Vernikos, 2007) and metabolic syndrome (Schram, et al., 2004) to arterial stiffness suggest impaired glucose metabolism and deterioration in insulin sensitivity. For one, hyperglycemia provokes changes in arterial walls due to protein glycation and the consequent formation of advanced glycation end products in the extracellular matrix, which compromise arterial distensibility (Avolio, 2013; Schram, et al., 2004). Sedentary time might furthermore increase pro-inflammatory cytokines (Jain, Khera, Corrales-Medina, Townsend, & Chirinos, 2014), which initiate a cascade of inflammatory mediators that target the vascular endothelium, thereby prompting the endothelium dysfunction (Jain, Khera, Corrales-Medina, Townsend, & Chirinos, 2014; Pavy-Le Traon, Heer, Narici, Rittweger, & Vernikos, 2007) and the migration and proliferation of smooth muscle cells (Jain, Khera, Corrales-Medina, Townsend, & Chirinos, 2014), impairing arterial distensibility (Laurent, & Boutouyrie, 2015). Since individuals with metabolic syndrome have elevated resting blood pressure, it is important to note that hypertension generates repeated pulsatile stress leading to biomechanical fatigue and related loss of well-ordered arrangement of smooth muscle cells and extracellular matrix (Avolio, 2013; Laurent, et al., 2006). As a consequence, there is a degeneration of elastic fibers, an increased in collagenous material, and often deposition of calcium in arterial walls (Avolio, 2013; Laurent, et al., 2006). Furthermore, the activation of the renin-angiotensin system might contribute to structural alteration of the arterial wall, promoting vascular smooth muscle cell proliferation, low-grade inflammation, increased collagen content, and advanced glycation end product formation, which ultimately augments arterial stiffness (Avolio, 2013; Jain, Khera, Corrales-Medina, Townsend, & Chirinos, 2014; Laurent, et al., 2006).

Our study has some limitations. As an observational, cross-sectional study, it hinders the establishment of causal inferences and suggests associations only. Moreover, the large percentage of potential participants who declined to participate might have skewed the prevalence of metabolic syndrome in our study. Although we adjusted all models for multiple variables, the influence of residual confounders cannot be excluded. The small sample size for the age range and the seasonality also influenced the total amount of daily PA, which

constitutes a study limitation for future research work to overcome. We have drawn the following conclusions from our findings. First, in those with metabolic syndrome, sedentary time leads to significantly higher cfPWV. Second, sedentary time is also positively associated with cfPWV, independent of age and metabolic risk factors.

Acknowledgements

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Original Study II

Objective measures of moderate to vigorous physical activity are associated with the frequency domain index LF/HF of heart rate variability independently of age, sex, heart rate and waist circumference in adults

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Abstract

Objectives: To evaluate the association of daily physical activity (PA) with heart rate variability in adults free from established cardiovascular disease.

Design: This is a cross-sectional study with 197 apparently healthy individuals (mean age 47 ± 13 years; 58% female), enrolled in a primary health care unit. Methods: Heart rate variability indices (time domain, frequency domain and nonlinear indices) were derived from 5 min resting RR-interval recordings while subjects breathed at 12 breaths per minute. Daily PA was assessed over 7 consecutive days with accelerometers. Data was reduced in sedentary time, light, and moderate to vigorous PA. Correlation and multivariate linear regression analyses were used to examine associations between daily PA and heart rate variability indexes.

Results: Sedentary time and light physical activity were not associated with any heart rate variability index ($p > 0.05$). After adjustments for age, sex and resting heart rate, moderate to vigorous PA was significantly correlated with the ratio between low frequency power and high frequency power ($r^2 = -0.18$, $p = 0.01$). Moderate to vigorous PA ($\beta = -0.14$, $p = 0.03$), along with sex ($\beta = 0.32$, $p = 0.001$), waist circumference ($\beta = 0.14$, $p = 0.04$) and age ($\beta = 0.05$, $p = 0.45$), is an independent predictor of the ratio between low frequency power and high frequency power, explaining 2.3% of its variance.

Conclusions: Daily moderate to vigorous PA negatively predicted the ratio between low frequency power and high frequency power.

Keywords: autonomic function, physical activity, cardiovascular risk factors, lifestyle

1. Introduction

Heart rate variability (HRV) is an indicator of intrinsic cardiac autonomic function (Tsuji, et al., 1996). Impaired autonomic function is a sign of cardiac electrical instability that augments the risk of cardiovascular events (Tsuji, et al., 1996). HRV reflect the modulation of both the sympathetic and parasympathetic branches of the autonomic nervous system on the sinus atrial node (Shaffer, McCraty, & Zerr, 2014). Low HRV indicates a shift toward sympathetic predominance and parasympathetic reduction (Task Force, 1996).

Physical activity (PA) is recommended for the prevention and treatment of cardiovascular disease (CVD) (Blair, Sallis, Hutber, & Archer, 2012). Appropriate amounts of moderate to vigorous PA (MVPA) are related to cardiovascular health benefits (Blair, et al., 2012), while sedentariness is related to deleterious effects on many health indicators (Blair, et al., 2012; Katzmarzyk, Church, Craig, & Bouchard, 2009).

There is an abundance of literature on PA and its effects on HRV, both in terms of exercise interventions (Buchheit, et al., 2004; Stein, Ehsani, Domitrovich, Kleiger, & Rottman, 1999) and large-scale cohort studies (Dietrich, et al., 2008; Soares-Miranda, Sandercock, et al., 2012). Generally, it is established that MVPA or aerobic exercise training are associated with improved cardiac autonomic modulation (Dietrich, et al., 2008; Sandercock, Bromley, & Brodie, 2005; Soares-Miranda, Sandercock, et al., 2012; Stein, et al., 1999). Despite that, the impact of lower-intensity PA, such as sedentary time and light PA (LPA), on cardiac autonomic function is not clearly established. Furthermore, most studies assessed PA through self-reporting questionnaires (Dietrich, et al., 2008; Garet, et al., 2005; Sandercock, Hardy-Shepherd, Nunan, & Brodie, 2008), which have been reported to overestimate total PA and time spent at different levels of intensity, and therefore represent a methodological constraint (Dyrstad, Hansen, Holme, & Anderssen, 2014). Consequently, studies with objective approaches to assess PA, such as accelerometers, might improve the accuracy of results (Buchheit et al., 2005; Soares-Miranda, Sandercock, et al., 2012).

Clinical trials have demonstrated that low-grade inflammation (Sloan, et al., 2007; Soares-Miranda, Negrao, et al., 2012) and metabolic risk factors (Koskinen, et al., 2009; Soares-Miranda, Sandercock, et al., 2012) are independent predictors of a less favorable HRV profile, which justify the consideration of those variables as possible confounders in the relationship between PA and HRV. Furthermore, PA can also modulate the association between "inflammation and HRV" and "metabolic risk factors and HRV" (Soares-Miranda, Sandercock, et al., 2012).

Therefore, the main purpose of this study was to investigate in adults the extent to which different intensities of daily PA are associated with HRV.

We hypothesized that daily time spent engaging in LPA and MVPA is associated with improved cardiac autonomic function, while the opposite will be expected for sedentary time.

2. Materials and methods

Study design

This cross-sectional study was conducted in a primary health care center (Porto, Portugal). Inclusion criterion was age ≥18 and ≤65 years old. Exclusion criteria were established CVD or cognitive disorders, neurological and orthopaedic impairments, arrhythmias, severe hypertension [systolic blood pressure (SBP) > 180 mmHg or diastolic blood pressure (DBP) > 100 mmHg], acute coronary syndromes and peripheral arterial disease, thyroid disorders, severe pulmonary and renal disorders, or infectious and chronic immunological diseases.

Participants

Participants were recruited from database including 8000 registered individuals. An age filter was applied to the database, leaving 4600 potential participants. From those, 1200 were random sampled in 6 unique sets of 200 numbers. This study enrolled the general population, because participants were randomly

selected from the registries of the primary health care center. In Portugal access to National Health Service is universal, and everyone is registered, even those who do not access healthcare services (Carreira, Pereira, Azevedo, & Lunet, 2012). The study was approved by the Ethics Committee of the North Regional Health Authority (I.P. 25/2010). All procedures were conducted according to the declaration of Helsinki, and participants signed informed consent to participate.

Data collection

Participants were invited through phone calls and those who accepted went twice to the health care center. Participants were instructed to refrain from strenuous exercise and to avoid consuming caffeine-containing products or alcohol for at least 24 hours before evaluation. During the first appointment, eligibility criteria, data on sociodemographics, pre-existing clinical conditions and medications were verified. In this appointment, anthropometrics, blood pressure and HRV were taken and each participant was given an accelerometer. After seven days, participants returned for the second appointment to return the accelerometers and to collect blood samples.

Anthropometry

For anthropometrics, participants wore light clothing without shoes. Height (m) was assessed using a standard wall-mounted stadiometer and weight (kg) using a scale (Tanita, Inner Scan BC-522, Tokyo, Japan). Body mass index (BMI, kg/m^2) was thereafter calculated. Waist circumference (cm) was measured at the midpoint between the lowest rib and the iliac crest; participant was standing with arms hanging freely.

Heart rate variability and blood pressure

For HRV assessment, participants were instructed to refrain from strenuous exercise and to avoid consuming caffeine-containing products or alcohol for at

least 24 hours before evaluation. The HRV assessment was made at rest in a quiet, semi-dark room with an average temperature of 21º C. Recording of R-R interval data was performed using the Polar RS800CX (Polar Electro OY, Kempele, Finland) with a temporal resolution of 1ms (Nunan, et al., 2009). The HRV assessment methodology is described in detail elsewhere (Oliveira, et al., 2014). In brief, assessments were performed in the supine position, controlling the breathing rate by matching it to a metronome-paced frequency of 12 breaths/min. After 20 min of recording, R-R interval data were downloaded into Polar Precision Performance Software SW (Polar Electro OY, Kempele, Finland). Using the Kubious Software 2.0 for Windows (The Biomedical Signal Analysis Group, University of Kuopio, Finland) ectopic beats or arrhythmias were excluded and R-R data were de-trended (Tarvainen, Ranta-Aho, & Karjalainen, 2002) and resampled at 4Hz. Finally, the last 5 minutes of recording were selected and used for calculating HRV indices. Time domain indices included standard deviation of the normal-to-normal R-R intervals (SDNN) and the square root of the mean of the squared between successive NN intervals (rMSSD). SDNN reflects global variability and rMSSD is liked to vagal activity (Task Force, 1996). Frequency domain indices were determined using the non-parametric method (Fast Fourier transform) and encompassed very low frequency (VLF, 0.0033 - 0.04 Hz), low frequency (LF, 0.04 - 0.15 Hz), high frequency (HF, 0.15 - 0.4 Hz) power. Absolute LF (ms^2), HF (ms^2), and the ratio between low frequency power and high frequency power (LF/HF) were used as frequency domain variables. LF is a doubtful measurement of sympathetic activation of the heart, while HF is related with vagal activity (Shaffer, et al., 2014). The ratio LF/HF might reflect sympatho-vagal interaction. An increase in the ratio might reflect a shift toward sympathetic dominance, and a decrease in the ratio correspond a parasympathetic dominance (Lombardi & Stein, 2011; Shaffer et al., 2014).

Nonlinear HRV indices were (i) short-term fractal-scaling exponent (DFA1) and (ii) the Poincare ratio (SD12), which is the ratio between the short (SD1) and the long (SD2) diameter ellipses. Nonlinear indices represent complexity measures

of biological signals (Lombardi & Stein, 2011). The mean resting heart rate was also calculated as part of this evaluation.

Three blood pressure measurements were made in the left arm using a Colin model BP 8800 monitor (Critikron, Inc., Tampa, USA) following the 20 minutes of HRV assessment. Additional readings were performed when differences between readings exceed 5 mmHg. Systolic, diastolic and mean blood pressures were computed as the average of the 3 measurements with 1-minute intervals in between.

Physical activity

Daily PA was assessed using accelerometers (Actigraph GT1M, Actigraph LLC, Pensacola, USA) worn over the right hip for 7 consecutive days. Accelerometers were worn during waking hours except while bathing and during water-based activities. ActiLife software version 6.9 (Actigraph, Pensacola, USA) was used to reduce the raw activity data into daily PA. The average number of minutes/day spent at sedentary, LPA and MVPA was determined according to cut points relating counts/min to PA intensity (Troiano, et al., 2008). To be considered as valid data, individuals must had a minimum of 4 days recorded with at least 8 wear-time hours per day. The average minutes/day spent at different categories of PA intensity was determined according to cut points that relate counts/min to PA intensity levels: sedentary time (≤ 99 counts/min), LPA (100 - 2019 counts/min) and MVPA (≥ 2020 counts/min) (Troiano, et al., 2008).

Blood sampling

Twelve-hour fasting blood samples were collected by venipuncture of the antecubital vein into a serum separator and EDTA coated tubes. Serum glucose, high-density lipoprotein (HDL) cholesterol and triglycerides were measured in an automated clinical chemistry Olympus AU5400 (Beckman-Coulter, Brea, USA). Low-density lipoprotein (LDL) cholesterol was calculated

using the Friedewald equation. Serum high-sensitivity C-reactive protein (hs-CRP) was determined using a particle-enhanced immunonephelometric assay in a Dimension Vista 1500 nephelometer (Siemens, Erlangen, Germany). To determine plasma levels of leptin, a high sensitive Milliplex map kit (Millipore, Darmstadt, Germany) was used and assayed in a Luminex 200™ analyser (Luminex Corporation, Austin, USA). Adiponectin plasma levels were determined using a commercial enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden). Leptin-to-adiponectin ratio was calculated by dividing leptin per adiponectin levels. All assays were performed in duplicate according to the manufacturers' instructions.

Metabolic risk score

The risk factors hypercholesterolemia, hypertension and diabetes were recorded as previously described (Mancia, et al., 2014). A metabolic risk score was computed as the sum of z-scores derived from continuous values of waist circumference, triglycerides, glucose level, and SBP, minus the HDH z score, and then divided by 5 in order to obtain the mean metabolic risk score.

Statistical analysis

Normal data distribution was verified by the Kolmogorov-Smirnov test. Variables not normally distributed were transformed to their natural logarithm or square root for subsequent analysis and then transformed back to the original scale for the purpose of clarity. Data are expressed as mean ± standard deviation. T-test and chi-square were used to compare differences between sexes.

Bivariate and partial correlations, controlling for age, sex and resting heart rate, were used between PA variables and HRV indices.

Multivariate linear regression was applied on the determination of the association between HRV indices, PA variables that showed significant correlation, and potential confounders. The "enter" method was elected for variables selection in order to ensure that age, sex and resting heart rate were

constantly present. Age dependency of HRV could be caused by a structural modification linked to a loss of sinoatrial pacemaker cells and a reduction of arterial distensibility (Voss, Schroeder, Heitmann, Peters, & Perz, 2015). Sex differences in the younger ages are probably caused by the different hormonal situation that tends to reduce and even disappear with age (Voss, et al., 2015). Adjustment for heart rate has been recommended due to the strong association between HRV and the heart rate at which they were measured (Monfredi, et al., 2014).

In order to sort out the contribution of potentials confounders we performed a clustered selection of variables (Briet, et al., 2006). Clusters were established as follows: morphometric (waist circumference and BMI), medications (antidepressants, antihypertensive- including beta-blockers, lipid lowering and oral anti diabetics), metabolic (triglycerides, HDL cholesterol, LDL cholesterol, total cholesterol, fasting plasma glucose and mean metabolic risk score), inflammation (leptin, adiponectin, leptin-to-adiponectin ratio and hs-CRP) and blood pressure (SBP, DBP, and mean blood pressure). Within each cluster, variables were included with age, sex and resting heart rate in a competitive manner in multivariate models. If covariance was too high within clusters, the variable with the highest univariate significance level with HRV indices was kept.

Statistical analysis was performed using IBM SPSS 20 software (SPSS, Chicago, USA). The significance level was set at 95%. Power analysis was calculated post hoc and it was higher than 0.8 for both partial correlation and multiple regression analysis.

3. Results

From the random sampling of 1200 individuals, 318 did not answer phone calls, 244 decline to participate, 348 meet exclusion criteria and 33 missed their appointments. A total of 257 individuals attended the first appointment and from those, 197 had valid data for HRV and PA measurements, and were considered

as the final sample size**.** Table 1 describes the participants' clinical characteristics along with comparisons between sexes.

Table 1. Clinical characteristics along with comparisons between sexes

	Total (n = 197)	Female (n = 116)	Male (n = 81)	p
Age, years	47.4 ± 12.9	47.3 ± 12.3	47.4 ± 11.6	0.93
Anthropometrics				
Waist circumference, cm	92.2 ± 11.7	90.5 ± 11.7	94.5 ± 11.3	0.01
BMI, kg/m^2	26.8 ± 4.3	26.6 ± 4.6	27.1 ± 3.9	0.26
Risk factors				
Smoking, %	29.4	21.9	39.8	0.005
Dyslipidemia, %	71.1	69.3	73.5	0.31
Hypertension, %	43.1	39.5	48.2	0.14
Diabetes, %	9.2	6.3	13.3	0.07
Medications				
Lipid-lowering, %	22.3	19.3	26.5	0.15
Antihypertensive, %	33.0	28.9	38.6	0.10
Oral anti-diabetic, %	8.1	6.1	10.8	0.17
Antidepressants, %	17.3	21.9	10.8	0.03
Blood pressure				
SBP, mmHg	126.8 ± 16.3	126.2 ± 17.7	127.6 ± 14.2	0.38
DBP, mmHg	74.7 ± 10.9	73.3 ± 11.1	76.5 ± 10.3	0.03
MBP, mmHg	94.5 ± 13.2	93.9 ± 14.0	95.0 ± 11.9	0.55
Metabolic biomarkers				
Triglycerides, mg/dL	111.4 ± 55.9	103.8 ± 48.6	121.6 ± 63.3	0.02
HDL cholesterol, mg/dL	55.9 ± 15.2	61.7 ± 15.3	48.0 ± 10.9	< 0.001
LDL cholesterol, mg/dL	118.9 ± 35.9	120.0 ± 36.6	117.4 ± 35.1	0.63

	Total (n = 197)	Female (n = 116)	Male (n = 81)	p
Total cholesterol, mg/dL	197.2 ± 38.6	203.1 ± 38.5	189.2 ± 37.5	0.01
Fasting glucose, mg/dL	96.2 ± 29.6	94.7 ± 35.7	98.1 ± 18.2	0.06
Mean metabolic risk score, a.u.	-0.0 ± 0.6	-0.1 ± 0.6	0.2 ± 0.5	< 0.001
Inflammatory biomarkers				
Leptin, mg/mL	13.6 ± 12.0	17.8 ± 11.8	8.0 ± 9.8	< 0.001
Adiponectin, mg/mL	10.8 ± 5.2	12.1 ± 4.1	8.9 ± 5.9	< 0.001
Leptin-to-adiponectin ratio	1.4 ± 1.4	1.6 ± 1.4	1.0 ± 1.1	< 0.001
hs-CRP, mg/dL	0.3 ± 0.6	0.3 ± 0.4	0.3 ± 0.8	0.04

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure; a.u.: arbitrary units. HDL: high-density lipoprotein; LDL: low-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein.

Table 2 shows descriptive statistics for PA and HRV indices and differences between sexes. On average, the number of days recorded with accelerometers was 6.4 ± 1.1 days. Accelerometers mean wear time for participants with valid data was 13.3 ± 1.5 hours/day, ranging from 8.7 to 17.5 hours/day. Compared to males, females exhibited significantly less sedentary (p = 0.001) and MVPA (p = 0.04), but higher LPA (p = 0.01). For heart rate-related variables, males exhibited a lower heart rate (p = 0.003) and higher mean R-R intervals (p = 0.008), LF (p = 0.01), LF/HF (p < 0.001), and DFA1 (p = 0.001) than females, who in turn had significantly higher SD12 (p = 0.03).

Table 2. Mean values (mean ± SD) of daily physical activity and heart rate variability and between sex comparisons

	Total	Female	Male	*p*
Physical activity				
Sedentary, min/day	460.4 ± 92.8	442.5 ± 90.3	485.0 ± 91.1	0.001
LPA, min/day	301.3 ± 99.2	315.1 ± 97.4	282.3 ± 99.1	0.01
MVPA, min/day	36.3 ± 26.2	33.1 ± 23.4	40.6 ± 29.2	0.04
Heart rate variability				
Resting heart rate, bpm	63.6 ± 9.4	65.2 ± 8.6	61.3 ± 10.1	0.003
Time domain				
Mean RR interval, ms	917.6 ± 130.3	896.3 ± 120.3	946.9 ± 138.4	0.008
SDNN, ms	41.5 ± 25.8	40.9 ± 26.4	42.4 ± 25.0	0.55
rMSSD, ms	43.2 ± 31.1	43.9 ± 33.1	42.4 ± 28.3	0.91
Frequency domain				
LF, ms^2	734.1 ± 1252.9	573.7 ± 822.8	954.4 ± 1653.6	0.01
HF, ms^2	1277.1 ± 1839.8	1412.7 ± 1963.1	1090.7 ± 1649.0	0.35
LF/HF	1.0 ± 1.4	0.7 ± 0.9	1.4 ± 1.8	< 0.001
Non-Linear				
DFA1	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	< 0.001
SD12	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.03

Note. LPA: light physical activity; MVPA: moderate to vigorous physical activity; SDNN: standard deviation of the normal-to-normal R-R intervals; RMSSD: square root of the mean of the squared differences between successive NN intervals differences; LF: low frequency power; HF: high frequency power; DFA1: short-term fractal scaling exponent; SD12: Poincare ratio; bpm: beats per minute.

Bivariate correlation between HRV indices and daily PA showed that MVPA was positively correlated with HF (r = 0.15, P = 0.02) and negatively correlated with LF/HF (r = -0.13, P = 0.05). However, in the partial correlation, only MVPA and LF/HF remains significantly associated (r = -0.18, P = 0.01). Correlations between sedentary time and LPA with HRV indices were not significant (Table 3).

Table 3. Bivariate correlations between daily physical activity and heart rate variability indices. Values are r (p)

	Sedentary time (min/day)	LPA (min/day)	MVPA (min/day)
SDNN, ms	0.08 (0.91)	0.04 (0.51)	0.12 (0.08)
rMSSD, ms	0.03 (0.67)	0.02 (0.77)	0.08 (0.07)
LF, ms^2	0.08 (0.25)	0.02 (0.72)	0.05 (0.44)
HF, ms^2	0.00 (0.99)	0.03 (0.62)	0.15 (0.02) [*]
LF/HF	0.10 (0.15)	-0.15 (0.84)	-0.13 (0.05) [*]
DFA1	-0.02 (0.78)	0.07 (0.32)	-0.06 (0.40)
SD12	0.02 (0.70)	-0.03 (0.63)	0.03 (0.62)

Note. LPA: light physical activity; MVPA: moderate to vigorous physical activity; SDNN: standard deviation of the normal-to-normal R-R intervals; rMSSD: square root of the mean of the squared differences between successive NN intervals LF: low frequency power, HF: high frequency power, LF/HF: ratio between low frequency power and high frequency power; DFA1: short-term fractal-scaling exponent; SD12: Poincare ratio. [*] p < 0.05.

Table 4 presents multivariate analyses for HRV variables. Age, resting heart rate, and mean metabolic risk score independently predicted time domain indices SDNN and rMSSD. Models demonstrated 34% and 33% of the variance of SDNN and rMSSD, respectively (Table 3). Regarding frequency domain, the predictors change across indices (Table 4). For instance, age and resting heart

rate were predictors of LF and HF. Furthermore, the mean metabolic risk score was a predictor only for HF. Regarding daily PA, MVPA was an independent predictor of LF/HF (β = -0.14, p = 0.03), controlling for sex, waist circumference, resting heart rate, and age. The best model demonstrated 16% of the total variance of LF/HF, with the contribution of MVPA being 2.3%.

Sex, waist circumference, resting heart rate, and anti-hypertensive medication were independent predictors as related to non-linear indices DFA1 and SD12 (Table 4).

Table 4. Predictors of frequency domain indices of heart rate variability

	R^2 increment (%)	β	*p*
Time domain indices			
Ln SDNN (R^2: 0.34)			
Age	22.2	-0.40	< 0.001
Ln Resting heart rate	9.9	-0.26	< 0.001
Mean metabolic risk score	1.8	-0.19	0.03
Sex	0.0	0.05	0.38
Ln rMSSD (R^2: 0.33)			
Age	16.5	-0.34	< 0.001
Ln Resting heart rate	13.9	-0.33	< 0.001
Mean metabolic risk score	2.6	-0.16	0.03
Sex	0.0	0.02	0.74
Frequency domain indices			
Ln LF ms^2 (R^2: 0.27)			
Ln Resting heart rate	18.5	-0.22	< 0.001
Age	4.9	-0.44	< 0.001
Sex	3.3	0.13	0.03
HF ms^2 (R^2: 0.36)			
Age	26.0	-0.43	< 0.001
Ln Resting heart rate	7.6	-0.25	< 0.001
Mean Metabolic syndrome score	2.8	-0.17	0.02
Sex	0.0	-0.06	0.32

	R^2 increment (%)	β	*p*
Ln LF/HF (R^2: 0.16)			
Sex	9.2	0.32	< 0.001
Waist circumference	4.1	0.14	0.04
Sqrt MVPA	2.3	-0.14	0.03
Ln Resting heart rate	0.05	0.07	0.30
Age	0.02	0.05	0.45
Non-linear indices			
DFA 1 (R^2: 0.20)			
Ln Resting heart rate	9.1	0.008	< 0.001
Sex	5.4	0.151	< 0.001
Anti-hypertensive treatment	3.4	-0.13	0.003
Waist circumference	1.8	0.004	0.01
Age	0.04	0.002	0.30
Sqrt SD12 (R^2: 0.15)			
Sex	5.0	-0.20	0.003
Ln Resting heart rate	4.5	-0.24	< 0.001
Anti-hypertensive treatment	3.0	0.28	0.001
Waist circumference	2.3	-0.15	0.04
Age	0.02	-0.05	0.42

Note. Ln SDNN: natural logarithm of standard deviation of the normal-to-normal R-R intervals; Ln RMSSD: natural logarithm of the square root of the mean of the squared differences between successive NN intervals; Ln LF: natural logarithm of the low frequency power; Ln HF: natural logarithm of the high frequency power; Ln LF/HF: natural logarithm of the ratio between low frequency power and high frequency power; DFA1: short-term fractal-scaling exponent; Sqrt SD12: squared root of the Poincare ratio; Ln: natural logarithm; Sqrt: squared root

4. Discussion

The main finding of this study is that in adults without established CVD, MVPA was negatively associated with LF/HF, explaining 2.3% of its variance. The observed association was independent of sex, waist circumference, and age. Conversely, sedentary time and LPA were not associated with any HRV indices.

Our result possibly highlights the positive effect of MVPA on cardiac autonomic modulation. In our study, the LF/ HF might potentially reflect the vagal control of the heart because recordings were based in short-term measurements under laboratory conditions (Shaffer, et al., 2014). In this regard, the higher MVPA associated with low LF/HF ratio, reflects a shift toward parasympathetic outflow, which is characteristic of a healthier cardiac autonomic modulation (Buchheit, et al., 2004; Dietrich, et al., 2008). Supporting this, we found a weak, but significant positive correlation between HF and MVPA.

However, our results must be interpreted with caution. MVPA was not an independent predictor of any single frequency domain index (LF ms^2 and HF ms^2), but was only a predictor when both were combined.

Additionally, the significance of the LF/HF is questionable, especially due to the inexactness of the origin of the LF index (that might reflect vagal sympathetic and baroreflex activity) (Shaffer, et al., 2014). Finally, cardiac autonomic deregulation is a risk factor for adverse cardiovascular events (La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998), but the LF/HF had an inferior capacity to predict deaths from CVD when compared to others indices of HRV, such as LF and HF (Bigger, Fleiss, Rolnitzky, & Steinman, 1993). Therefore, clinical meaning of the observed association between MVPA and LF/HF must be ascertained.

Physiological mechanisms by which MVPA could influences cardiac autonomic function are (i) the reduction of sympathetic neural outflow to the sinoatrial node and the attenuation of the heart rate in response to myocardial stretch (Smith, Hudson, Graitzer, & Raven, 1989); (ii) the reduction of GABAAergic neurotransmissions in the nucleus tractus solitary, involved in heart rate control, and therefore increasing vagal influences on cardiac pace maker activity

(Mueller & Hasser, 2006); and (iii) the improvement of cardiomyoctyes contractility (Wisloff, Ellingsen, & Kemi, 2009) and enhanced cardiac electrical stability (Billman, 2009).

Our sample was selected from a general population encompassing a wide age range and diverse health conditions (such as hypertension, dyslipidemia, and diabetes) and habitual medication. This fact makes it difficult to compare the study with other studies that were mainly performed with a restricted age range [adults (Sandercock, et al., 2008; Soares-Miranda, Sandercock, et al., 2012), middle-aged adults (Buchheit, et al., 2005) or elderly (Soares-Miranda, et al., 2014)] and with homogeneous health conditions, (Sandercock, et al., 2008; Soares-Miranda, Negrao, et al., 2012; Soares-Miranda, Sandercock, et al., 2012) which might limit the clinical utility of results for the general population as users of primary health care centers.

Contrary to what was previously described (Sandercock, et al., 2008; Soares-Miranda, Sandercock, et al., 2012), we did not find a significant association between MVPA and time domain indices that reflect circadian rhythms (Task Force, 1996). Possible methodological issues might explain these differences. We assessed HRV in short recordings and the accurate assessment of global cardiac autonomic function requires long-term R-R intervals recordings, lengthened to at least 18 hours, including day and night time (Min, Min, Paek, Cho, & Son, 2008). This reasoning can also be used to justify the lack of significant associations of MVPA with non-linear indices. In fact, the measuring of HRV by short-term electrocardiogram recording (20 minutes) is an acceptable method to assess the frequency domain indices of HRV (Task Force, 1996), but it can be questioned if it is as sensitive to non-linear indices as the 24-h recordings (Min, et al., 2008; Voss, et al., 2015).

Sedentary time has been related to deleterious effects on health indicators (Blair, et al., 2012; Chau, et al., 2013; Katzmarzyk, et al., 2009). Studies on the association between the amount of time spent in sedentary behaviors and cardiac autonomic function are only in the very early stages. In the present study, sedentary time was not associated with HRV indices. Recently, in a study

conducted in a sample with an identical age range to our study, sitting time at work was negatively associated with time and frequency HRV indices in blue-collar workers, regardless of MVPA (Hallman, et al., 2015). Nevertheless, in that study PA was assessed in a specific population (blue-collar workers), and measures of PA are not representative of total daily PA, because it was only considered the sitting time at work during workdays, and weekend days were not included. Furthermore, the valid number of days for PA measurement was 1.9, which is far below the recommendations for assessment of daily PA levels (Troiano, et al., 2008). Therefore, future studies are needed to ascertain the possible relationships between HRV and sedentary time.

In individuals with metabolic syndrome and obesity, autonomic nervous system impairment is related with chronic sympathetic over activation (Imai, et al., 2006). Our results corroborate this statement because waist circumference was an independent predictor of LF/HF, SD12, and DFA1. In the same direction, our results showed that the mean metabolic risk score represents an independent predictor of time-domain indices and HF, and this is also already described (Soares-Miranda, Sandercock, et al., 2012). Despite the observed relationships between the deregulation of autonomic function and metabolic abnormalities, still remains unclear which problem appears first (Vinik, Maser, & Ziegler, 2011).

Reference values for HRV indices are still not established, and huge discrepancies between studies due to methodological approaches make comparisons difficult (Nunan, Sandercock, & Brodie, 2010). Despite that, a value close to 1 for the non-linear index DFA1 is representative of a "healthy" area (Voss et al., 2015), and our mean for both sex were close to it.

The present study presents several strengths. According to our knowledge, this is the first time that short-term HRV, objective measurement of daily PA, and traditional risk factors were simultaneously assessed in a primary health care setting. Furthermore, in this study we have considered in the analysis multiple potential confounders of the relationship between HRV and PA, including the adjustments for resting heart rate, which have been strongly suggested (Monfredi, et al., 2014). Furthermore, we assessed daily PA through

accelerometers, which overcome the expected overestimation of total PA and time spent in each category of intensity (Dyrstad, et al., 2014) and might contribute to a better understanding of the association between exposure (daily PA) and outcome (HVR indices).

Limitations of the present study should be outlined. First, we adjusted all models for multiple confounders but the influence of residual confounding cannot be excluded. Second, it should be recognized that short-term ECG recordings did not present the same stability and reproducibility as 24-h recordings, especially for the assessment of time domain and non-linear indices of HRV (Task Force, 1996). Since we have included participants with a wide age range and general health conditions, generalization of the results should be made with caution because of the study sample size.

5. Conclusion

Results of this study did not outline the cardio-protective effect of MVPA in a robust way in HRV indices, despite the inverse association with one frequency-domain index (LF/HF). MVPA is inversely associated with frequency domain index LF/HF. Conversely, sedentary time and LPA are not associated with cardiac autonomic function.

Acknowledgements

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Original Study III

Effects of a health education intervention on physical activity, arterial stiffness and cardiac autonomic function in individuals with moderate-to-high cardiovascular risk

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Under review

Abstract

Background: This study evaluated the effects of a health education and counseling intervention program, in a primary health care setting, on daily physical activity (PA), arterial stiffness, and cardiac autonomic function in individuals with moderate-to-high risk of cardiovascular disease.

Methods: This was a parallel-group study with a 4-month-long intervention, plus 8 months of follow-up. Participants were 164 individuals with moderate-to-high cardiovascular risk, allocated to either an intervention (n = 87) or a control group (n = 77). The intervention consisted by 3 walking and face-to-face group sessions plus text messages. The primary outcome was daily PA (sedentary time, light and moderate to vigorous PA); the secondary outcomes were arterial stiffness [carotid-femoral pulse wave velocity (cfPWV)] and cardiac autonomic function [standard deviation of all N-N intervals (SDNN, ms) and absolute high frequency (HF, ms^2)].

Results: There were not significant group*time interactions for sedentary time [-7.4 (7.6); p = 0.331], light [4.4 (6.4); p = 0.491] or moderate to vigorous PA [0.1 (2.6); p = 0.938]. Considering secondary outcomes, there were not significant group*time interactions for cfPWV [0.09 (0.18); p = 0.592], SDNN [0.09 (0.06); p = 0.148], or HF [0.16 (0.14); p = 0.263].

Conclusion: The health education and counseling program did not improve daily PA, arterial stiffness, or the autonomic cardiac function of participants with moderate-to-high cardiovascular risk.

Keywords: education, counseling, primary health care, cardiovascular risk, daily physical activity, arterial stiffness, cardiac autonomic function

1. Introduction

Physical activity (PA) confers health benefits (Pate, et al., 1995), with evidence indicating that any amount of PA is healthy (Eckel, et al., 2014). The increment of daily PA levels is recommended in primary and secondary prevention of cardiovascular disease (CVD) (Graham, et al., 2007). Despite the recommendations, 31.1% of the adults worldwide fail to meet the PA guidelines (Hallal, et al., 2012).

Given that the incidence of CVD remains high (Graham, et al., 2007), the early detection of patients at risk is an important strategy to prevent the onset of CVD (Perk, et al., 2012). In developed countries, 70-80% of adults visit their general practitioner at least once a year (van Doorslaer, Masseria, & Koolman, 2006), which makes the primary care health services the best setting to assess cardiovascular risk (Perk, et al., 2012), manage risk factors (Perk, et al., 2012), and promote a healthy lifestyle, including the promotion of PA (Perk, et al., 2012).

Studies aiming at improvement of PA in primary health care (Armit, et al., 2009; Elley, Kerse, Arroll, & Robinson, 2003; Hardcastle, Taylor, Bailey, & Castle, 2008; Koelewijn-van Loon, et al., 2010; Richards, Hillsdon, Thorogood, & Foster, 2013) have produced divergent results, suggesting the necessity of further research (Lin et al., 2014; Richards et al., 2013). The large variation in the intervention designs (e.g. provider, mode, frequency, duration of the intervention, and follow-up) is a barrier to the determination of the best approach (Lin, et al., 2014). Furthermore, methodological constraints [e.g., trials generally enroll people with inadequate levels of PA (Armit, et al., 2009; Elley, et al., 2003) and subjective assessment of PA through questionnaires (Armit, et al., 2009; Elley, et al., 2003; Hardcastle, et al., 2008; Koelewijn-van Loon, et al., 2010; Orrow, Kinmonth, Sanderson, & Sutton, 2012)] limit the generalization of the results and represent potential source of bias.

Primary care health education and counseling interventions to promote PA can have secondary positive effects in other outcomes such as CVD risk factors (Armit, et al., 2009; Elley, et al., 2003; Hardcastle, et al., 2008) but long-term

health benefits have not been consistently reported (Lin, et al., 2014). Arterial stiffness and cardiac autonomic function are understood as CVD risk factors associated with increased cardiovascular risk (Laurent, et al., 2001; Tsuji, et al., 1996) Furthermore, arterial stiffness and impaired cardiac autonomic function are both negatively associated with daily PA (Andersson, et al., 2015; Soares-Miranda, et al., 2014). However, according to the best of our knowledge, neither of these secondary outcomes has been assessed in primary care interventions aiming to improve daily PA, as secondary outcomes.

Hence, the aim of this study was to evaluate the effects of a 4-month health education and counseling intervention in primary care, and a 8-month follow-up period, on daily PA (primary outcome) and on arterial stiffness and cardiac autonomic function (secondary outcomes) in adults with moderate to high cardiovascular risk.

2. Methods

Study design, and participant's allocation

This study was a parallel group with a non-probabilistic sample conduced from March 2012 to July 2013 at the primary health care center.

The study consisted of a health education and counseling intervention aiming to promote the increase in daily PA levels. The intervention consisted of three group sessions, followed by mobile text messages to encourage and reinforce PA adherence. Two general practitioners and a PA specialist delivered the health educational and counseling program.

Participants were selected from the registries of a primary health care center. Inclusion criteria were age between 18 and 65 years old, and participants should have at least moderate cardiovascular risk, calculated according to the 2013 European Society of Hypertension and European Society of Cardiology guidelines (Mancia, et al., 2014). Exclusion criteria included established diagnosis of cognitive, pulmonary, renal, neurological or thyroid disorders, orthopedic impairments, arrhythmias, severe hypertension [systolic blood

pressure (SBP) > 180 mmHg or diastolic blood pressure (DBP) > 100 mmHg], history of acute coronary syndrome or abnormal hemodynamic response both at rest or at exertion, peripheral arterial disease, and cancer.

The invitation to participants was made through phone calls and those who accepted went to the primary care center twice to perform assessments. In the first appointment, participants received information about the study before providing information about their sociodemographics and current medication. Then assessment of anthropometrics, blood pressure, arterial stiffness and cardiac autonomic function were completed. Moreover, accelerometers to measure daily PA were delivered to the participants. In the second appointment, blood samples were collected, and the participants returned the accelerometers. With this data, the investigators calculated the cardiovascular risk of each individual. Only those with moderate or high CVD risk were selected and invited to participate in the study.

Allocation to the intervention group (IG) was made by convenience according to the will and availability to participate in educational and counseling group sessions and to receive text messages on their mobile phones. Those who agreed to participate in the evaluations but were not available to participate in the health education and counseling IG were allocated to the control group (CG). Eight months after the four-month intervention was completed, a follow-up evaluation was performed in which participants were re-evaluated with the same sequence and at the same time of day, with the exception of the variables determined from the blood samples. In this study, the primary outcome was daily PA and the secondary outcomes were arterial stiffness and cardiac autonomic function.

All participants provided written informed consent. The study was approved by the Ethics Committee of the North Regional Health Authority (I.P. 25/2010) and all procedures were conducted according to the Helsinki declaration.

Measures

Daily physical activity levels

Daily PA was assessed using accelerometers (Actigraph GT1M, Actigraph LLC, Pensacola, FL) over the right hip, for 7 consecutive days, during the waking hours, except while bathing and water-based activities. ActiLife software (Actigraph, Florida, USA, version 6.9) was used to reduce raw activity data into daily PA. The accelerometer measures the intensity of movement, which was averaged for 1-min sampling intervals (counts/min). For data analysis, non-wear periods were defined as $\geq$ 10 consecutive one-minute sampling intervals with "zero" counts. To be considered as valid data, individuals must have had a minimum of 4 days recorded with at least 8 wear-time hours per day. The average minutes/day spent at different categories of PA intensity was determined according to cut points that relate PA to counts/min: sedentary time ($\leq$ 99 counts/min), light PA (LPA) (100 - 2019 counts/min) and moderate to vigorous MVPA (MVPA) ($\geq$ 2020 counts/min) (Troiano, et al., 2008).

Cardiac autonomic function and arterial stiffness

Participants were instructed to refrain from strenuous exercise and to avoid consuming caffeine-containing products or alcohol for at least 24 hours before evaluation. The evaluation room was kept quiet, semi-dark, at an average temperature of 21ºC.

Cardiac autonomic function was measured as heart rate variability (HRV). Recording of R-R interval data was performed using the Polar RS800CX (Polar Electro OY, Kempele, Finland) with a temporal resolution of 1ms (Nunan, et al., 2009). The HRV assessment methodology was described in detail elsewhere (Oliveira, et al., 2014). In brief, assessments were performed in the supine position, controlling the breathing rate by matching it to a metronome-paced frequency of 12 breaths/min. Following 20 min of recording, R-R interval data were downloaded into Polar Precision Performance software SW (Polar Electro OY, Kempele, Finland). Using Kubious software 2.0 for Windows (the Biomedical Signal Analysis Group, University of Kuopio, Finland) ectopic beats

or arrhythmias were excluded and R-R data were de-trended (Tarvainen, Ranta-Aho, & Karjalainen, 2002) and resampled at 4 Hz. Finally, the last five minutes of each recording were selected and used for calculating HRV indices. The time domain selected index was standard deviation of the normal-to-normal R-R intervals (SDNN, ms). Frequency domain indices were determined using the non-parametric method (Fast Fourier transform) and encompassed very low frequency (VLF, 0.0033 - 0.04 Hz), low frequency (LF, 0.04 - 0.15 Hz), high frequency (HF, 0.15 - 0.4 Hz). Absolute HF power (ms^2) was used as frequency domain variable. When performed for a short-term interval under controlled laboratory conditions, SDNN and HF predominantly reflect the cardiac vagal modulation (Martinmaki, Rusko, Kooistra, Kettunen, & Saalasti, 2006). The mean resting heart rate was also calculated in this evaluation.

Afterwards, at least three blood pressure measurements were made in the left arm using Colin model BP 8800 monitor (Critikron, Inc., Tampa, USA) with the arm well supported and relaxed at heart level. Measurements were taken at intervals of one minute.

Subsequently, arterial stiffness was measured as carotid-femoral pulse wave velocity (cfPWV) using the SphygmoCor device (AtCor Medical, Australia) according to international guidelines (Boutouyrie, & Vermeersch, 2010). In brief, sequential and consecutive carotid (i.e., right) and femoral (i.e., right) pressure waves were registered with parallel electrocardiogram recording. The electrocardiogram served as a reference to calculate the wave transit time between the two recordings sites (i.e., foot-to-foot method). The distance travelled by the pressure wave was the direct distance between the recording points at the femoral and carotid arteries (Boutouyrie, & Vermeersch, 2010). The value of cfPWV was calculated as the direct distance (in meters) divided by the transit time (in seconds). All measurements were performed in duplicate by the same trained researcher. The mean value was calculated and implemented.

Medication and anthropometrics

Medication was assessed by interview.

Height (cm) was assessed with a standard wall-mounted stadiometer and weight (kg) using a scale (Tanita, Inner Scan BC-522, Japan). Body mass index (BMI; kg/m^2) was thereafter calculated. Waist circumference (cm) was measured at the midpoint between the lowest rib and iliac crest. Patients were barefoot and wearing light clothing.

Blood sampling

At baseline, twelve-hour fasting blood samples were collected by venipuncture of the antecubital vein into serum separator and EDTA coated tubes. The following parameters were assessed: fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol and triglycerides. Assays were assessed in duplicated.

Determination of cardiovascular risk

Total cardiovascular risk was calculated according to the 2013 European Society of Hypertension and European Society of Cardiology International Guidelines for the Management of Hypertension (Mancia, et al., 2014). Cardiovascular risk was stratified in different categories (i.e., low, moderate, high and very high risk) based on blood pressure, cardiovascular risk factors, asymptomatic organ damage, and the presence of diabetes, symptomatic CVD or chronic kidney disease (Mancia, et al., 2014). Participants with low cardiovascular risk were excluded.

Delivery of Intervention: health education and counseling program

The health education and counseling program consisted of three sessions, lasting approximately 90 minutes each. A maximum of 10 participants were included in each session group. Sessions were composed of a 30-minute group

walk at moderate intensity in the city park, followed by 60 minutes of face-to-face intervention. In the first 60-minute session, a general practitioner presented information about the CVD risk concept, how to identify personal risk factors that influence the CVD risk, and insights about the impact of a moderate and high CVD risk on health status and quality of life. A general practitioner conducted the second session and the content was targeted at healthy behaviors and lifestyle (i.e., diet; tobacco cessation; salt intake; adherence and compliance with medication; stress management; and PA) as a path to diminish CVD risk. A PA specialist conducted the third session. The session contents were: how to reduce sedentary time, what to do to become physically active (frequency, intensity, duration, and type of activity) and achieve PA recommendations; development of personal goals; how to overcome barriers for PA practice; strategies to improve PA during daily living activities; procedures to monitor exertion during practice; best periods of the day to exercise; risk of injuries; and emergency procedures. In the third session, participants received a booklet with all the information presented during the sessions and a PA plans for each week of the four-month period. After the sessions, participants in the IG received 12 mobile text messages to encourage and reinforce PA adherence. The texts messages were delivered once a week during the first two months, and twice a month in the last 2 months. During the follow-up IG and CG only received the usual care.

The intervention program followed the recommendations and standards of the American College of Sports Medicine (Garber, et al., 2011).

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 21 (SPSS, Chicago, USA). Heart rate variability indices (SDNN and HF) were transformed in its natural logarithm due to its skewed distribution.

An intention-to-treat analysis was conducted, with the inclusion of all participants assessed and allocated into groups at baseline. Subsequently, a per protocol analysis was performed including only the participants who fully

accomplished the health education and counseling intervention and who undertook all the evaluations over the three assessment periods.

For comparisons between groups at baseline (CG vs. IG), the chi-squared test (categorical variables), t-test and Mann Whitney (U) test (quantitative variables) were used as appropriate. Changes in groups over time (group*time interaction) in daily PA, arterial stiffness and cardiac autonomic function indices were modeled using a linear mixed-model regression with random-effects statements on the slope and intercept of each participant. The covariance type used for the random-effects was the unstructured option (completely general covariance matrix). Other covariance types (e.g. first order autoregressive) were also used but presented less accurate results (higher Akaike's Information Criterion values). Normality of residuals was visually verified. Data were expressed as mean ± SD.

Vales of P less than 0.05 were considered significant and tests were two-sided.

3. Results

Figure 1 shows the flow chart of recruitment, selection, and allocation of participants into groups. A total of 828 participants were initially listed from the general practitioners files, of which 425 were within the age range and 109 did not meet inclusion criteria. A total of 164 participants completed the baseline assessment, of whom 87 were allocated to the IG and 77 to the CG. Two participants in the IG did not receive the intervention (one dropped out because of failure to attend sessions and the other due to labor limitations).
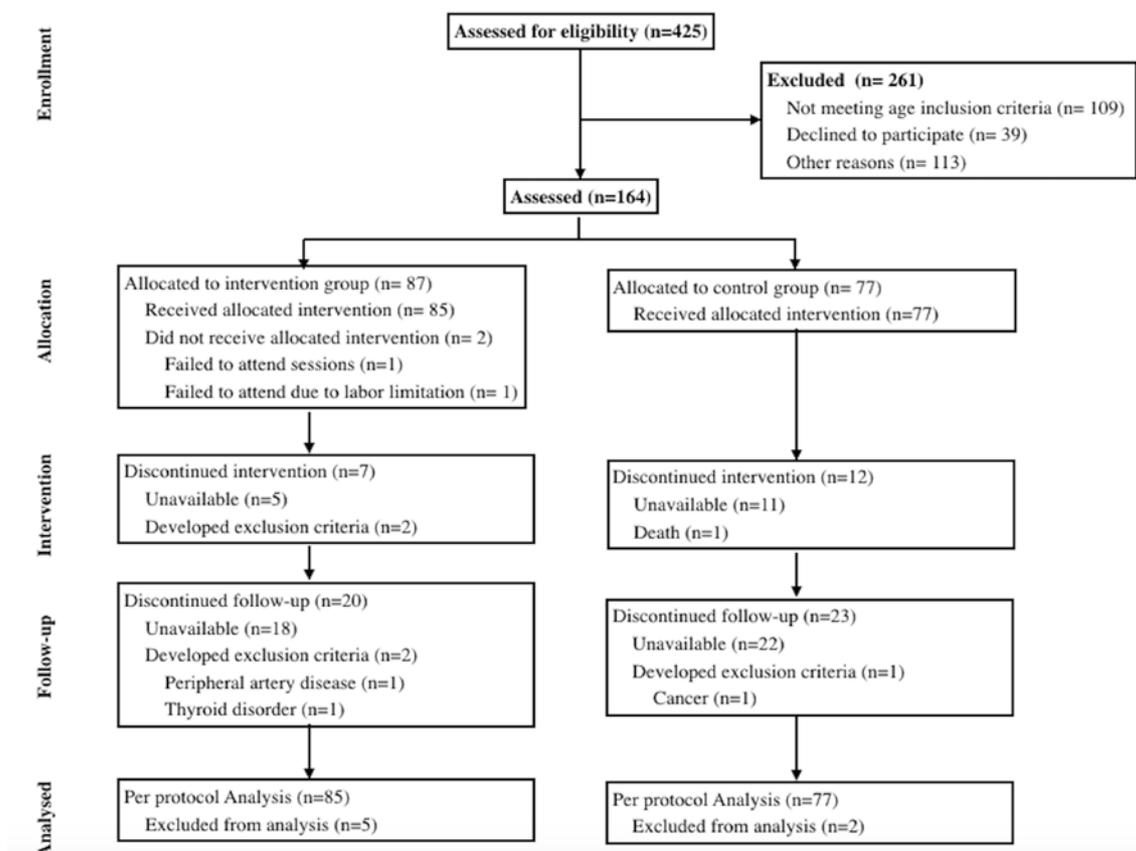
Figure 1. Study eligibility

Baseline characteristics

The study included 85 participants in the IG (57.16 ± 6.61 years old) and 77 in the CG (55.42 ± 7.34 years old) with moderate-to-high CVD risk. Their characteristics at baseline are summarized in Tables 1 and 2, and in supplemental material 1. Significant differences between groups were observed in the prevalence of dyslipidemia (X^2 = 5.4; P = 0.020), antihypertensive (X^2 = 4.1; P=0.042); and antidepressant/anxiolytic (X^2= 4.4; P = 0.036) medications. Considering daily PA, the IG showed significantly higher sedentary time (U = 2456.5; P = 0.040) and lower LPA (U = 2220.5; P = 0.004) than the CG.

Table 1. Overall sample characteristics and between groups comparisons at baseline

		Total (n = 162)	IG (n = 85)	CG (n = 77)	Statistical inference
Sociodemographics, n (%)					
Sex	Female	89 (54.9)	46 (54.1)	43 (55.8)	X^2 (1): 0.049; p = 0.825
	Male	73 (45.1)	39 (45.9)	34 (44.2)	
Cardiovascular risk score and cardiovascular risk factors prevalence, n (%)					
ESH/ESC Risk	Moderate	86 (53.1)	46 (54.1)	40 (51.9)	X^2 (1): 0.076; p = 0.782
	High	76 (46.9)	39 (45.9)	37 (48.1)	
Familial history	No	102 (63.8)	51 (60.0)	51 (68.0)	X^2 (1): 1.103; p = 0.294
	Yes	58 (36.3)	34 (40.0)	24 (32.0)	
Smoking	No	126 (77.8)	69 (81.2)	57 (74.0)	X^2 (1): 1.195; p = 0.274
	Yes	36 (22.2)	16 (18.8)	20 (26.0)	
Dyslipidemia	No	16 (9.9)	4 (4.7)	12 (15.6)	X^2 (1): 5.371; p = 0.020
	Yes	146 (90.1)	81 (95.3)	65 (84.4)	
Hypertension	No	14 (8.6)	9 (10.6)	5 (6.5)	X^2 (1): 0.858; p = 0.354
	Yes	148 (91.4)	76 (89.4)	72 (93.5)	
Diabetes	No	120 (74.1)	62 (72.9)	58 (75.3)	X^2 (1): 0.120; p = 0.730
	Yes	42 (25.9)	23 (27.1)	19 (24.7)	
Medication, n (%)					
Anti-hypertensive	No	39 (24.1)	26 (30.6)	13 (16.9)	X^2 (1): 4.152; p = 0.042
	Yes	123 (75.9)	59 (69.4)	64 (83.1)	
Lipid lowering	No	81 (50.0)	41 (48.2)	40 (51.9)	X^2 (1): 0.223; p = 0.637
	Yes	81 (50.0)	44 (51.8)	37 (48.1)	

		Total (n = 162)	IG (n = 85)	CG (n = 77)	Statistical inference
Antiplatelet agents	No	150 (92.6)	77 (90.6)	73 (94.8)	X^2 (1): 1.047; p = 0.306
	Yes	12 (7.4)	8 (9.4)	4 (5.2)	
Anti-diabetic	No	124 (76.5)	64 (75.3)	60 (77.9)	X^2 (1): 0.155; p = 0.693
	Yes	38 (23.5)	21 (24.7)	17 (22.1)	
Antidepressant or anxiolytics	No	125 (77.2)	60 (70.6)	65 (84.4)	X^2 (1): 4.383; p = 0.036
	Yes	37 (22.8)	25 (29.4)	12 (15.6)	

Note. IG: intervention group; CG: control group; ESH/ESC: European society of hypertension/ European society of cardiology

Table 2. Parameters at baseline, after 4 months and follow-up (intention-to-treat analysis)

		Baseline (T1)		4 months (T2)		Follow up (T3)		Statistics results	Changes between T1 and T2	Changes between T2 and T3
		n	Mean ± sd	n	Mean ± sd	n	Mean ± sd		Mean ± sd	Mean ± sd
Age, years old	IG	85	57.16 (6.61)	76	57.89 (6.23)	56	58.23 (6.34)	F_{time} (1; 244.67)= 0.505; p < 0.001	-0.36 (0.48)	-0.70 (0.49)
	CG	77	55.42 (7.34)	65	55.77 (7.56)	42	56.38 (6.48)		-0.29 (0.46)	-0.69 (0.47)
Anthropometrics										
BMI, kg/m^2	IG	85	29.27 (3.91)	76	28.87 (3.91)	56	28.92 (3.79)	F_{time} (1; 125.89)= 4.402; p = 0.038	0.32 (0.79)	-0.02 (1.10)
	CG	77	29.89 (4.32)	65	30.19 (4.08)	42	28.96 (3.64)		-0.03 (0.74)	0.16 (0.89)
Waist circumference, cm	IG	85	98.13 (9.81)	76	96.51 (10.07)	56	96.32 (10.24)	F_{time} (1; 122.35)= 0.217; p = 0.642	1.22 (0.34)	-0.07 (2.21)
	CG	77	99.35 (10.19)	65	101.42 (10.40)	42	98.56 (8.71)		-1.29 (3.85)	-0.01 (3.01)
Hemodynamics										
SBP, mmHg	IG	85	134.69 (15.47)	74	126.18 (14.27)	55	130.00 (14.85)	F_{time} (1; 126.01)= 17.143; p < 0.001	7.77 (11.88)	-4.59 (15.04)
	CG	77	136.02 (17.31)	63	130.74 (14.87)	41	128.39 (18.10)		5.61 (14.72)	1.05 (13.79)
DBP, mmHg	IG	85	79.14 (10.61)	74	75.64 (10.05)	55	76.74 (8.09)	F_{time} (1; 137.48)= 9.821; p = 0.002	2.95 (7.69)	-1.59 (9.71)
	CG	77	78.51 (9.71)	63	75.65 (10.07)	41	75.34 (9.20)		2.70 (8.74)	0.85 (9.08)
MBP, mmHg	IG	85	101.11 (12.17)	74	94.93 (11.06)	55	97.20 (10.40)	F_{time} (1; 130.11)= 15.884; p < 0.001	6.20 (8.07)	-3.61 (11.07)
	CG	77	101.31 (12.69)	63	96.87 (11.55)	41	96.78 (13.64)		4.20 (11.25)	1.97 (10.23)
PP, mmHg	IG	85	55.54 (9.07)	74	50.54 (8.96)	55	53.34 (10.69)	F_{time} (1; 117.03)= 10.808; p = 0.001	4.50 (8.54)	-2.20 (10.11)
	CG	77	57.51 (11.93)	63	55.11 (9.77)	41	53.02 (12.54)		2.94 (8.84)	0.52 (8.79)

			Baseline (T1)		4 months (T2)		Follow up (T3)	Statistics results	Changes between T1 and T2	Changes between T2 and T3
Resting heart rate, bpm	IG	84	65.82 (10.43)	69	64.53 (10.13)	55	63.07 (10.76)	F_{time} (1; 119.62)= 4.609; p = 0.034	1.38 (6.24)	1.55 (7.62)
	CG	76	64.32 (8.67)	59	63.03 (10.17)	37	63.91 (9.43)		0.40 (7.45)	-1.11 (7.02)
Daily physical activity										
Sedentary time, min/day*	IG	82	472.2 (85.6)	71	452.2 (89.3)	54	454.4 (102.2)	F_{time} (1; 237.3)=0.093; p = 0.761	13.9 (59.1)	-9.1 (69.2)
	CG	74	435.4 (100.2)	63	426.2 (109.2)	37	441.3 (101.8)		6.6 (93.7)	-13.8 (74.3)
LPA, min/day*	IG	82	289.8 (92.4)	71	299.1 (94.4)	54	294.4 (92.5)	F_{time} (1; 108.4)= 1.363; p = 0.246	-2.3 (52.9)	13.4 (55.9)
	CG	74	337.0 (103.7)	63	331.2 (102.1)	37	320.1 (86.1)		8.8 (72.0)	1.9 (57.2)
MVPA, min/day	IG	82	32.9 (25.8)	71	41.0 (29.9)	54	34.3 (27.4)	F_{time} (1; 117.4)= 0.367; p = 0.546	-5.2 (26.0)	6.6 (19.0)
	CG	74	38.3 (31.4)	63	41.0 (30.5)	37	42.5 (39.3)		-0.9 (21.7)	-1.8 (36.7)
Arterial stiffness										
cfPWV, m/s	IG	84	10.3 (2.1)	65	10.0 (2.3)	54	10.1 (2.4)	F_{time} (1; 91.2)= 0.318; p = 0.574	0.3 (1.5)	-0.5 (1.8)
	CG	74	9.9 (1.9)	58	9.9 (2.3)	34	9.7 (2.1)		0.5 (1.5)	-0.2 (1.1)
Heart rate variability										
Ln_SDNN	IG	84	3.2 (0.6)	67	3.3 (0.7)	54	3.3 (0.6)	F_{time} (1; 263.9)=0.198; p = 0.657	-0.16 (0.75)	0.06 (0.62)
	CG	75	3.3 (0.6)	58	3.3 (0.6)	36	3.1 (0.6)		-0.004 (0.66)	0.25 (0.63)
Ln_HF, ms^2	IG	84	5.5 (1.3)	67	5.6 (1.4)	54	5.7 (1.4)	F_{time} (1; 223.8)= 1.586; p = 0.210	0.03 (1.39)	0.53 (1.43)
	CG	75	5.7 (1.4)	58	5.6 (1.5)	36	5.4 (1.4)		-0.18 (1.49)	0.53 (1.43)

Note. IG: intervention group; CG: control group; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure, PP: pulse pressure; LPA: light physical activity; MVPA: moderate to vigorous physical activity; cfPWV: carotid femoral pulse wave velocity; Ln_SDNN: natural logarithm of the standard deviation of the normal-to-normal R-R intervals; Ln_HF: natural logarithm of high frequency power. * Groups were significantly different at baseline P < 0.05.

Daily PA parameters during intervention and follow-up

After the intervention and follow-up period, no significant change was observed in sedentary time, LPA or MVPA in either group (Table 3 and Figure 2). After adjustments for age, sex, BMI, and variables that were different between groups at baseline, the results remained similar (Table 3, Models = 1, 2 and 3). During the four-month intervention and in the follow-up period, the IG had reduced sedentary time (-7.4 ± 7.6; p = 0.331) and increased LPA (4.4 ± 6.4; p = 0.491) and MVPA (0.1 ± 2.6, p = 0.938) compared to CG, although this lacked statistical significance (Table 3).

Table 3. Linear mixed model regression for sedentary time, light and moderate to vigorous physical activity

		Slope (SE); statistical inference
Sedentary time		
Unadjusted model	Group	41.4 (17.9); p = 0.021
	Time	1.83 (5.8); p = 0.752
	Group * Time	-5.99 (7.6); p = 0.433
Model 1	Group	42.4 (18.2); p = 0.021
	Time	2.10 (5.7); p = 0.715
	Group * Time	-7.4 (7.6); p = 0.331
LPA		
Unadjusted model	Group	-50.0 (18.9); p = 0.009
	Time	-6.1 (4.9); p = 0.213
	Group * Time	4.7 (6.5); p = 0.468
Model 2	Group	-50.5 (19.0); p = 0.009
	Time	-6.6 (4.8); p = 0.168
	Group * Time	4.4 (6.4); p = 0.491

		Slope (SE); statistical inference
MVPA		
	Group	-2.8 (5.9); p = 0.640
Unadjusted model	Time	1.0 (1.9); p = 0.617
	Group * Time	-0.4 (2.5); p = 0.878
	Group	-2.7 (5.9); p = 0.651
Model 3	Time	1.2 (1.9); p = 0.553
	Group * Time	0.1 (2.6); p = 0.938

Note. Model 1: adjusted for age, sex, body mass index, dyslipidemia, antihypertensive and antidepressants/ anxiolytic medication, and light physical activity. Model 2: adjusted for age, sex, body mass index, dyslipidemia, antihypertensive and antidepressants/ anxiolytic medication, and sedentary time. Model 3: adjusted for age, sex, body mass index, dyslipidemia, antihypertensive and antidepressants/ anxiolytic medication, sedentary time, and light physical activity.
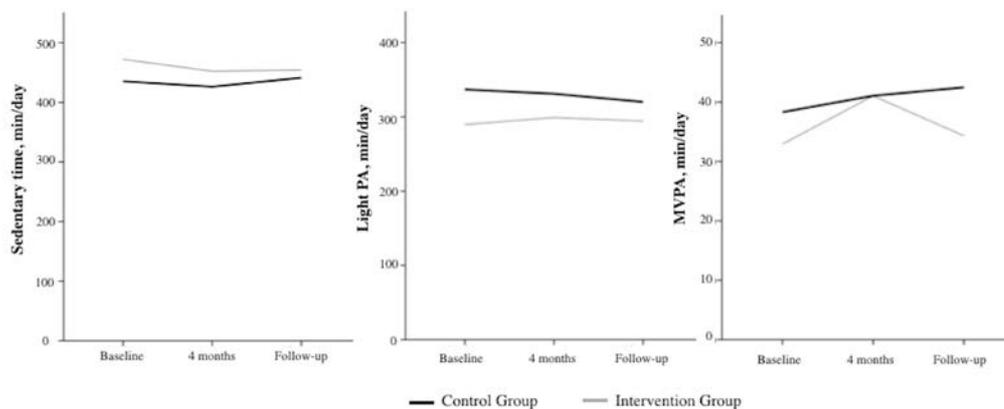
Figure 2. Daily physical activity intensities over time for intervention and control groups

Arterial stiffness parameters during intervention and follow-up

There was no significant group*time interaction for cfPWV, even after adjustments for variables that were different between groups at baseline, mean blood pressure, resting heart rate, and daily PA intensities (Table 4, model 1).

Heart rate variability parameters during intervention and follow-up

For unadjusted models, there was as significant time*group interaction, showing that the IG increased Ln_SDNN in 0.13 ± 0.06 (p = 0.040) compared to the CG (Table 4, unadjusted model). However, after adjustments for age; sex; BMI; dyslipidemia; antihypertensive and antidepressants /anxiolytic medications; resting heart rate; mean blood pressure; and daily PA, the group*time interaction lost statistical significance (0.09 ± 0.06; p = 0.148) (Table 4, model 2).

For the frequency domain index HF, no significant group*time interaction was observed along the intervention and follow-up periods (Table 4; model 3).

Table 4. Linear mixed model regression for arterial stiffness and heart rate variability

		Slope (SE); statistical inference
Arterial stiffness		
cfPWV, m/s		
	Group	0.23 (0.34); p = 0.491
Unadjusted model	Time	0.02 (0.12); p = 0.833
	Group * Time	0.03 (0.16); p = 0.812
	Group	0.12 (0.33); p = 0.704
Model 1	Time	0.00 (0.14); p = 0.985
	Group * Time	0.09 (0.18); p = 0.592
Heart rate variability		
Ln_SDNN		
	Group	-0.21 (0.13); p = 0.099
Unadjusted model	Time	-0.08 (0.04); p = 0.100
	Group * Time	0.13 (0.06); p = 0.040

		Slope (SE); statistical inference
Model 2	Group	-0.15 (0.13); p = 0.258
	Time	-0.06 (0.05); p = 0.217
	Group * Time	0.09 (0.06); p = 0.148
Ln_HF		
	Group	-0.43 (0.30); p = 0.153
Unadjusted model	Time	-0.20 (0.11); p = 0.063
	Group * Time	0.23 (0.14); p = 0.114
	Group	-0.32 (0.31); p = 0.304
Model 3	Time	-0.15 (0.11); p = 0.178
	Group * Time	0.16 (0.14); p = 0.263

Note. Model 1, 2 and 3: Adjustments for age, sex, body mass index, dyslipidemia, antihypertensive and antidepressants/ anxiolytic medication, mean blood pressure, resting heart rate, sedentary time, light and moderate to vigorous physical activity

For the per protocol analysis, which was restricted to 86 participants (GE: 51 and GC: 35), the results were similar to those observed in the intention-to-treat analysis (supplemental material 2).

4. Discussion

The main findings of this study were that the health education and counseling program conducted in the primary care setting did not promote significant changes in daily PA, arterial stiffness and cardiac autonomic function, both after the intervention and at the end of the 8-month follow-up period. In the IG group, minimal benefits were observed on reduction of sedentary time and increase in LPA and MVPA after four months, but this was reversed during the follow-up (Table 3 and Figure 2).

Regarding the lack of significant changes in daily PA in the IG, it is important to note that at baseline this group had significantly higher levels of sedentary time, and lower LPA than the CG. Furthermore, the observed mean values for MVPA

indicate that both groups were minimally active at baseline, in light of the current recommendations, suggesting that 30 min/day of MVPA should be performed (Haskell, et al., 2007). Therefore, it is possible that the minimal improvements observed in the IG were influenced by the aforementioned observations because they started the intervention with a poorer PA profile compared to controls; on the other hand, they were not too inactive, which could have influenced the rate of improvement (diminished returns from the intervention). In addition, it is also possible that the length of the four-month intervention was insufficient to enlarge the improvements in the IG beyond the observed mean values at four months in a way that allowed them to achieve significant differences over the CG.

Despite the minimal improvements in daily PA in the IG, the observed trend for the reversion at the end of follow-up possibly indicates that the intervention was insufficient to change the beliefs in the value of PA as a lifestyle risk factor influencing health and well-being, or the readiness and /or self-motivation for PA (Ingledew, & Markland, 2008). Future studies should evaluate the motives and self-regulatory skills associated with adherence to health-related PA.

Previous intervention studies such as the Hoorn Prevention Study (Lakerveld, et al., 2013) and the IMPALA study (Koelewijn-van Loon, et al., 2010) aimed to promote lifestyle modifications through health education and counseling programs, including daily PA levels, but these studies also reported no changes in PA. However, comparability between studies is difficult for a number of reasons: the differences in the health education and counseling programs; differences in the strategies of communication to deliver the interventions; length of intervention and duration of follow-up periods; the skills of caregivers or lifestyle facilitators who deliver the program (physicians, nurses, physiotherapists, or PA specialists); and the participant characteristics in intervention and control groups (Lin, et al., 2010; Orrow, et al., 2012).

The present study did not find any group*time interaction for arterial stiffness or cardiac autonomic function variables. It was previously reported that daily PA might directly impact cardiac autonomic function (i.e., down and up-regulation of

parasympathetic and sympathetic, respectively (Soares-Miranda, et al., 2014). and indirectly on arterial stiffness (Andersson, et al., 2015), probably due to its effects on arterial nitric oxide bioavailability. Given that PA didn't change significantly, the lack of changes in secondary outcomes in this study should be expected, in light of the hypothesis that daily PA has a role as a modulator or moderator of arterial stiffness and cardiac autonomic function.

Strengths

First, this study used objective measurement for PA, which likely improved the accuracy of assessments over time. Indeed, the use of self-report measures of PA is the most common method in previously published trials, which might inflate estimates of interventions effects (Orrow et al., 2012), once respondents tend to report less sedentary behaviours and more MVPA (Lin, et al., 2010; Orrow, et al., 2012), especially after interventions designed to promote PA.

Second, arterial stiffness and cardiac autonomic function were simultaneously assessed as secondary outcomes, making it the first time this has been done.

Third, this study performed baseline, four-month and eight-month assessments at the same period of the year in both groups, avoiding the seasonality effect on daily PA levels.

Limitations

Several limitations of this study should be noted. First, the allocation of patients into groups was made by convenience. Second, this study did not assess self-regulation for PA and compared this between the groups. Given that allocation was made by convenience, it is possible that those included in the IG were those who were more conscious of and motivated bout the importance of lifestyle changes. Third, the sample size, and the participant's retention at one year, was small.

5. Conclusion

In conclusion, this study did not provide evidence for the efficacy of a health education and counseling program in a primary care setting to improve daily PA levels and modification of arterial stiffness and cardiac autonomic function in individuals with moderate to high cardiovascular risk. These conclusions should not be generalized to other health settings and sample characteristics.

Conflict of Interest

Authors have no conflict of interest with companies or manufactures.

Results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Supplemental material 1

Supplemental Table 1. Lipid and metabolic profile at baseline

	Group	n	Mean (sd)
Lipid and metabolic profile			
Triglycerides, mg/dL*	IG	83	138.02 (67.32)
	CG	73	116.83 (55.30)
HDL-cholesterol, mg/dL	IG	83	54.71 (14.34)
	CG	73	58.61 (15.82)
LDL-cholesterol, mg/dL	IG	83	126.81 (40.12)
	CG	73	119.94 (36.12)
Total cholesterol mg/dL	IG	83	209.13 (39.30)
	CG	73	201.93 (41.26)
Fasting plasma glucose, mg/dL	IG	83	117.63 (52.39)
	CG	73	101.19 (18.43)

Note. IG: intervention group; CG: control group;

* Groups were significantly different at baseline $P < 0.05$.

Supplemental material 2

Supplemental Table 2. Per protocol linear mixed models regression for physical activity, arterial stiffness and heart rate variability

		Slope (SE); statistical inference
Physical activity		
Sedentary time		
	Group	42.23 (22.22); p= 0.061
Unadjusted model	Time	8.22(5.91); p=0.168
	Group * Time	-13.07(7.70); p=0.094
LPA		
	Group	-41.26 (24.95); p=0.102
Unadjusted model	Time	-10.27 (5.41); p=0.061
	Group * Time	6.33 (7.05); p=0.372
MVPA		
	Group	-7.04 (8.08); p=0.386
Unadjusted model	Time	-0.77 (2.25); p=0.731
	Group * Time	1.03 (2.93); p=0.726
Arterial stiffness		
cfPWV		
	Group	0.16 (0.38); p=0.668
Unadjusted model	Time	0.11 (0.17); p=0.509
	Group * Time	-0.00 (0.22); p=0.988
Heart rate variability		
Ln_SDNN		
	Group	-0.79 (0.41); p=0.058
Unadjusted model	Time	-0.18 (0.12); p=0.152
	Group * Time	0.31 (0.16); p=0.056
Ln_HF		
	Group	-1.76 (0.73); p=0.019
Unadjusted model	Time	-0.40 (0.30); p=0.184
	Group * Time	0.55 (0.38); p=0.152

Note. PA: physical activity; MVPA: moderate to vigorous physical activity; Ln_SDNN: natural logarithm of standard deviation of all N-N intervals; Ln_HF: natural logarithm of high frequency

CHAPTER IV

GENERAL DISCUSSION

Methodological discussion

The present thesis had two main aims. The first, consisted in the determination of possible associations between daily PA and sedentary time with arterial stiffness and cardiac autonomic function, in adults without established CVD. The second, consisted in the evaluation of the effects of a 4-month health education and counselling intervention in primary health care and 8-month follow-up period, on daily PA (primary outcome) and on arterial stiffness and cardiac autonomic function (secondary outcomes), in adults with moderate to high cardiovascular risk.

The rationale supporting this thesis was based on the assumption that CVD are preventable through a set of actions, both at the population and at individual levels, aiming to eliminate and control risk factors (Piepoli et al., 2016).

Determining the risk factors underlying CVD and the development of estimation risk scores to identify individuals at moderate to high risk for CVD are therefore essential to set up preventive interventions (Cooney, Dudina, & Graham, 2009; Mancia et al., 2007; Mancia et al., 2014).

Beyond the well-established risk factors such as obesity, type II diabetes, and hypertension (Danaei et al., 2011; Finucane et al., 2011; Sundstrom et al., 2014), CVD might also be triggered by prolonged exposure to lifestyle risk factors such as smoking, poor quality diet, and physical inactivity (WHO, 2011a, 2011b). Indeed, PA at certain level has been pointed out as a cardio protective behavior (Nocon et al., 2008; Paffenbarger et al., 1993; Shiroma & Lee, 2010) that also mitigates the progression of pathologies (Hambrecht et al., 2003; Manson et al., 2002; Seals, 2014).

Sedentariness is also recognized as an independent CVD risk factor (Held et al., 2012; Katzmarzyk, Church, Craig, & Bouchard, 2009) with a strong impact in different health related outcomes. However, the objective assessment of time spent in sedentary behaviors and the quantitative determination of its association with other cardiovascular risk factors, namely arterial stiffness and cardiac autonomic function has not yet been established (Andersson et al., 2015; Gomez-Marcos et al., 2014; Hallman et al., 2015). The cross-sectional

studies supporting this thesis (presented separately as original studies I and II) are therefore relevant, original, and appropriate to appraise the extent at which a broad range a PA intensities, even sedentary behaviors, are associated and independent predictors of health-related outcomes as are arterial stiffness and cardiac autonomic function (Vlachopoulos, Aznaouridis, & Stefanadis, 2010; Hillebrand et al., 2013; Tsuji et al., 1996).

Lifestyle interventions should be applied prior or in combination with drug therapies to reduce the burden of CVD morbidity and mortality (Piepoli et al., 2016). Health promotion and modification of unhealthy lifestyle behaviors are an essential part of CVD prevention in adults at moderate to high cardiovascular risk (Piepoli et al., 2016). There is evidence that prevention is effective: the elimination of health risk behaviors would prevent at least 80% of CVDs (Piepoli et al., 2016).

Health education and counseling intervention is an important path for CDV prevention, contributing to the improvement of individual, group, institutional, community and systemic strategies to expand health knowledge, attitudes, skills and behaviors (WHO, 2012). It aims to enhance health literacy, behavior and lifestyles changes conducive to health (WHO, 2012).

Studies aiming at improving PA (Armit et al., 2009; Elley, Kerse, Arroll, & Robinson, 2003; S. Hardcastle, Taylor, Bailey, & Castle, 2008; Koelewijn-van Loon et al., 2010) have produced divergent results, suggesting the need for further research (Lin et al., 2014; Richards, Hillsdon, Thorogood, & Foster, 2013), although it has been concluded in a recently systematic review that health education and counseling interventions improve daily PA, cardiovascular risk factors and risk score (Ramoa Castro, Oliveira, Ribeiro, & Oliveira, 2017). However, in the majority of the studies included in the systematic review, PA was not the main outcome, and arterial stiffness and cardiac autonomic function were not assessed (Ramoa Castro, Oliveira, Ribeiro, & Oliveira, 2017).

In the present thesis, we have undertaken a parallel-group and pragmatic study, enrolling individuals recruited from a primary health care setting who were allocated either to a 4-month health education and counseling intervention,

aiming to improve health literacy, daily PA levels and reduce time spent in sedentary behaviors, or to a CG. This study also adds a follow-up period after the intervention. Using this study design we would be able to assess the intervention's effectiveness and sustainability after cessation.

According to the The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Piepoli et al., 2016), prevention should be delivered at the general population level by promoting healthy lifestyle behavior. In Portugal access to the public National Health Service is universal, and everyone is registered, even those who do not access healthcare services (Carreira, Pereira, Azevedo, & Lunet, 2012). In this sense, the samples enrolled in our studies are within the broad classification of general population targeted in both cross-sectional and intervention studies.

Given that we aimed to undertake studies in the general population within the scope of primary prevention of CVD, individuals with history of established CVD were excluded. Moreover, we want to establish the associations between PA and arterial stiffness, and cardiac autonomic function, as well as to improve PA through an education and counseling intervention. We therefore excluded any individuals that presented conditions to which PA was an absolute contraindication (e.g. severe hypertension) and those with other diseases impacting capability to perform PA (e.g. pulmonary, renal, infectious and chronic immunological disorders) (Fletcher et al., 1995; Working Group, 2001).

The studies included in this thesis were conducted in adults aged over 18 years old. Elders aged over 65 years old were excluded. Young, middle-aged, and advanced aged adults are the largest portion of the demographic pyramid in Portugal, as is in many other countries (Eurostat, 2017). Considering that the elderly is a part of the population in which the cardiovascular risk is increased primarily, and largely, by the effect of age itself. Moreover, even considering that the elderly is the demographic stratum where the prevalence and incidence of CVD is higher, this might be partially due to the exposure to lifestyle risk factors in the precedent years of life (young adults and middle-aged adults).

Therefore, determination of risk factors for CVD and study interventions on health education and promotion of a healthy lifestyle should encompass this large-spectrum age cohort.

Another important methodological issue is the sample composition regarding sex. For all the outcomes we have considered, there is evidence that sex is an important covariate (Dishman, Sallis, & Orenstein, 1985; Laurent et al., 2006; Voss, Schroeder, Heitmann, Peters, & Perz, 2015). Having said that, our studies have enrolled both men and women. Moreover, for the cross-sectional study sample recruitment was random to avoid sampling bias, but not randomly selected to leverage sample diversity (Sugden & Moulson, 2015). However, in the intervention study, allocation into groups was made by convenience, which is an important limitation because adherence can be biased. However, as we have mentioned before, the intervention study is a pragmatic one, which means that its nature is more descriptive than experimental, for which a randomized controlled design is more convenient and acceptable (Thiese, 2014).

Being PA a complex behavior, its accurate evaluation is a challenging task (Dyrstad, Hansen, Holme, & Anderssen, 2014). Physical activity and sedentariness are frequently assessed by questionnaires (Troiano et al., 2008). Accelerometry represents a huge advance in the field of PA assessment due to its objective results (Troiano et al., 2008). Accelerometry is considered a better methodology to assess PA, compared to questionnaires (Dyrstad et al., 2014), but it is still far from getting comprehensive information in terms of type, duration, intensity and context in which PA occur (Chastin, Palarea-Albaladejo, Dontje, & Skelton, 2015). Almost all evidence regarding the study of PA as risk factor and/or as a predictor of other risk factors and CVD morbidity, as well as in the study of the changes on daily PA as a result of interventions to promote PA, came from self-report measurements (Armit et al., 2009; Cochrane et al., 2012; Elley et al., 2003; S. J. Hardcastle, Taylor, Bailey, Harley, & Hagger, 2013; Harris et al., 2012; Koelewijn-van Loon et al., 2009; Lakerveld et al., 2013; Parra-Medina et al., 2011; Reid et al., 2014), representing a limitation in many studies (Dyrstad et al., 2014). According to the best of our knowledge, in what

concerns the study of interventions to promote PA, only one has used accelerometry to assess PA (Griffin et al., 2014).

From a methodological point of view, PA assessed through accelerometry strengthens the studies included in the present thesis. However, there are important considerations to be done. First, accelerometry data analysis use cut-off points to categorize captured body accelerations (counts per min) into intensities of daily PA. Intensities are therefore only based in one unique numeric value of counts per minute. It is possible to discuss if a specific value of count per minute is related with certain intensity, and the next one with more/less intense PA. It might occur that values in proximity of cut off points are misclassified. This is especially important for light, moderate and vigorous PA (Chastin et al., 2015). The evidence related with sedentary activities associated with less than 100 counts per min is more robust despite not giving information about the body position (sitting or standing) (Healy, Matthews, Dunstan, Winkler, & Owen, 2011).

Second, the accelerometer does not capture information about the context in which PA is performed. Indeed, there is evidence that the protective effects of PA on CVD is not equal at occupational or leisure-time contexts (Krause, Brand, Arah, & Kauhanen, 2015; Li, Loerbroks, & Angerer, 2013). This should be considered a limitation of studies included in the present thesis. Despite that, in our samples, educational attainment (data not shown) reveals that almost 72% of the individuals have a maximum nine years of education. Low levels of educational attainment is linked with worse economic outcomes characteristic potentially linked with more physically demanding work (American Psychological Association, 2007). Unfortunately, we did not register information on occupational status.

Participants at the IG were encouraged to reach optimal levels of PA as well as to replace sedentary time by LPA such as standing up and walking. In previous interventions with similar aims, reduction of sedentary time further than increment of MVPA was either not adopted or was not described as a goal (Armit et al., 2009; Cochrane et al., 2012; Davies et al., 2008; Elley et al., 2003;

S. J. Hardcastle et al., 2013; Harris et al., 2012; Koelewijn-van Loon et al., 2009; Lakerveld et al., 2013; Parra-Medina et al., 2011; Reid et al., 2014; Wister et al., 2007).

The intervention study was important because it was conducted in a primary health context by a multidisciplinary team. This represents an innovation in Portugal, where physicians give little or no information on PA, and there is no data about this topic already published.

The delivered strategy was based on two approaches: face-to-face group sessions plus reinforcements using mobile text messages. Combined approaches were already used in randomized controlled studies for the modification of lifestyle behaviors (Ramoa Castro, Oliveira, Ribeiro, & Oliveira, 2017). Additionally, the use of mobile text messages to deliver and reinforce health messages is promising due to its simplicity, cost-effectiveness and easily tactics to approach participants (Richards, Thorogood, Hillsdon, & Foster, 2013).

None of the intervention deliverers had training on motivational techniques for behavioral change, and motivational interviewing, which have been pointed out as good practices to promote behavioral modification (Perk et al. 2012; Artinian et al., 2010). Indeed, interventions combining cognitive behavioral strategies (e.g. goal-setting, self-monitoring, face-to face contacts, feedback, and reinforcement) are more likely to promote changes in lifestyle behaviors (Artinian et al., 2010). The motivation or self-determination for PA, and readiness for change behaviors are intra-personal correlates or determinants to achieve real lifestyle modifications (Ingledew & Markland, 2008) and unfortunately, it was not possible to include this in our studies.

The assessment of arterial stiffness with cfPWV strengthens the methodology of this thesis. There is evidence that cfPWV is an independent predictor for cardiovascular events (Vlachopoulos et al., 2010), and it has already been introduced as a risk factor for organ damage that leads to at least moderate cardiovascular risk (Mancia et al., 2014). The careful procedure during data collection, following strict guidelines (e.g. two valid measurements, utilization of

the direct distance and correction by the factor 0.8) (Boutouyrie & Vermeersch, 2010), and all measurements performed by one unique researcher allowed the reduction of errors, and simultaneously comparability with other studies.

The assessment of cardiac autonomic function by short recordings under laboratory conditions was the best methodological approach available according to our setting. For longitudinal data, participants were assessed in the same time of the day, avoiding variations from the circadian rhythm that potentially leads to differences in HRV measurement (Task et al., 1996).

Discussion of the Results

Physical activity encompasses a continuous broad range of intensities and energy expenditures, ranging from sedentariness up to extremely high exertion PA (very vigorous intensities) (Caspersen, Powell, & Christenson, 1985). It is well established that MVPA is associated with health benefits (Nocon et al., 2008; Paffenbarger, Hyde, Wing, & Hsieh, 1986). In agreement, our results from the cross-sectional study suggest that MVPA is associated with a better cardiac autonomic function. However, appropriate amounts of MVPA can co-exist with high quantities of sedentary time in the same individual. There is evidence that time spent in sedentary behaviors is inversely associated with health outcomes (Owen, Healy, Matthews, & Dunstan, 2010; Pate, O'Neill, & Lobelo, 2008; Patel et al., 2010), and its deleterious effect might not be nullified by accomplishing PA guidelines for MVPA (Chastin et al., 2015; Patel et al., 2010). Indeed, the data from the cross-sectional study we have conducted showed that MVPA *per se* is not a guarantee to prevent risk factors and their clustering, given that individuals accomplishing 30-minute day of MVPA also had metabolic syndrome. In addition, individuals with metabolic syndrome and more sedentary time, exhibit significantly higher arterial stiffness, regardless the amount of MVPA. It was already shown that individuals with metabolic syndrome have higher arterial stiffness (Scuteri et al, 2014; Scuteri et al, 2004). Our study therefore adds something novel that is the contribution of sedentary activity as risk factor and an independent predictor of cfPWV.

The relevant clinical and practical message from our cross-sectional studies is that public health policies and clinical practice, at the levels of primary health care, should emphasize the need to adhere and comply with MVPA recommendations, and concomitantly, making a strong appeal to the reduction of time spent in sedentary behaviors, as well as providing cues on how to operationalize this (Chastin et al., 2015; Healy et al., 2011; Patel et al., 2010).

From the cross-sectional studies, we have concluded that both sedentary time and MVPA are related with cardiovascular risk factors. We have therefore conducted an intervention study in individuals with moderate to high risk of CVD, to evaluate the effects of a health education and counselling program on the modification of daily PA levels. The secondary outcomes were arterial stiffness, and cardiac autonomic function.

Overall, the IG did not significantly change daily PA levels when compared to the CG. However, it is important to highlight that more participants from the IG started meeting the international PA guidelines, compared to CG (data not shown). Indeed, when we consider only those participants that had valid PA measurements at baseline, 4-months and follow up period, it was observed that five participants in the IG that did not meet the guidelines for PA at baseline, having reached this goal after 4-months intervention and sustaining this after the follow-up. In the CG, none of participants changed their PA, both after 4-months and follow-up. In addition, in the IG, five participants started at baseline with less than 30 minutes day of MVPA and have reached this minimal target for PA at the end of the follow-up period. Conversely, in the CG this was only observed for one participant. Having said that, and based on the data of the group*time interaction, we can't state that the intervention was effective to improve LPA and MVPA and to reduce significantly sedentary time, but we cannot disregard the clinical relevance of the observed trend for positive changes in the IG. Indeed, beyond the increased frequencies in the participants that have meet the PA guidelines at 4-months and/or at the end of the follow-up period, we also noticed that in the IG, along the period of the study, sedentary time was reduced on average in 7 minutes per day, which was possibly replaced by PA of any intensity. Therefore, given that PA has been identified as

protective for general health status (Paffenbarger et al., 1986; Paffenbarger et al., 1993), and for CVD (Nocon et al., 2008; Shiroma & Lee, 2010), it is possible that the improved PA profile in some of the participants might have diminished their global risk for disease.

A phenomenon observed in the intervention study was an increased interest of general practitioners and nurses over PA prescription, which might have to some extent, influenced the prescription of PA in their usual medical care appointments with participants allocated to the CG. We do not have control over this potential results confounder. Indeed, there is evidence that short counseling might impact PA behavior (Cochrane et al., 2012). However, if this happened and impacted somehow our results, the clinical implication is of interest and should be further investigated.

Regarding arterial stiffness and cardiac autonomic function, it is possible that the lack of changes in the intervention period and follow-up was due to minimal sedentary time reduction, and insufficient replacement by higher PA intensities. Despite that, is also possible that the length of time spent at higher PA levels, should be above 4-month to induce significant changes on arterial stiffness and cardiac autonomic function. Therefore, future intervention studies should be designed to appraise these research gaps.

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CHAPTER V

CONCLUSIONS

Conclusions

From this thesis, we draw the following conclusions:

In individuals with metabolic syndrome, sedentary time leads to significantly higher cfPWV. Sedentary time is also positively associated with cfPWV, independent of age and metabolic risk factors.

MVPA is inversely associated with LF/HF ratio.

A health education and counseling program conducted in primary health care setting did not promote significant group*time interaction in daily PA, arterial stiffness and cardiac autonomic function, both after the intervention and at the end of the 8-month follow-up period. However, in the IG group, minimal benefits were observed on reduction of sedentary time and increase in LPA and MVPA after four months, but this was reversed during the follow-up.

Health education and PA promotion for cardiovascular disease prevention, particularly in individuals with moderate to high cardiovascular risk, should target the reduction of sedentary behaviors and their replacement by any PA intensity above 1,5 METS, but ideally by MVPA (above 3,9 METS).