ANTÓNIO JOSÉ VIDEIRA DA SILVA ASCENSO

THE RELATIONSHIP BETWEEN SEDENTARY BEHAVIOR, PHYSICAL ACTIVITY AND CARDIORESPIRATORY FITNESS, WITH CAROTID INTIMA-MEDIA THICKNESS IN OBESE ADOLESCENTS

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Universidade Lusófona de Humanidades e Tecnologias

Faculdade de Educação Física e Desporto

Lisboa

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Dissertação apresentada para obtenção do grau de Mestre em Exercício e Bem-Estar no curso de mestrado em Exercício e Bem-Estar, conferido pela Universidade Lusófona de Humanidades e Tecnologias

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"Walking is man's best medicine"

Hippocrates (460-370 B.C.)

AGRADECIMENTOS

A realização deste trabalho só foi possível devido à colaboração, participação, compreensão e apoio, de inúmeras pessoas que de uma forma ou de outra, num determinado momento ou durante todo o processo, desempenharam um papel importante nesta etapa de formação académica e desenvolvimento pessoal.

Assim, ao Professor Doutor António Palmeira, os meus mais sinceros agradecimentos por ter aceite a orientação desta dissertação, bem como por ter acreditado no empenho e dedicação com a qual esta dissertação seria concebida.

À Professora Doutora Helena Fonseca agradeço o voto de confiança e o facto de ter viabilizado o presente estudo. Agradeço ainda a esta especial professora, o contacto com a Faculdade de Medicina da Universidade de Lisboa, essa tão reconhecida instituição.

À minha "nova" família agradeço o irrepreensível e incondicional apoio, não só neste processo como em todas as outras áreas e projectos pessoais.

À Professora Doutora Andreia Ascenso, docente da Faculdade de Farmácia da Universidade de Lisboa, e minha mulher, agradeço de todo o coração o facto de ter assumido, ainda que inconscientemente, um papel determinante na minha decisão de iniciar este percurso académico, que ambiciono não findar por aqui. A esta, agradeço ainda todo o apoio prestado na redacção desta dissertação.

Para concluír, a todos os colegas que participaram activamente nas avaliações e sessões do TOP (Liliana, Viviane, entre muitos outros), bem como aos participantes, que de forma voluntária se deslocaram às várias instalações onde o programa se realizou, deixo também um imenso agradecimento.

RESUMO

O Espessamento do complexo Intima-Média da artéria Carótida (EIM) é um indicador fiável do desenvolvimento aterosclerótico primário, estando associado à obesidade e inversamente associado à Aptidão Cardiorespiratória (ApCR). Desta forma, o Comportamento Sedentário, bem como a Actividade Física (AF) podem estar relacionados com o EIM.

O principal objectivo desta dissertação foi analizar a relação entre o Comportamento Sedentário, a AF, a ApCR e o EIM em adolescentes obesos, uma vez que estes podem representar o grupo pediátrico de maior risco de desenvolvimeto do EIM e aterosclerose primária.

Foram realizados, uma revisão sistemática de literatura e um estudo transversal de forma a analizar esta relação. Na revisão sistemática de literatura foram identificados estudos experimentais que se focassem a AF e o EIM. Para o estudo transversal foram recrutados 54 adolescentes obesos com idades compreendidas entre os 13 e os 17 anos. Os dados antropométricos, o Comportamento Sedentário, a AF, a ApCR (VO_2 max) e o EIM foram avaliados.

Na revisão sistemática de literatura foram incuídos seis estudos, num total de 313 participantes com idades entre os 7 e os 16 anos. Dos seis estudos incluídos, dois não reportaram melhorias no EIM após a intervenção, possivelmente devido à duração da intervenção ou à intensidade do exercício prescrito. Por outro lado, os grupos de controlo revelaram um aumento tendêncial do EIM. Adicionalmente, o estudo transversal mostrou uma correlação positiva entre o EIM e a AF leve ($r(38)$=.36, p=.024) , bem como com a AF moderada ($r(38)$=.37, p=.018), mas não com o tempo em Comportamento Sedentário. A ApCR mostrou-se inversamente correlacionada com o EIM ($r(40)$=-.36, p=.019).

O efeito benéfico da AF no EIM em adolescentes obesos parece ser fortemente moderado pela AptCR.

Palavras-chave: Comportamento Sedentário, Actividade Física, Aptidão Cardiorespiratória, Espessamento Intima-Média, Obesidade, Adolescentes.

SUMMARY

Carotid Intima-Media Thickness (CIMT) is a reliable marker of primary atherosclerosis associated with obesity, and inversely associated with Cardiorespiratory Fitness (CRF). Thus, Sedentary Behavior, as well as Physical Activity (PA) may be associated with CIMT.

The main objective of this dissertation was to analyze the association between Sedentary Behavior, PA, CRF and CIMT in obese adolescents, since these may represent the pediatric highest risk group of CIMT and primary atherosclerotic development.

A systematic review of the literature and a cross-sectional study were performed in order to analyze this association. In the systematic review, prospective studies were identified focusing in PA and CIMT. For the cross-sectional study were recruited 54 obese adolescents between 13 and 17 years old. Anthropometrics, Sedentary Behavior, PA, CRF (VO_2 max) and CIMT were assessed from the participants.

Six studies were included in the systematic review, on a total of 313 participants between 7 and 16 years old. Of the six included, two studies did not report CIMT improvement after the intervention, possibly due to the duration of the intervention or intensity of the exercise prescribed. On the other hand, control groups showed a trend to increase CIMT. In addition, the results in the cross-sectional study reported that Mean CIMT correlated positively with Light PA ($r(38)$=.36, p=.024) and Moderate PA ($r(38)$=.37, p=.018), but not with sedentary time. CRF showed to be inversely correlated with CIMT ($r(40)$=-.36, p=.019).

The beneficial effect of PA on CIMT in obese adolescents seems to be strongly moderated by CRF.

Keywords: Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness, Carotid Intima-Media Thickness, Obesity, Adolescent.

ABBREVIATIONS

BFM – Body Fat Mass

BMI – Body Mass Index

CCA – Common Carotid Artery

CIMT – Carotid Intima-Media Thickness

CRF – Cardiorespiratory Fitness

CRP – C-Reactive Protein

CV - Cardiovascular

CVD – Cardiovascular Disease

FFM – Fat-Free Mass

HDL-C – High Density Lipoprotein Cholesterol

IR – Insulin Resistance

LDL-C – Low Density Lipoprotein Cholesterol

LPA – Light Physical Activity

MPA – Moderate Physical Activity

MS – Metabolic Syndrome

MVPA – Moderate-to-Vigorous Physical Activity

NO – Nitric Oxide

PA – Physical Activity

SAT – Subcutaneous Adipose Tissue

VAT – Visceral Adipose Tissue

VPA – Vigorous Physical Activity

WC – Waist Circumference

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ORGANIZATION OF THE DISSERTATION

This dissertation is organized in four main chapters.

The first chapter, *Introduction*, makes a primary approach to childhood obesity and associated comorbidities. In this chapter we also provide an overview of the relationship between childhood obesity and Carotid intima-Media Thickness (CIMT) (chapter 1.1), and of those conditions with Sedentary Behavior, Physical Activity (PA) and Cardiorespiratory Fitness (CRF) (chapter 1.2).

In the second chapter, *Method*s, the methodology used to analyze the main purpose of this dissertation is briefly presented.

The third chapter, *Results*, is composed by two scientific articles submitted to international peer-reviewed journals. 3.1) The Relationship between Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness, with Carotid Intima-Media Thickness in Obese Children and Adolescents: A Systematic Review; 3.2) Physical Activity and Cardiorespiratory Fitness, but not Sedentary Behavior are associated with Carotid Intima-Media Thickness in Obese Adolescents.

In the last chapter, two sub-chapters are included, *Discussion* and *Conclusion*, which pretend to discuss and summarize all the information and knowledge about the relationship between Sedentary Behavior, PA and CRF with CIMT in obese adolescents. Intended future work is mentioned at the end of this chapter.

Chapter 1

Introduction

Chapter 1.1 – Carotid Intima-Media Thickness and Childhood Obesity

Chapter 1.2 – Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness, and Carotid Intima-Media Thickness

INTRODUCTION

Obesity is characterized by excessive fat accumulation in adipose tissue and other organs. Overweight and obesity among children and adolescents is spreading and is now considered an epidemic disease worldwide (Wang & Lobstein, 2006). In Portugal, 21.8% of children between 10 and 18 years old are overweight and another 9.9% are obese (Sardinha et al., 2011).

Childhood obesity is a health issue with many associated comorbidities. Obesity-related chronic disease, such as, high blood pressure, hyperlipidemia, and elevated insulin levels are common in obese children and adolescents (Deckelbaum & Williams, 2001; Woo et al., 2004b; Wunsch, de Sousa, Toschke, & Reinehr, 2006). These conditions lead to a poor health status during childhood that can be dragged into adulthood (Davis, Dawson, Riley, & Lauer, 2001; Eisenmann, 2004; Wright, Parker, Lamont, & Craft, 2001). Moreover, 80% of obese children and adolescents are in risk of becoming obese adults (Cali & Caprio, 2008).

Regarding the adverse metabolic outcomes of obesity, some authors associate pediatric obesity with Metabolic Syndrome (MS), suggesting that MS and its pathological mechanisms observed in adults are already operative in early ages (Huang, Zou, Yang, Chen, & Liang, 2010; Weiss et al., 2004). MS is defined as a cluster of components associated with Cardiovascular Disease (CVD), such as, high abdominal adiposity, atherogenic dyslipedemia, raised blood pressure, insulin resistance and/or glucose intolerance, pro-inflammatory and pro-thrombotic states (Grundy et al., 2004). In youngsters, the prevalence of MS increases directly with the obesity level (Weiss et al., 2004), thus the biomarkers of a potential risk of CVD are already observed in childhood.

Although genetic factors can affect body weight, obesity in childhood is mainly caused by "obesogenic" environment where the increase in energy intake is cluster to a decrease in energy expend, over a prolonged period (Carlson, Crespo, Sallis, Patterson, & Elder, 2012; Crocker & Yanovski, 2011; Deckelbaum & Williams, 2001). In fact, obese children and adolescents present lower levels of Physical Activity (PA), sports participation and Cardiorespiratory fitness (CRF) (Farpour-Lambert et al., 2009), as well as, higher levels of Sedentary Behavior compared to non-obese (Tremblay et al., 2011).

Furthermore, weight control in adolescence has additional importance in the prevention of many adverse health issues, once it represents the last critical period of adipocyte differentiation (Dietz, 1994), and there is no consensus about the unchangeably on the number of adipocytes thereafter (Daniels et al., 2005).

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Introduction

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Carotid Intima- Media Thickness and Childhood Obesity

Atherosclerosis is a chronic immunoinflammatory and fibroproliferative disease that occurs in medium and large size arteries and is characterized by a development of atheromatous plaques (Falk, 2006) by influence of oxidized Low-Density Lipoprotein (ox-LDL).

Carotid artery Intima- Media Thickness (CIMT) is an important endothelial structural parameter of an earlier stage of atherosclerosis (Davis et al., 2001), preceding the plaque formation, once it can predict myocardial infarction and stroke. In addition, CIMT is the only atherosclerotic marker from child to adulthood (Bauer et al., 2012). For the general population, an increment of 0,1mm in CIMT may be associated with an enhanced relative risk of 15% and 18% for myocardial infarction and stroke, respectively (Lorenz, Markus, Bots, Rosvall, & Sitzer, 2007). Furthermore, it is a reliable (Currie, Proudfoot, Timmons, & MacDonald, 2010), noninvasive and inexpensive method that can reflect atherosclerotic development in children and adolescents, even without major abnormalities of the classic Cardiovascular (CV) risk factors (Atabek, Pirgon, & Kivrak, 2007).

There is no consensus if CIMT increase linearly with age in healthy children and adolescents (Doyon et al., 2013; Jourdan et al., 2005; Lenard, Studinger, Mersich, Kocsis, & Kollai, 2004). However, similarly with MS, obese children (Giannini et al., 2009) and adolescents present increased CIMT, which correlates positively with relative Body Mass Index (BMI) (Beauloye et al., 2007; Doyon et al., 2013; Fang, Zhang, Luo, Yu, & Lv, 2010; Iannuzzi et al., 2004; Stabouli, Kotsis, Karagianni, Zakopoulos, & Konstantopoulos, 2012), and waist circumference (WC) (Elkiran et al., 2013; Hacihamdioğlu et al., 2011; Huang et al., 2010). In addition, CIMT is intensified in the presence of MS (Fang et al., 2010; Huang et al., 2010; Iannuzzi et al., 2004). Furthermore, adult CIMT has a stronger relation with obesity levels in childhood, than with other CV risk factors in the same ages, with the exception of LDL cholesterol (Davis et al., 2001; Freedman et al., 2008; Raitakari et al., 2003). Thus, childhood obesity seems to be the main source of those adverse mechanisms.

Increased CIMT may be explained by the combination of negative profile of MS components (high abdominal adiposity, dyslipedemia, raised blood pressure, insulin

resistance and pro-inflammatory state) and altered concentration levels of resistin and adiponectin observed in the obese state (Beauloye et al., 2007).

Abdominal adiposity is composed by subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). VAT seems to have most adverse and strongly relation to health than SAT. Research has already shown that VAT is associated with MS and CV risk factors in adults. In children the impact of VAT may be small, once it represents only 10% of abdominal adiposity, however this percentage tend to increase with age (Suliga, 2009). Thus, in adolescence, high proportion of VAT compared to SAT is associated with marked insulin resistance (Jiménez-Pavón et al., 2011) and triglycerides, and decreased HDL-C and adiponectin levels, showing a positive association of VAT with MS. In addition, the risk of MS development is five times higher in adolescents with VAT compared to SAT deposition (Cali & Caprio, 2008). Although WC is an undifferentiated measure of central adiposity, it can be a simple and effective way to estimate central adiposity in children, and it seem to be an independent risk factor of increased CIMT (Hacihamdioğlu et al., 2011). Furthermore, the measurement of WC may be more reliable than BMI for the prediction of an early stage of atherosclerosis (Elkiran et al., 2013).

Total cholesterol measured in early ages can predict CIMT in young adulthood (Davis et al., 2001). Moreover, high levels of LDL cholesterol are an independent risk factor of increased CIMT, by itself (Freedman et al., 2004; Raitakari et al., 2003).

Primary hypertension can result in vascular abnormalities in childhood, observed by an increment of CIMT. However, this relationship is often hidden by obesity (Lande, Carson, Roy, & Meagher, 2006; Stabouli et al., 2012), once higher blood pressure levels are associated with obesity, as well as with CIMT. Obesity seems to be, however, the only factor that correlates independently with CIMT in children and adolescents (Stabouli et al., 2012).

Insulin resistance (IR) is associated with obesity and can lead to other health complications, such as diabetes type 2, even in childhood. IR has an important role in early atherosclerosis development. There is no consensus, however, if it acts directly on the endothelium (Iannuzzi et al., 2004), or trough chronic inflammation and oxidative stress mechanisms, that are also present in obese children and adolescents (Giannini et al., 2009; Giannini et al., 2008). Furthermore, IR can impair the impact of PA on CIMT

in obese children, even on the presence of similar weigh reductions (Sanches et al., 2012).

Inflammation might affect endothelium function, promoting CIMT (Kelishadi, Hashemi, Mohammadifard, Asgary, & Khavarian, 2008; Meyer, Kundt, Steiner, Schuff-Werner, & Kienast, 2006). The degree of inflammation can be assessed by pro-inflammatory markers, such as Tumor Necrosis Factor-α (TNF- α), Isoleucine-6 (IL-6), C-reactive protein (CRP) or fibrogen. Those pro-inflammatory markers are increased in obese population associated with the amount of adipose tissue and more specific to central adiposity (Balagopal et al., 2005; Visser, Bouter, McQuillan, Wener, & Harris, 2001). Of all inflammation markers, CRP show, however, to has the most reliable association with increased CIMT (Elkiran et al., 2013; Roh, Lim, Ko, & Cheon, 2007) Body Fat Mass (BFM), VAT and CRF in children and adolescents (Barbeau et al., 2002).

Adiponectin and resistin are cytokines (cell signaling proteins) secreted by adipose tissue – adipokines. It seems that resistin does not have an independent effect on CIMT but only in inflammation (Kunnari, Ukkola, Päivänsalo, & Kesäniemi, 2006). Adiponectin has, on the other hand, insulin-sensitizing and antiatherogenic properties (Grohmann et al., 2005). The levels of adiponectin are negatively correlated with obesity (Beauloye et al., 2007; Grohmann et al., 2005), insulin resistance and triglycerides (Beauloye et al., 2007). Furthermore, adiponectin is also negatively associated with CIMT, suggesting that adiponectin may decrease the expression of adhesion molecules on endothelial cells, suppress foam cell formation by the macrophages and inhibit smooth cell migration, as was already showed in vitro studies (Pilz et al., 2005).

Regardless obesity and MS being a cluster of CV risk factors with mechanisms related to increased CIMT, adiponectin (Pilz et al., 2005), triglycerides levels, IR and VAT seems to be, however, the strongest predictors of CIMT (Fang et al., 2010).

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Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness, and Carotid Intima- Media Thickness

Sedentary Behavior may be defined as any waking behavior, where the bodily movement is minimal, and in which the energy expenditure is ≤ 1,5 METs (Sedentary Behaviour Research Network, 2012). Sedentary Behavior should not be understood, however, as the opposite of PA, once each Sedentary Behavior may have a different impact in health (Dietz, 1996). Screen time is one type of Sedentary Behavior, such as watching television and videos, playing videogames and working on the computer, and is known to be the behavior in which youngsters spend more time. Moreover, increased screen time is associated with worse health-related outcomes, mainly in body composition and CRF, due to, not only, the replacement of PA for Sedentary Behavior, but also to a decrease in basal metabolism and increase of energy intake (Burke, 2006). Furthermore, some authors (Martinez-Gomez et al., 2010) report a positively association of excessive TV viewing with some CV risk factors, independently of body weight. In addition, sedentary time represents the largest portion of youngsters day (up to nine hours), compared with PA (Tremblay et al., 2011).

PA may be defined as any bodily movement produced by the contraction of skeletal muscle that increases energy expenditure. The amount of energy expended depends on the muscle mass used in the activity, duration, intensity and frequency of the muscular contractions. On the other hand, exercise is one type of PA that is planned, structured, repetitive and implies the objective of improving or maintaining one or more components of physical fitness (Caspersen, Powell, & Christenson, 1985).

CRF is one of five components of physical fitness related to health. The others are muscular endurance, muscular strength, body composition and flexibility. CRF represents the ability to uptake, deliver, and use oxygen to produce energy, and is expressed in maximal oxygen consumption (VO_2max) (Caspersen et al., 1985).

In fact, CRF is inversely correlated with WC and BMI (Ortega et al., 2007). Rather, Sedentary Behavior is associated with higher levels of WC, and BMI (Ortega et al., 2007), and lower levels of CRF (Ruiz, Ortega, Warnberg, & Sjöström, 2007) and HDL-C (Byun, Dowda, & Pate, 2012). On the other hand, lower levels of CRF are associated with morphologic and biochemical parameters reflecting early stage of

atherosclerotic process (Meyer, Kundt, Lenschow, Schuff-Werner, & Kienast, 2006). Furthermore, adolescent lower CRF is associated with adult body fatness. On the contrary, a better CRF is inversely associated with adult body fatness. In turn, adolescent body fatness is moderately associated with adult CV risk factors (Eisenmann, 2004) and increased CIMT (Oren et al., 2003). This relationship is more strongly when obesity levels remain or increases from adolescence to adulthood (Oren et al., 2003). In addition, improvements in CRF from adolescence to adulthood seem to have a positive impact on arterial stiffness (Ferreira, Twisk, Stehouwer, van Mechelen, & Kemper, 2003) (Fig.1).

It is well known that acute and chronic PA has a beneficial effect on CV risk factors. In healthy population this benefit is extensible to endothelial function and structure (Pahkala et al., 2011) and in obese subjects this beneficial effect may be raised.

In general, PA programs with obese children and adolescents indicates beneficial effects of exercise on body composition (reduction on whole body and abdominal fat, and an increase of body fat-free mass), insulin resistance, blood pressure, lipid profile, and CRF (Chae et al., 2010; Farpour-Lambert et al., 2009; Sanches et al., 2012; Thijssen, Cable, & Green, 2012). These effects are evident even without major changes in weight or BMI (Balagopal et al., 2005; Kelishadi, Hashemi, et al., 2008). Furthermore, regular PA may increase the activity of antioxidant enzymes and reduce the levels of pro-inflammatory markers (Kelishadi, Hashemi, et al., 2008; Wilund, 2007), as well as increase adiponectin levels (Sanches et al., 2012), which can be explained by its association with CRF (Ruiz et al., 2007) and major weight losses (Masquio et al., 2013; Sanches et al., 2012), respectively. In addition, CRF may be associated with an increase of endothelial progenitor cells (Arnold, Wenta, Müller-Ehmsen, Sreeram, & Graf, 2010), and acute exercise with a increase of NO availability (Green, Maiorana, O'Driscoll, & Taylor, 2004), which represents functional improvements of the endothelium . On the other hand, Sedentary Behavior seems to be the ignition point of many health problems, even in early ages, being obesity, usually the first of those.

Thus, there may be a possible inverse correlation between PA/CRF and CIMT, therefore lifestyle interventions, focusing in PA may be the main nonpharmacological

way to reverse or delay CIMT development (Balagopal et al., 2005; Kelishadi, Hashemi, et al., 2008).

The main purpose of this dissertation is to analyze the association between Sedentary Behavior, PA and CRF with CIMT in obese adolescents, a high risk group of primary atherosclerotic development.

Figure 1 may represent the objective of this dissertation.

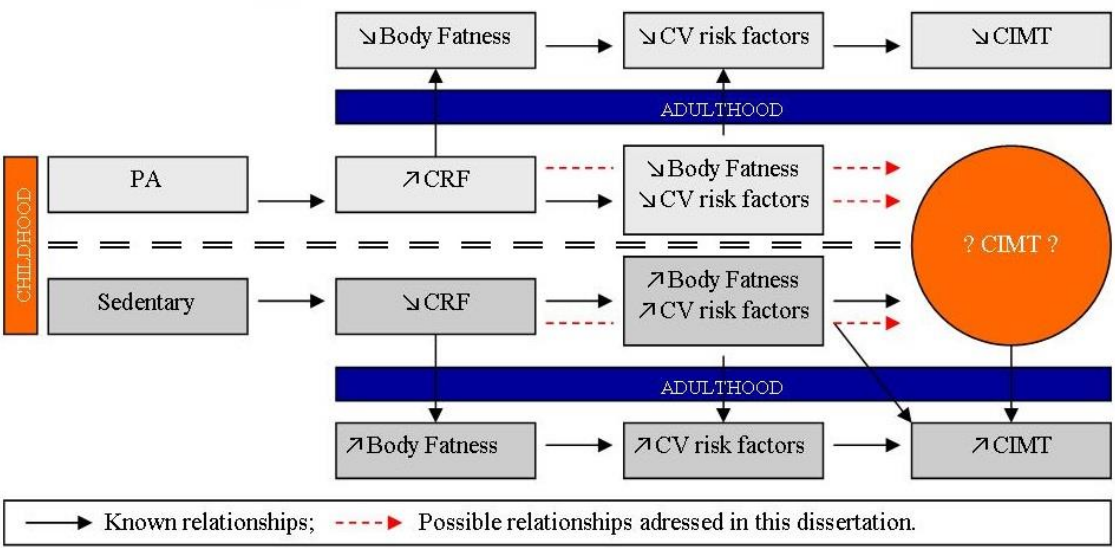

Figure 1. Relationship between PA and Sedentary Behavior during childhood, and CRF, body fatness, CV risk factors and CIMT in childhood and adulthood.

Chapter 2

Methods

METHODS

In order to analyze the association between Sedentary Behavior, PA, CRF and CIMT, two manuscripts were elaborated.

Firstly, a systematic review of the existing literature was performed including all the prospective studies published since 2000, from PubMed, Google scholar, and SportDiscus databases. This allowed the collection and analysis of the published knowledge subjected on this dissertation. The PRISMA statement for reporting systematic reviews was used in the process (Chan et al., 2013).

Then, a cross-sectional study in obese adolescents recruited to the 12-months intervention program TOP (Treatment of Pediatric Obesity) was elaborated. In order to ensure study methodological quality, recommendations for interventional trials were adjusted and used (Liberati et al., 2009).

Due to methodological heterogeneity of the performed manuscripts, methods are specified and described in each paper.

Chapter 3

Results

Chapter 3.1 – The Relationship between Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness, with Carotid Intima-Media Thickness in Obese Children and Adolescents: A Systematic Review

Chapter 3.2 – Physical Activity and Cardiorespiratory Fitness, but not Sedentary Behavior are associated with Carotid Intima-Media Thickness in Obese Adolescents

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The Relationship between Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness, with Carotid Intima-Media Thickness in Obese Children and Adolescents: A Systematic Review

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The present study was submitted to European Journal of Pediatrics
The original format was not maintained in order to satisfy University format recommendations

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ABSTRACT

Carotid Intima-Media Thickness (CIMT) may represent a primary inflammatory process, preceding the atheromatous plaque formation, which can be explained by the combination of negative profile of Metabolic Syndrome (MS) and obesity. CIMT is already observed in obese children and adolescents, especially in the presence of high levels of Sedentary Behavior and impaired Cardiorespiratory Fitness (CRF). On the other hand, Physical Activity (PA) is known to have a beneficial effect on CRF, MS and obesity, suggesting an inverse correlation with CIMT.

Thus, we aimed to analyze the associations between Sedentary Behavior, PA and CRF, with CIMT in obese children and adolescents.

For this systematic review relevant studies were identified, published since 2000, from PubMed, Google scholar and SportDiscus databases, searched between December 2013 and February 2014. The search strategy and inclusion criteria followed the PICOS approach. Were included prospective studies focusing in PA, and reporting CIMT development. The methodological quality of the studies included was assessed using the levels of evidence of the Oxford Center for Evidence-Based Medicine.

Six studies were included. The age of the participants ranged from 7 to 16 years old, on a total of 313 participants. The duration of the interventions ranged from 6 weeks to 12 months. Almost all studies presented information about type, frequency, time and intensity of exercise. Improvements in CRF, CIMT and Metabolic Syndrome (MS) /Cardiovascular (CV) risk factors were reported.

Conclusion: The beneficial effect of PA on CIMT seems to be moderated by CRF. Moreover, 240 minutes/week (three/80 minutes sessions) of Moderate-to-Vigorous Physical Activity during three months seem to be enough to improve CIMT, independent of the type of PA.

Keywords: Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness, Carotid Intima-Media Thickness, Childhood Obesity.

INTRODUCTION

Obesity is one of the greatest health concerns of the 21st century (World Health Organization, 2004). Excessive fat accumulation in adipose tissue and other organs starts in early ages. Therefore, obesity-related adverse metabolic outcomes, such as Metabolic syndrome (MS) and its comorbidities are already operative in children and adolescents (Huang et al., 2010; Weiss et al., 2004). Thus, it is of major importance to analyze the pathological mechanisms related to obesity and MS, which in turn are associated with CIMT (Fang et al., 2010; Iannuzzi et al., 2004; Stabouli et al., 2012; Woo et al., 2004b), an important vascular parameter of an earlier stage of atherosclerosis (Davis et al., 2001).

Increased CIMT may correspond to an impairment of the structural endothelial properties preceding the atheromatous plaque formation (Bauer et al., 2012). Furthermore, CIMT is the only atherosclerotic marker up to adulthood, being also, a reliable (Currie et al., 2010), noninvasive and inexpensive method that can reflect atherosclerotic development, before emergence of major abnormalities of the classic Cardiovascular (CV) risk factors (Atabek et al., 2007).

Sedentary Behavior is associated with increased Body Mass Index (BMI) (Mitchell, Pate, Beets, & Nader, 2013), whole body fat and abdominal adiposity (Ortega et al., 2007), which may be explained by a decrease in basal metabolism (Burke, 2006) and in Cardiorespiratory Fitness (CRF), a well-known parameter of physical fitness associated to health (Ortega, Ruiz, Castillo, & Sjöström, 2008). In addition, lower levels of CRF are associated with morphologic and biochemical conditions that may reflect a primary atherosclerotic development (Meyer, Kundt, Steiner, et al., 2006).

On the other hand, higher levels of CRF may compensate the adverse consequences of obesity (Ortega et al., 2008), even regarding CIMT development. This effect is however evident even without major changes in Weight or BMI (Balagopal et al., 2005; Kelishadi, Hashemi, et al., 2008). Endothelial functional improvements associated to an increase in endothelial progenitor cells (Arnold et al., 2010), and to a increase of Nitric Oxide (NO) availability (Green et al., 2004) may explain this mechanism.

To our knowledge, this is the first systematic review which aimed to analyze the associations between Sedentary Behavior, PA and CRF on CIMT in obese children and adolescents.

METHODS

Databases and Search Strategies

PubMed, Google Scholar and SportDiscus databases were searched between December 2013 and February 2014. The keywords "obesity" (obes*) and "physical activity" or "exercise" or "sedentary" (sedentar*) and "cardiorespiratory fitness" or "cardiorespiratory capacity" and "carotid intima-media thickness" were searched on titles and abstracts from articles published since the year 2000.

The search strategy and inclusion criteria followed the PICOS (Population/Patient Problem, Intervention, Comparison, Outcomes, and Study Design) approach (Liberati et al., 2009) (Figure 2). For this search, population was defined as obese children or adolescents. To be included, the intervention protocols must have any type of PA on the design. However, because few studies were found with interventions based only on PA, we included multidisciplinary interventions, such as nutritional counseling or behavioral therapy approaches. Our controls were obese children or adolescents, who underwent CIMT evaluation, without participating in the PA intervention. Main outcomes were defined as CIMT and CRF. Nonetheless we report all health and MS-related outcomes from the studies included in this review, as secondary outcomes. In addition, only studies published in English in peer-reviewed journals were included.

Data extraction

Data from each paper, such as study design, participant characteristics (number, mean age and mean BMI), intervention characteristics (strategy and duration), information specific-related to PA intervention protocol and outcomes, were extracted. Descriptive data and characteristics of PA protocol (type, frequency, time and intensity) were emphasized as well as data from the outcomes (main and secondary outcomes).

Inclusion/exclusion criteria

The first inclusion criteria for this systematic review included (i) pediatric obese participants in school ages. A second criteria was (ii) PA being part of the study protocol, even if was not the only intervention strategy. In our original search we did not included studies with multidisciplinary strategies. In result, we only found two studies to review, so,

our next step was to extend the inclusion criteria to interventions that had nutrition approaches as well. Only studies with (iii) measures of CIMT development fit in the inclusion criteria. At last, only (iv) prospective studies were accepted.

Quality assessment

The methodological quality, of all included studies was assessed using the levels of evidence of the Oxford Center for Evidence-Based Medicine (OCEBM, 2011).

Population / Patient Problem	Obese children or Adolescents
Intervention	Physical activity
Comparison / Control	Sedentary (without increment of physical activity)
Outcomes	Carotid intima-media thickness and Cardiorespiratory fitness
Study Design	Prospective studies

Figure 2. PICOS framework.

RESULTS

Were identified 514 references. Of these, 493 did not meet the inclusion criteria. The full text of the remaining articles (21) was analyzed. We excluded 17 cross-sectional studies, one review article including all ages, and one study that did not present measures of CIMT. Furthermore, four more articles were included by manual search (Figure 3).
Characteristics and intervention outcomes of the included studies are summarized in Table 1.

Study design

Three studies included in this systematic review are Randomized Controlled Trials (RCT) with the participants (obese children or adolescents) divided into an exercise-only intervention group or a non-exercise control group (Farpour-Lambert et al., 2009; Meyer, Kundt, Lenschow, et al., 2006; Park et al., 2012). Of these studies, the first two recruited a lean control group for baseline comparison. Two other studies included are Randomized Trials (RT), with the participants randomized to a diet plus exercise or a diet-only intervention groups (Woo et al., 2004a); and with the participants randomized to exercise and diet (Kelishadi, Hashemi, et al., 2008). The other study included is a Control Trial (CT) using a lean control group at baseline, and data from a relevant interventional study (Wunsch et al., 2006).

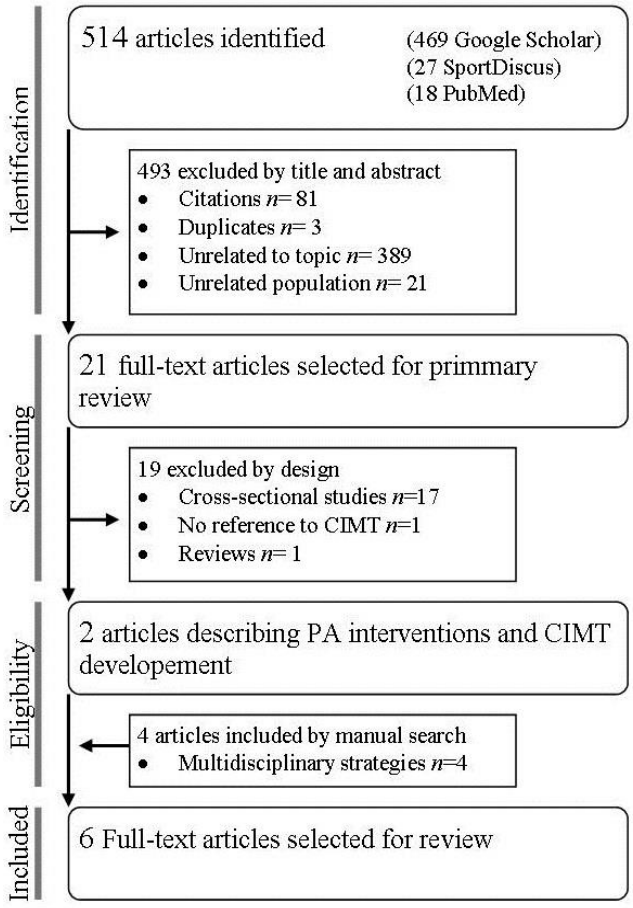

Figure 3. Flow diagram of the selection process.

Sample size and recruitment

Samples size of the included studies ranged from 29 to 82 participants (52±30). Only two studies report power calculations to detect statistically significant differences at $p < 0.05$ on blood pressure (Farpour-Lambert et al., 2009), and endothelial progenitor cells (Park et al., 2012). Both recruited the intended number of participants. Two studies recruit their participants in health institutions (children's hospital and healthcare centers) (Farpour-Lambert et al., 2009; Meyer, Kundt, Lenschow, et al., 2006). Two other studies conducted their recruitment in schools (Park et al., 2012; Woo et al., 2004a). In only one study, recruitment was made in both health institutions and schools (Kelishadi, Hashemi, et al., 2008; Wunsch et al., 2006). Finally, in one study, a sample of children who participated in one-year obesity intervention program was used (Wunsch et al., 2006).

Participants

The age of the participants ranged from 7 to 16 years old (11.4±5.5), on a total of 313 participants. Only the studies of Farpour-Lambert et al. (2009) and Woo et al. (2004a) refer the pubertal stage of the participants at baseline (<1 and ≤2 Tanner stage, respectively). All the studies recruited girls and boys, and there were no reported significant sex differences between groups in all studies. Although the studies did not use the same criteria to define obesity, almost all the participants recruited were obese. Indeed, only one study (Woo et al., 2004a) recruited also overweight children (BMI, 21-23) (Ogden et al., 2002), however the mean BMI did not differ from the other studies (25±3.0) (Table 1).

Control groups

Four studies, of the six reviewed, used control groups (Farpour-Lambert et al., 2009; Meyer, Kundt, Lenschow, et al., 2006; Park et al., 2012; Wunsch et al., 2006). One used two different intervention groups for comparison (Woo et al., 2004a), and another used neither a control nor a comparison group (Kelishadi, Hashemi, et al., 2008). There were no significant differences in BMI, Age or Sex between control and intervention groups, in those studies. Only one study recruited a control group with significant differences in number (C n=10/I n=56) for baseline comparison (Wunsch et al., 2006). Furthermore, two studies (Farpour-Lambert et al., 2009; Meyer, Kundt, Lenschow, et al., 2006) recruited two control groups, one of lean subjects for baseline comparison, and another of obese children for

comparison after the intervention. In both studies the control group was asked to maintain the current level of PA during the intervention; only in Meyer et al. (2006) both control and intervention groups were instructed to participate in one consultation about healthy nutrition with a nutritionist.

Interventions

Two, of the six reviewed articles, used exercise as the only intervention strategy (Farpour-Lambert et al., 2009; Park et al., 2012). Meyer et al (2006) used a similar exercise strategy with the addition of nutritional counseling. Two studies (Kelishadi, Hashemi, et al., 2008; Woo et al., 2004a) followed exercise and diet advice as intervention strategy, and another study (Wunsch et al., 2006) added behavior therapy to exercise and diet. The intervention period of the studies ranged from six weeks to twelve months. In two studies (Farpour-Lambert et al., 2009; Woo et al., 2004a), modified designs from the beginning to the end of the study were used. Farpour-Lambert et al (2009) used a three months intervention protocol followed by other three months in which the control group was asked to participate. Woo et al (2004a) prescribed a six weeks intervention before dividing the intervention group into those stopping exercise or continuing exercise until twelve months.
References to type, frequency, time and intensity of the exercise protocol were collected from all studies, except two, which did not report intensity (Meyer, Kundt, Lenschow, et al., 2006; Wunsch et al., 2006) or time (Wunsch et al., 2006). Three studies (Kelishadi, Hashemi, et al., 2008; Meyer, Kundt, Lenschow, et al., 2006; Wunsch et al., 2006) used protocols of multi-activities, such as fitness, sport or ball games, running or walking, swimming and/or trampoline. The other three (Farpour-Lambert et al., 2009; Park et al., 2012; Woo et al., 2004a) used a combined exercise protocol (aerobic and resistance training) allowing more objective measurement of intensity. Intervention groups from half of the studies (Farpour-Lambert et al., 2009; Kelishadi, Hashemi, et al., 2008; Meyer, Kundt, Lenschow, et al., 2006) performed exercise three times/week (beyond physical education in school) for 60 minutes. In Park et al. (2012), the exercise protocol contemplated three 80 minutes sessions/week. Only Woo et al. (2004a) and Wunsh et al. (2006) intervention groups performed exercise two 75 minutes times/week, and one time/week, respectively. In the two studies with modified designs (Farpour-Lambert et al., 2009; Woo et al., 2004a), the frequency decreased (-1 session/week) from first to the second phase of the intervention. As written previously, only three studies reported objective measures of exercise intensity using

different parameters, 55-65% VO_2 max, 50-70% Heart Rate Reserve (HHR), and 60-70% of predicted Maximum Heart Rate (Max HR) , respectively. One study reports a subjective intensity measure (Kelishadi, Hashemi, et al., 2008). Normalizing to a subjective scale of PA intensity, two studies used Light-to-Moderate Physical Activity (LMPA) intensities (Farpour-Lambert et al., 2009; Woo et al., 2004a) and two others used MVPA intensities (Kelishadi, Hashemi, et al., 2008; Park et al., 2012).

Main outcomes

Carotid Intima-Media Thickness

One study used both Mean and Maximum values of CIMT measured at the far and near walls of the Common Carotid Artery (CCA) and Carotid Bifurcation segments for statistical purposes (Meyer, Kundt, Lenschow, et al., 2006; Wunsch et al., 2006). Another study used only Maximum values of CIMT (Wunsch et al., 2006), and all the others used Mean values for statistical purposes. To the exception of Meyer et al. (2006), and Park et al. (2012) who did not specify the measured segment, all the other studies performed the CIMT measurement at the far walls of the CCA.

Only two studies did not report statistically significant improvements in CIMT after the intervention (Farpour-Lambert et al., 2009; Kelishadi, Hashemi, et al., 2008). One study reported Mean CIMT decrease (-0.04 mm) at 3 months (Park et al., 2012). All the other studies report similar results up 6 months (Meyer et al, 2006 [Mean CIMT -0.04 mm] month 6^{th}; Woo et al, 2004 [Mean CIMT -0.02 mm] month 12^{th}; Wunsch et al, 2006 [Max CIMT -0.07 mm] month 12^{th}). Differences between intervention and control groups were reported at the end of intervention. In all cases intervention groups showed to have lower CIMT than the controls (Farpour-Lambert et al.; Meyer, Kundt, Lenschow, et al., 2006; Park et al., 2012).

Table 1. Characteristics and outcomes of the studies that evaluated exercise interventions on CIMT, CRF, and other health-related indicators in obese children and adolescents.

Study	Design	Participants			Intervention		PA intervention				Intervention Outcomes		
		n	Age	BMI	Strategy	Duration	Type	Frequency	Time	Intensity	CIMT	CRF	Secondary
Farpour-Lambert, N. et al (2009)	RCT	44 (Exerc *n* =22) (Control *n* =22)	8.9 (±1.5)	25.2 (±4.6)	Exercise	3 months	Combined exercise	3 times /week	60 min (30min Aerobic, 20min Strengh, 10min Cool down)	55-65% VO_2 max (LMPA)	NS Mean CIMT	(1.9 ml/kg/min) VO_2 max	BMI z-score↘ ABDfat↘ BFM↘ TC↘ HDL-C↘ LDL-C↘ SBP↘ DBP↘
		(BCG *n* =22)				(plus 3 months both groups)		(2 times /week)			NS Mean CIMT	(2.8 ml/kg/min) VO_2 max	MBI z-score↘ BFM↘ HDL-C↗ Glucose↘ IR↘
Kelishadi, R. et al (2008)	RT	35 (No control Group)	14.1 (±2.2)	25.3 (±4.06)	Exercise and diet	6 weeks	Multisports (fitness, sport games and running)	3 times /week	60 min (30min fitness 30min games and running)	MVPA	NS Mean CIMT	NR	Weight↘ BMI↘ BMIsd↘ WC↘ W/Hip ratio↘ BFM↘ TC↘ LDL-C↘ ox-LDL↘ IR↘ C-RP↘
Meyer, A. et al (2006)	RCT	67 (Exerc *n* =33) (Control *n* =34) (BCG *n* =35)	14.7 (±2.2)	29.8 (±5.93)	Exercise and nutritional counseling	6 months	Multisports (swimming, sport games, or walking)	3 times /week	60min (swimming/walking or 90min games)	NR	(-0.04 mm) Mean CIMT (-0.05 mm) Max CIMT	(0.32 w/min) Wmax	BMI↘ BMIsd↘ W/Hip ratio↘ LDL↘ TG↘ SBP↘ IR↘ Insulin↘ C-RP↘ Fibrogen↘
Park, J. et al (2012)	RCT	29 (Exerc *n* =15) (Control *n* =14)	12.2 (±0.1)	24.4 (±0.6)	Exercise	3 months (12 Week)	Combined exercise	3 times /week	80 min (10min Warmup, 30min Aerobic, 30min Strengh, 10min Cool down)	50-70% HRR (MVPA)	(-0.04mm) Mean IMT	(3.7 ml/kg/min) VO_2 max	BMI↘ WC↘ e-Selectin↘ NO↗
Woo, K. et al (2004)	RT	82 (DE *n* =41) (D *n* =41)	9.9 (±1.0)	25.0 (±3.0)	Exercise and diet	6 weeks	Combined exercise	2 times /week	75 min (10min Warmup, 30min Strengh, 10min Aerobic, 10min Agility, 5min Cool down)	60-70% Max HR (LMPA)	NS Mean CIMT	NR	W/Hip ratio↘ TC↘ LDL-C↘ Glucose↘
						untill 12 months for half of the ED group (*n* = 21)		1 time /week			(-0.02 mm) Mean CIMT	NR	BFM↘ LDL-C↘ HDL-C↗ LDL/HDL ratio↘
Wunsch, R. et al (2006)	CT	56 (BCG *n* =10)	8.7 (±0.4)	24.8 (±0.7)	Exercise diet and behavioral therapy	12 months	Multisports (ball games, jogging and trampoline)	1 time /week	NR	NR	Only in substantial weight losses (-0.7 BMI SD) *n* =24 (-0.07 mm) Max CIMT	NR	BMI↘ BMIsd↘ HDL-C↗ TG↘ SBP↘ DBP↘ IR↘ Insulin↘

ABD fat- Abdominal fat; BCG- Baseline Control Group; BFM- Body Fat Mass; BMI- Body Mass Index; C-RP- C-Reactive Protein; CT- Controlled Trial; D- Diet only; DE- Diet and Exercise; DBP- Diastolic Blood Pressure; HDL- High Density Lipoprotein; HRR- Heart Rate Reserve; IR- Insulin Resistance; LDL- Low Density Lipoprotein; LMPA- Light-to-Moderate Physical Activity; Max HR- Predicted Maximum Heart Rate; MVPA- Moderate-to-Vigorous Physical Activity; NR- No Reference; NS- No statistical Significant; NO - Nitric Oxide; ox-LDL- Oxidized Low Density Lipoprotein; RCT- Randomized Control Trial; RT- Randomized Trial; SBS- Systolic Blood Pressure; TC- Total Cholesterol; TG- Triglycerides; WC- Waist Circunference; W/Hip ratio- Waist/Hip ratio.

Cardiorespiratory fitness

All the studies who measured CRF, trough VO_2 max (Farpour-Lambert et al., 2009; Park et al., 2012), or exercise capacity, by W max (Meyer, Kundt, Lenschow, et al., 2006), reported improvements on these parameters. In VO_2 max the improvements ranged from 1.9 to 3.7 ml/kg/min both using direct gas analysis. Furthermore, Farpour-Lambert et al (2009) reported an increment in VO_2 max (0.9 ml/kg/min) from the first to the second stage of the intervention. The study which evaluated exercise capacity with W max reported an increase of 0.32 W/kg. On the contrary, all the control groups showed a stabilization (Meyer, Kundt, Lenschow, et al., 2006) or a decrease on CRF (Farpour-Lambert et al., 2009; Park et al., 2012).

Secondary outcomes

Metabolic syndrome

All studies, except one (Woo et al., 2004a), reported significant improvements in BMI or BMI z-score. Although Woo and colleagues (2004a) did not report improvements in BMI, they presented a decrease in percentage of Body Fat Mass (BFM) at 12 months. Identical results were presented by other authors from all the reviewed studies, who measured BFM. The decrease on BFM is achieved at different moments in all those studies (Kelishadi et al. – week 6th; Farpour-Lambert et al. – month 3rd; Meyer et al. – month 6th; Woo et al. – month 12th). The decrease of abdominal adiposity (-2.1±2.5 %) is reported, by Dual-energy X-ray Absorptiometry (DXA) in just one study (Farpour-Lambert et al., 2009). Four other studies, only report estimated measures of central adiposity, such as WC or W/Hip ratio. Kelishadi et al. (2008), and Park et al (2012) presented improvements in WC of -2.2 and -2.6 cm, respectively. Similar results are presented in W/Hip ratio (-0.05; -0.05; -0.03) by Kelishadi et al, (2008), Meyer et al (2006) and Woo et al (2004a). Only one study did not measure or estimate abdominal or central adiposity (Wunsch et al., 2006).

Total Cholesterol (TC) decreased after intervention, in almost all studies which presented statistical analysis of this parameter (Farpour-Lambert et al., 2009; Kelishadi, Hashemi, et al., 2008; Woo et al., 2004a). The improvements ranged from -0.2 to -1.1 mmol/L (-0.51±0.5). All these studies, also, reported the same trend for Low Density Lipoprotein Cholesterol (LDL-C) (-0.18 mmol/L; -0.4 mmol/L; -0.3 mmol/L). In addition, Meyer et al (2006) reported similar results on LDL-C (-0.14 mmol/L). Farpour-Lambert et al

(2009) reported a statistically significant decrease in High Density Lipoprotein Cholesterol (HDL-C) (-0.06 mmol/L) at 3 months. Contradictory results are reported however, by Woo et al (2004a) and Wunsch et al (2006) with improvements at 12 months of 0.2 and 4.0 mmol /L, respectively on HDL-C. Furthermore, the same author (Farpour-Lambert et al., 2009) reported improvements in HDL-C in the control group after they underwent exercise training for 3 months. Only one study (Park et al., 2012) did not report any statistically difference in cholesterol parameters after intervention, within or between groups. Additionally, one study (Kelishadi, Hashemi, et al., 2008) also presented results of intervention on ox-LDL (-3.3 U/L).

A decrease in Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) is reported in two studies (Farpour-Lambert et al., 2009; Wunsch et al., 2006). Meyer et al (2006) also reported a decrease in SBP, but did not present the results of DBP. SBP improvements ranged from -1.9 to -8.0 mm Hg (5.97±2.03) and DBP from -1.3 to -7 mm Hg. Furthermore, two studies did not verify any significant differences in SBP or DBP (Kelishadi, Hashemi, et al., 2008; Park et al., 2012), and another study (Woo et al., 2004a) did not present any results of those parameters.

Insulin resistance, evaluated by Homeostasis Model Assessment (HOMA-IR), decrease in four, of the six reviewed articles (Farpour-Lambert et al., 2009; Kelishadi, Hashemi, et al., 2008; Meyer, Kundt, Lenschow, et al., 2006; Wunsch et al., 2006), with the outcomes ranging from -0.82 to -1.6 (-1.15±0.45). Additionally, Farpour-Lambert et al (2009) (at six months) and Woo et al (2004a) reported improvements in glucose metabolism, ranging from -0.2 to -0.4 mmol/L. Improvements in insulin metabolism (-8.0 mmol/L) are also reported by Wunsch et al (2006).

Pro-inflammatory markers, such as C-RP, fibrogen or e-selectin were analyzed in only three studies. Both, Kelishadi et al (2008) and Meyer et al (2006) reported a decrease in C-RP of -0.29 and -2.79 mg/L. Meyer et al (2006) also reported improvements in Fibrogen of -0.33. In addition, Park et al (2012) reported a decrease in e-selectin of -1.0 mg/L within group and -4.0 mg/L between groups.

Evaluation of quality assessment

None of the studies included meet the Level 1 of evidence-based medicine. Four, of the six, studies had a significant effect of the intervention on CIMT, meeting the 2nd level of evidence (Meyer, Kundt, Lenschow, et al., 2006; Park et al., 2012; Woo et al., 2004a;

Wunsch et al., 2006). The two other meet the level 3 (Farpour-Lambert et al., 2009; Kelishadi, Hashemi, et al., 2008).

DISCUSSION

Time spent in Sedentary Behavior represents the largest portion of waking time among children and, more significantly, among adolescents (Brodersen, Steptoe, Boniface, & Wardle, 2007; Tremblay et al., 2011). This is associated with a decrease in PA. The created energy imbalance may lead to obesity, and obesity leads to all the others CV risk factors (Brodersen et al., 2007; Carson & Janssen, 2011). In addition, the absence of MVPA may lead to a decrease in CRF. The combination of these two factors represents the worse metabolic profile (Eisenmann, Welk, Ihmels, & Dollman, 2007), and may aggravate CIMT development.

In accordance to the present review, and despite the heterogeneity of studies characteristics, PA seems to have a beneficial effect on the development of CIMT in obese children and adolescents. It is not possible, however, to make direct associations with type, frequency, time or intensity of the PA.

In our review two studies did not achieve statistically significant improvements in CIMT (Farpour-Lambert et al., 2009; Kelishadi, Hashemi, et al., 2008). Regarding the study of Kelishadi et al. (2008),we suggest two possible reasons for this fact. One is from statistical nature, since these authors used Mean CIMT for statistical purposes, which is not consensual. Maximum CIMT may reflect a more advanced stage of thickness (Touboul et al., 2007), therefore, it may be more sensitive to change (Stein et al., 2008), and possibly to show more impressive reductions in response to intervention. Thus, Maximum CIMT may be more reliable to study interventions efficacy (Bots, Evans, Riley, & Grobbee, 2003; Hurwitz & Netterstrom, 2001). In accordance to this, the studies of Meyer et al (2006) and Wunsch et al (2006), who used Maximum CIMT values, presented higher decrease in CIMT and higher *p*-values compared with other authors for similar intervention time periods. The second reason is the short-duration of the intervention. As Woo et al. (2004a) also reported, at six weeks, there were no statistically differences in CIMT. Thus, we can assume that significant improvements in CIMT are improbably achieved at six weeks, independently of time or intensity of PA. A decrease in CIMT is possible to achieve only at three months (Park et al., 2012). These results seem to be, however, dependent of the exercise intensity

and volume, since Farpour-Lambert et al. (2009), using LMPA intensity, 180 minutes/week (versus MVPA, 240 minutes/week), did not report similar results at three months.

Accordingly with these results, it seems that the type of exercise is not as important on CIMT as the duration of the intervention, frequency, time or intensity of the PA.

Also, it is not possible to establish a causal inference between increasing CRF and decreasing CIMT, since few studies included in this systematic review measured CRF. It is possible, however, to compare the studies of Farpour-Lambert et al. (2009) and Park et al. (2012) who presented improvements in VO_2 max with similar PA interventions. In those cases, the major improvements in VO_2 max (3.7 mm/kg/min) matched to the greatest improvements in CIMT (-0.04 mm) (Table 1). Indeed, Farpour-Lambert et al. (2009) merely reported a stabilization of CIMT after the intervention. This result acquires significance however when compared to the control group, which showed increase CIMT (CIMT difference between groups= 0.02 mm, p=.0.045). The literature suggests that higher levels of CRF may compensate the adverse consequences of obesity (Ortega et al., 2008) even in obese state (Eisenmann et al., 2007; Nassis, Psarra, & Sidossis, 2005). Thus, the improvement reported in CIMT may be due to higher levels of exercise intensity and time, which may lead, not only to improvements in CV risk factors, but also, to a major increase of CRF (Buchan et al., 2011; Denton et al., 2013; Green et al., 2003; Gutin et al., 2002; Hay et al., 2012; Hägg, Wandt, Bergström, Volkmann, & Gan, 2005).

In addition, Park et al (2012) also reported increased levels of NO and endothelial progenitor cells after the intervention, compared to the control group. These two parameters represent improvements in endothelial function, and is one possible reason for the beneficial effect of exercise on CIMT, independently of body Weight, BMI (Watts, Beye, Siafarikas, Davis, et al., 2004; Watts, Beye, Siafarikas, O'Driscoll, et al., 2004), or other conventional CV risk factors (Green et al., 2003; Kelly et al., 2004). Acute exercise increases systemic blood flow, causing shear stress in vascular walls. This may lead to a response of endothelial NO Synthase (NOS) enzyme by increasing NO bioavailability, which in turn may promote the relaxation of the smooth muscle cells, and consequently the vasodilatation of the vessels, increasing blood flow (Maiorana, O'Driscoll, Taylor, & Green, 2003; Prior, Yang, & Terjung, 2004). Moreover, NO may also inhibit platelet adhesion (Lüscher & Barton, 1997). Additionally, endothelial progenitor cells may mediate the vessels response to exercise, by promoting angiogenesis and vascular repair (Laufs et al., 2004). In obese children and adolescents, the increase number of endothelial progenitor cells is positively associated with

CRF (Arnold et al., 2010). The NO-related improvements in endothelial function may be achieved with short-duration interventions (Green et al., 2004; Kelly et al., 2004). However as already mentioned, improvements in CIMT may be achieved only after 3 months of structured MVPA. This suggests that functional improvements on the endothelium may precede structural improvements.

In summary, although it was not possible to associate directly PA characteristics (type, frequency, time and intensity) to CIMT in obese children and adolescents, it was here suggested that PA may have a novel role on improvement of many parameters associated with the atherosclerotic process. Short-duration exercise interventions (six weeks) of LMPA intensity improve some MS/CV risk factors, such as cholesterol profile and glucose metabolism. PA acquires more importance with the increment of intensity (MVPA), improving, in many cases, Weight, Fatness, Inflammatory Markers, CRF and Endothelial Function (eight weeks - assessed by NO and endothelial progenitor cells). The extension of the exercise program enhances all the parameters mentioned before, and at 3 months with MVPA, improvements in CIMT are achieved. On the other hand, the control groups showed a trend to decrease CRF, and to increase CIMT as well as other MS components. Thus, it seems that PA has a beneficial effect on CIMT. This benefit may be moderated by CRF, independently of other CV risk factors. Consequently, exercise time and intensity may be the major contributors for the beneficial effect of PA on CIMT.

Limitations of the review

This systematic review has some limitations. Almost all limitations are based on the few (six) studies addressed to this issue. The low number (three) of studies presenting CRF measures did not allow feasible comparisons between CRF, frequency, time or intensity of the PA and CIMT. In addition, the heterogeneity of study protocols and measures used for statistical analysis of CIMT development of those studies, impair possible direct associations between PA, CRF and CIMT.

Moreover, although CIMT is a novel marker of an early stage of atherosclerosis, it can also reflect a compensatory nonatherosclerotic enlargement of the arteries (Touboul et al., 2007), caused by the remodeling of endothelial cells, smooth muscle cells and fibroblasts (Prior et al., 2004). Furthermore, this nonatherosclerotic enlargement may be increased by exercise, through NO- arteriogenesis-related mechanisms (Prior et al., 2004). In our opinion,

this is the most important limitation since it may hide the true impact of exercise, not only in CIMT but also in CV risk.

Future directions

To better understand the relationship that we aimed to analyze, future studies must be as descriptive as possible regarding the exercise protocol. Duration of the intervention (and data for compliance), type, frequency, time spent in exercise (normalized by energy expenditure within intervention group) and intensity (accessed by cardiofrequencymeters) must be part of the exercise protocol information.

Regarding the measurement of intensity, combined exercise seem to be the best choice, since it is a type of exercise in which intensity is easier to measure, compared to many others activities, such as swimming or ball games.

Furthermore, measures of CRF (VO_2 max) and daily PA (accelerometry) may be contemplated as well.

Finally, in order to realize the range of exercise impact on CV risk, it is crucial to include both functional and structural parameters of endothelial health. Functional parameters can be accessed by Flow-Mediated Dilatation (FMD), related to NO availability and its mechanisms or number of progenitor cells ($CD34^+/CD133^+$) related to angiogenesis and vascular repair. Structural health may continue to be accessed through CIMT. However, we and other authors (Stein et al., 2008) recommend the use of both Mean and Maximum values of CIMT for statistical purposes due to more reproducibility and sensitivity, respectively.

CONCLUSION

Increased CIMT is common among obese children and adolescents compared to lean ones. Furthermore, CIMT is associated with obesity and other MS/CV risk factors, and inversely associated with CRF. The few prospective studies relating Sedentary Behavior, PA, CRF and CIMT, and the heterogeneity between them, do not allow causal inferences, which enhance the importance of future work on this issue. Nonetheless, regular PA shown to has a beneficial effect on CIMT. However, if this beneficial effect is directly associated with a better CRF, independently of the improvements on the other risk factors and besides Weight, remains to be confirmed. Nonetheless, according to the existing literature, 240

minutes/week (three/80 minutes sessions) of MVPA during three months seem to be enough to improve CIMT, independent of the type of PA.

ACKNOWLEDGEMENTS

AA was the responsible for the database search and implementation of the inclusion/exclusion criteria. AP and HF were responsible for the revision of all the methodological and intellectual content. All the authors had access to all data and had a substantial contribution in this paper. The integrity and accuracy of the data is assumed by all the authors. The authors also declare no conflict of interest.

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Chapter 3

Results

Chapter 3.1 – The Relationship between Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness on Carotid Intima-Media Thickness in Obese Children and Adolescents: A Systematic Review

Chapter 3.2 – Physical Activity and Cardiorespiratory Fitness, but not Sedentary Behavior are associated with Carotid Intima-Media Thickness in Obese Adolescents

Physical Activity and Cardiorespiratory Fitness, but not Sedentary Behavior are associated with Carotid Intima-Media Thickness in Obese Adolescents

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The present study was submitted to International Journal of Pediatric Obesity
The original format was not maintained in order to satisfy University format recommendations

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ABSTRACT

The main objective of this study was to analyze the associations between Sedentary Behavior, Physical Activity (PA), and Cardiorespiratory Fitness (CRF), with Carotid Intima-Media Thickness (CIMT), an early marker of atherosclerosis often observed in obese adolescents.

Weight, Height, Body Mass Index, Waist Circumference, Trunk and Whole Body Fat, as well as Trunk and Whole Body Peripheral Fat were assessed from 54 Caucasian obese adolescents. The association of anthropometric measures, Sedentary time and PA determined by accelerometry, CRF determined by the shuttle-run test, and CIMT was analyzed using partial correlations (controlling for Age and Sex) and multiple linear regressions. Further correlations between CRF and all the other variables controlling for Age, Sex and Sedentary time, were also performed. Differences between participants with healthy and unhealthy CRF, was analyzed with independent-sample *t* test.

Sedentary time was not correlated with any other variable. Light Physical Activity correlated positively with Mean CIMT ($r(38)$=.36, p=.024), and Moderate Physical Activity (MPA) correlated positively with both Mean ($r(38)$=.37, p=.018) and Maximum ($r(38)$=0.33, p=.039) CIMT. On the other hand, CRF showed to be inversely associated with Mean CIMT ($r(40)$=-.36, p=.019), even when controlling for Sedentary time ($r(37)$=-.35, p=.030). MPA and Weight showed to be the best predictors of CIMT. Independent-sample t-test did not show statistically significant differences in CIMT between participants with healthy/unhealthy CRF.

Conclusion: Lower levels and intensities of PA may impair the possible beneficial effect of CRF, and emphasize the adverse effect of Weight on CIMT in obese adolescents. Thus, the enhancement of Vigorous Physical Activity in order to improve CRF among obese adolescents seems to be crucial to avoid CIMT development.

Keywords: Cardiorespiratory Fitness, Carotid Intima-Media Thickness, Physical Activity, Sedentary Behavior, Obese Adolescents.

INTRODUCTION

Childhood obesity remains a worldwide concern. In Portugal, near 30% of children between 10 and 18 years old are overweight or obese (Sardinha et al., 2011). This numbers are worrisome once 80% of obese children and adolescents run the risk of becoming obese adults (Cali & Caprio, 2008).

Obesity is a health condition characterized by excessive fat accumulation in adipose tissue and other organs, which may be associated with Metabolic Syndrome (MS) and its pathological mechanisms, even early in life (Weiss et al., 2004). Therefore, increased insulin and lipid levels, high blood pressure and/or pro-inflammatory factors are common among obese adolescents (Deckelbaum & Williams, 2001; Huang et al., 2010; Woo et al., 2004b; Wunsch et al., 2006). These conditions are also associated with atherosclerotic development (Fang et al., 2010; Huang et al., 2010; Iannuzzi et al., 2004). Thus, study Carotid Intima-Media Thickness (CIMT), the only atherosclerotic indicator from child to adulthood (Bauer et al., 2012) may be crucial to avoid or delay atherosclerotic development in obese adolescents.

It is well known that CIMT correlates positively with Body Mass Index (BMI) (Doyon et al., 2013; Fang et al., 2010) and with Waist Circumference (WC) in obese adolescents (Hacihamdioğlu et al., 2011; Huang et al., 2010). WC may be however, more reliable than BMI for the prediction of an early stage of atherosclerosis, since it represents an indicator of central adiposity, the worse metabolic and health-related fat depot (Elkiran et al., 2013).

Sedentary Behavior, such as watching television and videos, are primary causes for the development of obesity, mainly due to Physical Activity (PA) decrease (Carlson et al., 2012; Crocker & Yanovski, 2011). The lack of PA is associated with a lower Cardiorespiratory Fitness (CRF), which in turn is associated with morphologic and biochemical parameters that may induce an early stage of atherosclerosis (Meyer, Kundt, Lenschow, et al., 2006). On the other hand, a higher CRF may be protective against the adverse health-related consequences of obesity (Ortega et al., 2008), even among obese subjects (Eisenmann et al., 2007; Nassis, Papantakou, et al., 2005).

The combination of both lower CRF and excessive time spent in Sedentary Behavior may represent a major unhealthy cluster associated to the development of

CIMT, and consequently to atherosclerosis. To our knowledge, few studies in obese adolescents were focused on this issue, which enhances the importance of this study.

In this cross-sectional study, we aimed to analyze the association between Sedentary Behavior, PA, CRF, and CIMT in obese adolescents, a group who spend most of their waking time in Sedentary Behavior (Brodersen et al., 2007; Tremblay et al., 2011) and present lower levels of CRF than lean ones (Aires et al., 2010).

These aims were based on our initial hypothesis according which 1) there is a positive association between the time spent in Sedentary Behavior and CIMT; 2) higher levels of CRF are associated with a lower CIMT, independently of time spent in Sedentary Behavior; 3) Measures of central adiposity are more reliable on the prediction of CIMT than BMI.

METHODS

Participants

54 Caucasian adolescents (13 - 17 years-old) with a BMI over the 95th percentile (Barlow & Committee, 2007) were recruited to participate in the 12-months intervention program TOP (Treatment of Pediatric Obesity), described elsewhere (Fonseca, Palmeira, Martins, Falcato, & Quaresma, 2014). All participants were recruited from the pediatric outpatient obesity clinic of the Hospital de Santa Maria, Lisbon, and from schools in Lisbon area. Informed consent was signed from both participants, and their parents. For this study, subjects with major diseases or inability to perform regular PA, or involved in other weight loss program were excluded. This study was approved by the research ethics committee of the Faculty of Medicine of the Universidade de Lisboa, Lisbon, Portugal, and it is registered in clinicaltrials.gov (NCT02024061).

Measurements

At baseline, a variety of anthropometric and clinical parameters were assessed.

Anthropometrics

Body weight (bioimpedance Scale OMRON BF-511, Japan), was measured to the nearest 0.1 kg, in the anthropometric position (with the palms turned into thighs). The subjects should wear fewer clothes as possible, and stay without shoes.

Height (height stadiometer, SECA 217, Hamburg, Germany), was accessed in the anthropometric position, without shoes, with the participant backs to the stadiometer. Height was registered to the nearest 0.1 cm, after an expiratory phase.

BMI was calculated as body weight in kilograms divided for the square of height in meters [BMI= weight (kg)/height^2 (m)] (Cole, Bellizzi, Flegal, & Dietz, 2000).

WC (circumference measuring tape, SECA 203, Hamburg, Germany) was measured at the half distance between the edge of the lower ribs and the edge of the supra-iliac crest (WC1) and one cm above the iliac crest (WC2). Both measures were taken at the end of a common expiration, with the subject standing.

Body composition was accessed using Dual-energy X-ray Absorptiometry (DXA) (DXA, HOLOGIC QDR 1500, Waltham, Massachusetts, USA). DXA exam was performed in accordance with the American College of Sports Medicine (ACSM) protocol (American College of Sports Medicine, 2005). Trunk and Whole Body Fat, as well as Trunk and Whole Body Peripheral Fat were measured. In order to assess trunk adiposity, upper limbs were kept way from the trunk in order to include all trunks, but not arm tissue in the analysis.

Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness

PA and time in Sedentary Behavior was assessed with accelerometers (ACTIGRAPH GT3X, Pensacola, Florida, USA). All subjects used one accelerometer above the right hip, near to the iliac crest, during seven consecutive days, except during sleep, bath or swimming. The accelerometer was programmed to use a 5-second cycle. Only days with more than 480 minutes (8h) of use, were considered for the analysis. Moreover, periods of 60 minutes with "zero activity" were interpreted as un-using equipment. Data upload was performed with the ActiLife software, version 6.8.0. The cut-points of Sedentary Behavior, Light Physical Activity (LPA), Moderate Physical

Activity (MPA), and Vigorous Physical Activity (VPA) were determined by age (Trost et al., 2002).

CRF was assessed with the Shuttle-Run test (SR) (FITNESSGRAM® battery test, version 8.0, Cooper institute for aerobic research, Dallas, Texas, USA), conducted accordingly with the FITNESSGRAM® test battery guidelines (Cooper Institute for Aerobic Research, 1994). The choice to assess CRF with the SR test was based on three main reasons: 1) the SR test is an ecological and cost-effective test, allowing multiple participants to perform the test simultaneously; 2) all the participants are familiarized with the SR test, once FITNESSGRAM® test battery is part of the Portuguese physical education curriculum, since the fifth grade; 3) it is possible and reliable to estimate the maximal oxygen uptake (VO_2 max) from the SR test, using an equation [VO_2 max= 43.313+4.567*sex–0.560*BMI+2.785*stage] validated for Portuguese youth (Silva et al., 2012). In addition, FITNESSGRAM® guidelines were also used for healthy/unhealthy classification of VO_2 max.

Carotid Intima-Media Thickness

CIMT exam was performed in the Vascular Surgery Unit of the Hospital de Santa Maria, Lisbon, with an ultrasound imager using a 12-5 MHz linear transducer (Philips ultrasound imager HD15, Andover, Massachusetts, USA). CIMT may be defined as the distance between the lumen-intima and the media-adventitia interfaces. The measurement was performed in the longitudinal plane on both right and left common carotid arteries, about 2-3 cm proximal to the carotid bifurcation (Touboul et al., 2007). Three different places at the far walls were measured using an automatic technique. The participants were examined in the supine position with the neck slight extended and head tuned slight to the side. All the measurements were made for just one blinded observer, eliminating inter-observer variability.

Statistical analysis

Data was analyzed using the IBM SPSS statistics (IBM SPSS statistics, version 21.0, IBM, New York, USA).

Girls and boys were analyzed together due to the low number of the participants recruited. Thus, to evaluate the associations between all the variables was

computed a partial correlation controlled for Age and Sex, and for Age, Sex, and Sedentary time, when analyzed the associations of VO_2 max with the other variables. We used both Mean and Maximum values of CIMT for statistical purposes since the first may be more reproducible (Stein et al., 2008) and the second may represent an advanced stage of thickness (Touboul et al., 2007), which implies a higher risk of atherosclerosis. In order to better understand the associations between all the variables, multiple linear regressions (stepwise method) were performed with Mean and Maximum CIMT as dependent variables. Additionally, differences between sexes and healthy/unhealthy CRF groups were analyzed using independent-samples *t* tests. A $p<0.05$ was considered statistically significant.

RESULTS

From the 54 participants recruited (68.5% girls) only 29 (53.7%) performed all the assessments, therefore sample size varied in the various statistical procedures. Participant characteristics are presented in Table 2.

Correlations between Sedentary time, PA, anthropometrics, CRF and CIMT

Sedentary time did not correlate with CIMT or with any other variable. LPA correlated positively with Mean CIMT ($r(38)$=.36, p=.024). Moderate-to-Vigorous Physical Activity (MVPA), as well as MPA correlated positively with both Mean ($r(38)$=.37, p=.018; $r(38)$=0.37, p=.018) and Maximum CIMT ($r(38)$=0.33, p=.039; $r(38)$=0.32, p=.044), respectively. VPA was positively correlated with VO_2 max ($r(40)$=.46, p=.002), and VO_2 max correlated negatively with Mean CIMT ($r(40)$=-.36, p=.019). Additionally, when controlling for Sedentary time, VO_2 max remained inversely correlated with Mean CIMT ($r(37)$=-.35, p=.030). BMI ($r(44)$=.32, p=.033) and Whole Body Fat ($r(30)$=.35, p=.048) correlated positively with Mean CIMT. Moreover, Weight ($r(44)$=.39, p=.008; $r(44)$=.37, p=.012), Trunk Fat ($r(30)$=.36, p=.041; $r(30)$=.35, p=.048), and Trunk Peripheral Fat ($r(30)$=.36, p=.041; $r(30)$=.37, p=.036) correlated positively with both Mean and Maximum CIMT, respectively. Correlations are presented in Table 4.

Table 2. Participants characteristics at baseline.

	Boys		Girls		
	n	Mean (SD)	*n*	Mean (SD)	*p* value
Age (years)	17	15.24 (1.39)	37	15.08 (1.48)	.719
Weight (kg)	17	93.54 (14.49)	37	86.49 (13.14)	.082
Height (m)	17	1.69 (0.08)	37	1.59 (0.05)	<.001
BMI (kg/m^2)	17	32.72 (3.30)	37	34.14 (5.38)	.240
WC1 (cm)	17	99.27 (8.33)	36	96.18 (9.64)	.262
WC2 (cm)	17	103.02 (8.60)	36	100.27 (9.57)	.318
Trunk Fat (g)	13	14976.64 (4763.09)	24	17357.92 (4098.80)	.120
Trunk P Fat (g)	13	33.58 (6.08)	24	42.98 (4.50)	<.001
Whole Body Fat (g)	13	32387.55 (8984.31)	24	37001.37 (7455.47)	.103
Whole Body P Fat (g)	13	33.98 (5.60)	24	43.63 (3.63)	<.001
Sedentary (min)	15	580.32 (90.11)	32	590.14 (71.70)	.689
LVPA (min)	15	144.89 (30.16)	32	145.47 (38.82)	.959
MPA (min)	15	42.44 (21.08)	32	32.73 (13.73)	.065
VPA (min)	15	4.56 (3.10)	32	1.93 (1.64)	.006
VO_2 max (ml/kg/min)	15	41.98 (3.79)	35	30.79 (5.14)	<.001
CIMT mean (mm)	14	0.056 (0.002)	34	0.053 (0.007)	.100
CIMT max (mm)	14	0.060 (0.008)	34	0.056 (0.008)	.154

BMI - Body Mass Index; CIMT - Carotid Intima-Media Thickness; LPA- Light Physical Activity; MPA- Moderate Physical Activity; Trunk P fat - Trunk Peripheral fat; VPA- Vigorous Physical Activity; WC1 - Waist Circumference measured at the half distance between the edge of the lower ribs and the edge of Iliac crest; WC2 - Waist Circumference measured 1 cm above the Iliac crest; Whole body P fat - Whole body Peripheral fat.

Multiple linear regressions

MPA and Weight showed to be the two best predictors of both Mean and Maximum CIMT. Together MPA and weight could explain 31.2% (p=.001) and 23.6% (p=.007) of Mean and Maximum CIMT variance, respectively. Furthermore, LPA could explain 5.8% of BMI or Weight (both p=.056), and VO_2 max variance could be explained by VPA (40.5%, p<.001) and MVPA (15.7%, p=.005) (Data not shown).

Comparison between subjects with healthy and unhealthy CRF

Significant differences in anthropometrics, VPA and VO_2 max, but not in CIMT, were found between the two groups. The participants with a healthy VO_2 max showed to have less Weight (p=.011), and lower BMI (p=<.001), WC (1 p=.001; 2 p=.003), Trunk Fat (p=<.001), Trunk Peripheral Fat (p=<.001), Whole Body Fat (p=<.001) and Whole Body Peripheral Fat (p=<.001). Furthermore, they presented higher levels of VPA (p=.015) than the other participants. Comparison between groups is presented in Table 3.

Table 3. Comparison between subjects with healthy and unhealthy CRF.

	Healthy CRF		Unhealthy CRF		
	n	Mean (SD)	*n*	Mean (SD)	*p* value
Weight (kg)	15	81.46 (9.75)	35	91.13 (12.68)	.011
BMI (kg/m^2)	15	29.73 (1.84)	35	35.45 (4.63)	<.001
WC1 (cm)	14	90.92 (6.85)	35	99.60 (8.19)	.001
WC2 (cm)	14	95.46 (6.39)	35	103.36 (8.35)	.003
Trunk Fat (g)	10	12104.77(2344.44)	24	17859.45 (3742.55)	<.001
Trunk P Fat (g)	10	32.86 (6.01)	24	42.20 (5.46)	<.001
Whole Body Fat (g)	10	27118.40 (2234.65)	24	37907.43 (6948.39)	<.001
Whole Body P Fat (g)	10	33.91 (5.70)	24	42.68 (5.15)	<.001
Sedentary (min)	12	580.13 (65.26)	32	593.42 (80.69)	.612
LVPA (min)	12	143.24 (30.98)	32	147.51 (38.37)	.732
MPA (min)	12	41.69 (18.60)	32	35.04 (16.01)	.247
VPA (min)	12	4.27 (2.55)	32	2.21 (2.34)	.015
VO_2 max (ml/kg/min)	15	41.05 (4.57)	35	31.19 (5.69)	<.001
CIMT mean (mm)	13	0.051 (0.005)	31	0.054 (0.007)	.195
CIMT max (mm)	13	0.055 (0.006)	31	0.057 (0.008)	.239

BMI - Body Mass Index; CIMT - Carotid Intima-Media Thickness; LPA- Light Physical Activity; MPA- Moderate Physical Activity; Trunk P fat - Trunk Peripheral fat; VPA- Vigorous Physical Activity; WC1 - Waist Circumference measured at the half distance between the edge of the lower ribs and the edge of Iliac crest; WC2 - Waist Circumference measured 1 cm above the Iliac crest; Whole body P fat - Whole body Peripheral fat.

Table 4. Partial correlation between all the variables, controlled for Age and Sex, followed by partial correlation of VO_2 max with all the other variables, controlled for Age, Sex, and Sedentary time.

	Weight	Height	BMI	WC1	WC2	TF	TPF	WBF	WBPF	Sed	LPA	MPA	VPA	MVPA	VO_2 max	Mean CIMT	Max CIMT
Weight	1																
Height	.35 *	1															
BMI	.87 †	-.15	1														
WC1	.84 †	.07	.85 †	1													
WC2	.84 †	.06	.85 †	.98 †	1												
TF	.79 †	.07	.86 †	.87 †	.84 †	1											
TPF	.45 §	-.18	.68 †	.67 †	.61 †	.91 †	1										
WBF	.82 †	.07	.89 †	.81 †	.82 †	.95 †	.83 †	1									
WBPF	.42 *	-.26	.71 †	.56 †	-.56 †	.82 †	.88 †	.88 †	1								
Sed	-.06	.16	-.13	-.13	-.10	-.14	-.09	-.08	-.04	1							
LPA	.35	-.05	.41 §	.36 *	.33 *	.07	-.06	.13	-.03	-.12	1						
MPA	-.16	-.13	-.08	-.12	-.18	-.07	.07	-.10	.04	-.19	.27	1					
VPA	-.27	.08	-.32 *	-.36 *	-.37 *	-.30	-.30	-.30	-.38 *	.03	.11	.44 §	1				
MVPA	-.19	-.11	-.12	-.16	-.21	-.10	.03	-.13	-.00	-.18	.27	.99 †	.53 †	1			
VO_2 max	-.78 †	.30 *	-.91 †	-.83 †	-.79 †	-.92 †	-.86 †	-.96 †	-.92 †	.11	-.25	.23	.46 §	.27	1		
Mean CIMT	.39 §	.21	.32 *	.27	.23	.36 *	.36 *	.35 *	.33	-.20	.35 *	.37 *	.17	.37 *	-.36 *	1	
Max CIMT	.37*	.24	.28	.27	.24	.35 *	.37 *	.31	.29	-.15	.29	.32 *	.22	.33 *	-.30	.96 †	1
VO_2 max	-.79 †	.29	-.90 †	-.82 †	-.79 †	-.92 †	-.86 †	-.96 †	-.92 †	-	-.24	.25	.46 §	.29	1	-.35 *	-.29

* $p < .05$; § $p < .01$; † $p < .001$

BMI- Body Mass Index; CIMT- Carotid Intima-Media Thickness; LPA- Light Physical Activity; MPA- Moderate Physical Activity; MVPA- Moderate-to-Vigorous Physical Activity; Sed- Sedentary time; TF- Trunk fat; TPF- Trunk Peripheral Fat; VPA- Vigorous Physical Activity; WBF- Whole Body Fat; WBPF- Whole Body Peripheral Fat; WC1- Waist circumference measured at the half distance between the edge of the lower ribs and the edge of iliac crest; WC2- Waist Circumference measured 1 cm above the iliac crest.

DISCUSSION

The purpose of this study was to analyze the associations between Sedentary Behavior, PA and CRF, with CIMT in obese adolescents.

Firstly, we hypothesized a positive association between the time spent in Sedentary Behavior and CIMT, since high levels of Sedentary Behavior are associated with insulin resistance (Sardinha, Baptista, & Ekelund, 2008), and systolic blood pressure (Martínez-Gómez et al., 2010), which are predictors of CIMT development during adolescence (Iannuzzi et al., 2004; Stabouli et al., 2012). Data showed that participants spent around 76% of accelerometer wearing time in Sedentary Behavior (Table 2). However, no direct correlations between Sedentary time and both Mean and Maximum CIMT, or with any other variable measured in this study were observed (Table 3). In fact, to our knowledge only one author reported a positive correlation between Sedentary Behavior and CIMT, in an apparently healthy adult population (Kozàkovà et al., 2010). In addition, there is no consensus if higher levels of Sedentary time are associated with higher BMI, WC, or central adiposity (Carlson et al., 2012; Ortega et al., 2007; Ruiz et al., 2011).

We have also hypothesized that higher levels of CRF (VO_2 max) were associated with a lower CIMT independently of Sedentary time, once CRF may be associated with endothelial functional health (Arnold et al., 2010; Green et al., 2004), which in turn could be associated with endothelial structural health over time. Moreover, time spent in Sedentary Behavior seem to be unassociated with CRF (Denton et al., 2013). Indeed, we found that CRF was inversely associated with Mean CIMT, even when controlling for Sedentary Behavior time. Furthermore, although not statistically significant, when compared subjects with different CRF, those who had a healthy VO_2 max, presented lower values of CIMT (Table 3). CRF also was inversely associated with all adiposity measures performed in this study (Table 4), which is similar to the results obtained in other studies (Ortega et al., 2007).

In this study, a positive correlation was found between LPA with Mean CIMT, and with Weight, BMI and WC, suggesting also an negative association of LPA on CRF (Table 4), which is confirmed by other authors (Denton et al., 2013). Thus, high levels of LPA may represent an adverse effect in health in the presence of obesity. On the other hand, MVPA, often used to test associations between PA and health-related

outcomes, it is known to have a beneficial effect in health (Ekelund et al., 2012). In our study, MVPA explained 15.7% of VO_2 max variance, thus it would be expected that it could also be inversely correlated with CIMT. However MVPA showed a positive correlation with both Mean and Maximum CIMT, which seems contradictory.

When individually analyzed, both MPA and VPA presented substantial correlation differences with the other variables. MPA correlated positively with both Mean and Maximum CIMT, predicting 15% (p=.015) and 10.8% (p=.035) of CIMT variance, respectively. Whereas VPA correlated positively with CRF, predicting 40.5% of CRF variance (p<.001), which was the only variable inversely correlated with CIMT. Furthermore, VPA showed to be negatively associated with BMI, WC and Whole Body Peripheral Fat, which is in accordance with the results reported by other authors (Gutin, Yin, Humphries, & Barbeau, 2005). Although, VPA may have a major beneficial effect in health than MPA, both MPA and VPA tended to be positively associated with CIMT (Table 4). One possible explanation for this fact may be the lower levels of VPA measured in our sample, and in general, in obese adolescents (Trost, Kerr, Ward, & Pate, 2001). The lower levels of VPA may be insufficient to induce a great VO_2 max increase, not affecting CIMT, since higher PA levels and intensities are closely related to VO_2 max (Aires et al., 2010; Denton et al., 2013). Thus, the healthy values of CRF among our participants may be due to heritability, which may influence till 50% of CRF in sedentary state, even when controlled for Age, Sex, Weight, Body Fat Mass and Fat-Free Mass (Teran-Garcia, Rankinen, & Bouchard, 2008). Another possible explanation for this result, may be the nonatherosclerotic enlargement of carotid artery as an adaptive response to PA (Touboul et al., 2007), through a Nitric Oxide- arteriogenesis-related mechanisms (Prior et al., 2004). However, this endothelial adaptive response to PA may be unlikely in our population, since it would be dependent of time and intensity of the PA (Lewis, Dart, Chin-Dusting, & Kingwell, 1999).

Furthermore, we hypothesized that central adiposity would be more reliable in the prediction of CIMT than BMI (Elkiran et al., 2013). Other authors previously reported an association of BMI with CIMT in obese adolescents (Doyon et al., 2013; Fang et al., 2010), which is in line with our results. In this study, BMI as well as Whole Body Fat only correlated positively with Mean CIMT. On the other hand, the DXA measures of central adiposity correlated positively with both Mean and Maximum CIMT, which may represent a high risk of atherosclerosis in the last case (Touboul et al., 2007).

Interestingly, neither WC1 nor WC2 correlated positively with CIMT, which highlight the importance of assessing central adiposity using DXA exam to study CIMT development. In addition, although Weight may be a nondiscriminatory measure of adiposity it showed to correlate better with CIMT than all the measures of central adiposity, being the second best predictor of CIMT. Although it was not observed in this study, the developmental process in this age group may be associated with CIMT (Doyon et al., 2013), explaining in part, the association of Weight with CIMT.

Figure 4 may summarize the results of this study. PA, but not Sedentary Behavior seems to be associated with CIMT, however these relationships may be moderated by Weight and CRF, in an unhealthy and healthy way, respectively. Thus, this study highlights the importance of enhancing VPA in order to increase CRF and decrease Weight when targeting CIMT decrease in obese adolescents.

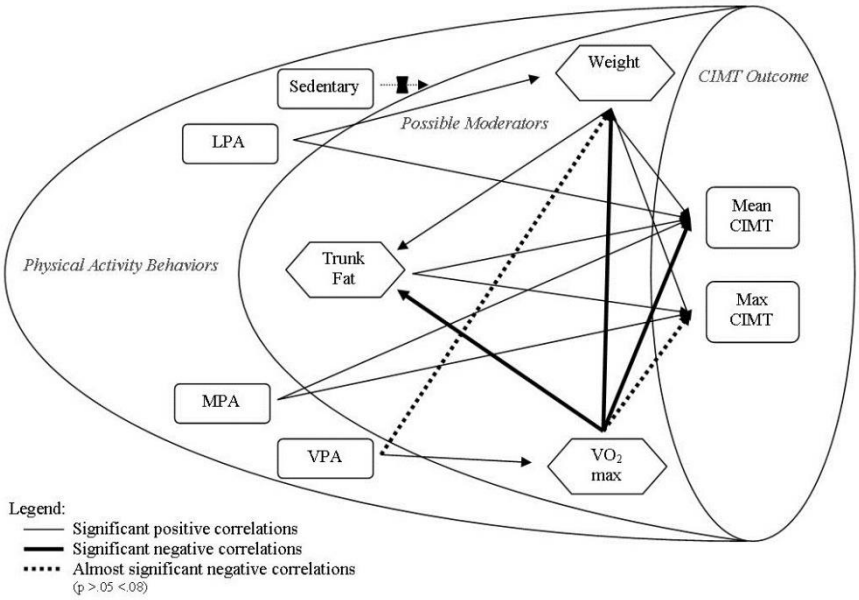

Figure 4. Associations between Sedentary and PA behaviors, possible moderators and CIM.

Design and the low number of participants are the two major limitations of this study, since cross sectional studies do not allow causal inferences. In addition, statistical procedures did not take into account for medication capable of influence the cardiovascular function and, glucose or lipid metabolism, since only three participants were medicated. Finally, we did not present other metabolic assessments such as blood pressure, insulin resistance, or inflammatory markers, which may also mediate CIMT development (Beauloye et al., 2007), since Weight seems to be the only factor that correlates independently with CIMT in children and adolescents (Stabouli et al., 2012).

In order to better understand the relationship between PA and CIMT, future studies may assess not only endothelial structural health trough CIMT, but also endothelial functional health trough Flow-Mediated Dilatation (FMD). Since it is possible that CIMT may be the result of adaptive response to PA (Prior et al., 2004) or to the developmental process (Doyon et al., 2013), and not necessarily, the result of atherosclerosis development.

Acknowledgements

The original Trial was funded by the Foundation of Science and Technology (FCT – Fundação para a Ciência e a Técnologia, Av. D. Carlos I, 126, 1249-074 Lisboa, Portugal. Tel. 213924300, Fax. 213907481) (PTDC/DES/113591/2009).

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Chapter 4

4.1. Discussion

4.2. Conclusion

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4.2. Conclusion

DISCUSSION

In obese adolescents, CIMT may be a reliable marker (Currie et al., 2010) of an earlier stage of atherosclerosis (Davis et al., 2001), even before major abnormalities of the classic CV risk factors (Atabek et al., 2007). On the other hand, CIMT may also reflect an adaptive response to developmental process (Denton et al., 2013), and to PA trough a Nitric Oxide- arteriogenesis-related mechanisms (Prior et al., 2004). Either way, Sedentary Behavior and PA may be associated with CIMT.

Initially we hypothesized that, Sedentary Behavior was associated with CIMT development, since it is associated with IR (Sardinha et al., 2008), and SBP (Martínez-Gómez et al., 2010), which are CIMT indicators (Iannuzzi et al., 2004; Stabouli et al., 2012). However, in the cross-sectional study (Ascenso, Palmeira, Pedro, Martins, & Fonseca, 2014), Sedentary Behavior showed no correlations with CIMT. In fact, to our knowledge only one author reported a positive association of Sedentary Behavior with CIMT in an apparently healthy adult population, using a sedentary/Light Physical Activity (LPA) ratio, as an index of Sedentary Behavior (Kozàkovà et al., 2010).

Unexpectedly, in our study all PA intensities, to the exception of Vigorous Physical Activity (VPA), correlated positively with obese adolescents Mean and/or Maximum CIMT at baseline (Ascenso, Palmeira, Pedro, et al., Submitted). On the other hand, VPA showed to be positively correlated with CRF, as already reported by other authors (Denton et al., 2013; Gutin et al., 2005). Additionally, CRF was the only variable inversely associated with CIMT, suggesting that exercise intensity and volume may moderate the relationship between CRF and CIMT (Ascenso, Palmeira, & Fonseca, Submitted).Thus, LPA and Moderate Physical Activity (MPA) although both associated with CIMT may represent substantial differences on CIMT development.

In the cross-sectional study, LPA tended to be inversely associated with CRF, which is confirmed by other authors (Denton et al., 2013). Moreover, LPA showed to be positively associated with BMI and WC, two variables often reported as associated with CIMT (Doyon et al., 2013; Fang et al., 2010; Hacihamdioğlu et al., 2011; Huang et al., 2010). On the contrary, MPA tended to be positively associated with CRF, which is consistent with other studies (Denton et al., 2013; Gutin et al., 2005; Ruiz et al., 2006). This association may be dependent however of PA volume and/or obesity condition, since in our population we registered 35.83 (±16.83) min/day of MPA (versus 39.3 ±

29.40 min/day, BMI 23.1 ± 5.1 - Gutin et al., 2005), which were not associated with CRF. Nevertheless, even higher levels of MPA (<170 min/day) do not seem to be inversely associated with obesity measures (Gutin et al., 2005; Ruiz et al., 2006), suggesting that MPA may also be unlikely inversely associated with CIMT at a short (Kelishadi, Hashemi, et al., 2008; Woo et al., 2004a) and medium time periods (Farpour-Lambert et al., 2009).

Thus, LPA may be associated with an atherosclerotic development of CIMT. Whereas, increments in MPA may promote a transitional stage, between the atherosclerotic development and an adaptive response to PA trough improvements in endothelial functional parameters, which may imply a slightly increase or maintenance of CIMT (Farpour-Lambert et al., 2009). Over time (around twelve months) this process may originate a decrease in CIMT (Woo et al., 2004a). Accordingly to the systematic review (Ascenso, Palmeira, & Fonseca, Submitted), MVPA associated with a major improvements in CRF (3.7 mm/kg/min), showed however capacity to accelerate this process, promoting a major decrease in CIMT (-0.04 mm) in just three months (Park et al., 2012).

We, and other authors reported a positive association of CIMT with BMI (Doyon et al., 2013; Fang et al., 2010), however in the cross-sectional study, Weight was the anthropometric measure better associated with CIMT, presenting a high positive correlation (Ascenso, Palmeira, Pedro, et al., Submitted). Neither measures of central obesity assessed by DXA correlated better with CIMT, confirming that the developmental process may be associated with CIMT (Doyon et al., 2013). In addition, substantial weight losses are associated with a major CIMT decrease (Wunsch et al., 2006). Although, Weight does not allow deductions about the cause of CIMT development, additional analysis showed that it remains high positively correlated with CIMT even when controlling for Height. Thus, Weight may be the most reliable anthropometric moderator of CIMT development.

This work suggests that CRF may be the healthy major moderator of CIMT, compensating the adverse consequences of obesity on CV risk factors (Hurtig-Wennlöf, Ruiz, Harro, & Sjöström, 2007; Ortega et al., 2008) and on CIMT. The novelty is that, even besides PA, a heritable healthy CRF may be associated with lower values of CIMT among obese adolescents (Ascenso, Palmeira, Pedro, et al., Submitted). Nevertheless, VPA is the most efficient way of increasing CRF (Buchan et al., 2011), being also

negatively associated with obesity-related outcomes (Ascenso, Palmeira, Pedro, et al., Submitted; Buchan et al., 2011; Gutin et al., 2005), which in turn are associated with CIMT. Furthermore, enhancing VPA in order to increase CRF seems to be imperative, since CRF tended to decrease over the years (Ferreira, Twisk, & Stehouwer, 2007). Thus, as written previously adolescence may be a critical period for intervention.

Chapter 4

4.1. Discussion

4.2. Conclusion

CONCLUSION

Globally, this work contributes to the understanding of the relationship between Sedentary Behavior, PA, CRF, and CIMT, mostly because it summarizes and brings to the light a new approach on this issue, suggesting also future directions.

In summary, 1) although CIMT may be a reliable indicator of primary atherosclerotic development, it may also reflect an adaptive response to the developmental process and to PA; 2) In order to specify the nature of CIMT development, endothelial function health may be assessed as well; 3) Weight seems to be the anthropometric measure which correlates better with CIMT, even when controlling for Height; 4) DXA measures of central adiposity correlates better with CIMT than WC, thus we suggest the use of those measures in CIMT studies; 5) Sedentary Behavior proved not to be associated with CIMT early in life; 6) Lower levels and intensities of PA seems to be associated with CIMT; 7) On the other hand, high levels and intensities of PA proved to be inversely associated with CIMT over time, since functional endothelial improvements may precede structural improvements; 8) Functional endothelial improvements may be associated with NO availability, resulting in improvements on CRF; 9) Although NO may be at the origin of this process, a heritable CRF may be also associated with lower values of CIMT; 10) Thus, CRF seems to be the major healthy moderator of CIMT process.

FUTURE DIRECTIONS

Future studies could be made in order to better understand the associations between Sedentary Behavior, PA, CRF and CIMT in obese adolescents.

Since participants of the cross-sectional study included in this dissertation were recruited for the 12 months interventional program TOP (Treatment of Pediatric Obesity), described elsewhere (Fonseca et al., 2014), the next step would be analyze all the outcomes at six and 12 months. Moreover, because in the TOP the Flow-Mediated Dilatation (FMD) was not assessed, which is a non-invasive indicator of endothelial functional health, we would analyze inflammatory markers such as CRP, as well as adiponectin levels. Therefore, we would be able to associate CIMT with inflammatory level, and to associate PA intensities with an atherosclerotic development, or with a compensatory nonatherosclerotic enlargement of CIMT (Prior et al., 2004).

Ahead, it would be of an incalculable value, perform a representative epidemiological study in order to develop age- and sex-specific CIMT reference values for Portuguese youth.

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Attachments

Carotid Intima-Media Thickness in Obese Adolescents: The link between Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness – Study Protocol

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Carotid Intima-Media Thickness in Obese Adolescents: The link between Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness – Study Protocol

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Trial registered in clinicaltrials.gov with the number NCT02024061

Protocol number 1, 21/03/2014

The original trial was funded by the Foundation of Science and Technology (FCT – Fundação para a Ciência e a Técnologia, Av. D. Carlos I, 126, 1249-074 Lisboa, Portugal. Tel. 213924300, Fax. 213907481) (PTDC/DES/113591/2009).

ABSTRACT

It is well known that childhood obesity is one of the greatest health issues worldwide. Many of the known adverse health-related mechanisms associated with obesity are already operative in early ages, presenting in adolescence, similar values to the adulthood. One of those mechanisms is atherosclerosis. From child to adulthood, the measurement of Carotid Intima-Media Thickness (CIMT) is the only procedure to assess a primary atherosclerotic development. The literature suggests a possible association between Sedentary Behavior, Physical Activity (PA) and CIMT independently of Body Mass Index (BMI). This relationship may be mediated by Cardiorespiratory Fitness (CRF), a well-known parameter of physical fitness related to health.

The main aim of this study is to analyze the relationship between Sedentary Behavior, PA and CRF on obese adolescents CIMT, the pediatric group with lower levels of CRF.

For this study we aim to recruit 54 participants from the pediatric outpatient obesity clinic of the Hospital de Santa Maria (HSM) and from schools in Lisbon area. To be included, all the participants have to be between 13 and 17 years-old and a BMI $\geq$ 95th percentile. Will be excluded all those who have major diseases or inability to perform regular PA, involved in other weight loss program, or with smoking habits. The participants will be divided into three groups to be evaluated in different times. Measures of Weight, Height, BMI, Body Fat Mass, central adiposity, Waist Circumference, Sedentary Behavior, PA, CRF, and CIMT will be made. In order to evaluate the relationship between all the measured variables we will use the IBM SPSS statistical.

Keywords: Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness, Carotid Intima-Media Thickness, Obese Adolescents.

INTRODUCTION

Background and rationale

Obesity has been pointed as one of the greatest concerns of the 21st century (World Health Organization, 2004). Excessive fat accumulation in adipose tissue and other organs starts in early ages. In Portugal, near 30% of children between 10 and 18 years old are overweight or obese (Sardinha et al., 2011). Therefore, obesity-related adverse metabolic outcomes, such as Metabolic Syndrome (MS), and its comorbidities are already operative in adolescents (Huang et al., 2010; Weiss et al., 2004). Thus, it is of major importance to analyze the pathological mechanisms related to obesity and MS, which in turn are associated with Carotid Intima-Media Thickness (CIMT) (Fang et al., 2010; Iannuzzi et al., 2004; Stabouli et al., 2012; Woo et al., 2004b), an important vascular parameter of an earlier stage of atherosclerosis (Davis et al., 2001).

Increased CIMT corresponds to an impairment of the structural endothelial properties that can be observed previously to the atheromatous plaque formation (Bauer et al., 2012). Furthermore, CIMT is the only atherosclerotic marker up to adulthood, being also, a reliable (Currie et al., 2010), noninvasive and inexpensive method that can reflect atherosclerotic development even without major abnormalities of the classic Cardiovascular (CV) risk factors (Atabek et al., 2007).

Sedentary Behavior is associated with increased Body Mass index (BMI) (Mitchell et al., 2013), whole body fat and abdominal adiposity (Ortega et al., 2007), in part due to a decrease on Cardiorespiratory Fitness (CRF), a novel indicator of health (Ortega et al., 2008). In addition, lower levels of CRF are associated with morphologic and biochemical conditions that may reflect a primary atherosclerotic development (Meyer, Kundt, Steiner, et al., 2006).

On the other hand, higher levels of CRF may compensate the adverse consequences of obesity (Ortega et al., 2008), even regarding CIMT development. The literature suggests that in obese adolescents, interventions focusing in exercise may lead to improvements in CIMT (Farpour-Lambert et al., 2009; Meyer, Kundt, Lenschow, et al., 2006; Woo et al., 2004a; Wunsch et al., 2006). However, it is not clear if the improvements in CIMT may be due to less time spent in Sedentary Behavior and higher levels of PA, which may be associated with improvements in BMI; or due to Moderate-

Vigorous Physical Activity (MVPA), which is known to be associated with major increases in CRF, independently of substantial changes in BMI (Balagopal et al., 2005; Kelishadi, Cook, et al., 2008; Kelishadi, Hashemi, et al., 2008).

To our knowledge, no other study aimed to analyze the associations between Sedentary Behavior, PA and CRF among obese adolescents, a high risk group for early atherosclerosis.

Furthermore, we believe that study CIMT of obese adolescents has an additional importance in the prevention atherosclerosis, once (i) it represents the last critical period of adipocyte differentiation (Dietz, 1994), and there is no consensus about the unchangeably on the number of adipocytes thereafter (Daniels et al., 2005); (ii) Obese adolescents present higher levels of Sedentary Behavior, and lower levels of PA, sports participation and CRF (Farpour-Lambert et al., 2009); (iii) The course of obesity may be changing, presenting a new trend to major increments in abdominal than in overall obesity (McCarthy, Ellis, & Cole, 2003) and central adiposity may be more reliable than BMI for the prediction of an early stage of atherosclerosis (Elkiran et al., 2013); And finally, because (iv) adolescent body fatness is associated with adult Cardiovascular (CV) risk factors (Eisenmann, 2004), and increased CIMT (Oren et al., 2003).

Objectives

The main objective of this study is to analyze the associations between Sedentary Behavior, PA, CRF and CIMT among obese adolescents, while controlling for central adiposity.

Main objectives and hypotheses

(i) Analyze if time spent in Sedentary Behavior is associated with CIMT development, since Sedentary Behavior is associated with insulin resistance (Sardinha et al., 2008), and Systolic Blood Pressure (SBP) (Martínez-Gómez et al., 2010), which are predictors of CIMT development during adolescence (Iannuzzi et al., 2004; Stabouli et al., 2012).

(ii) Analyze if CRF, assessed by VO_2 max, can predict CIMT independently of time spent in Sedentary Behavior, once CRF may be

associated with endothelial functional health (Arnold et al., 2010; Green et al., 2004), which in turn may be associated, over time, with endothelial structural health. Furthermore, increments in CRF are more closely associated with time and intensity of PA than with time spent in Sedentary Behavior (Denton et al., 2013).

(iii) Analyze if the first two associations may be independent of BMI, but not of the central adiposity, since central adiposity seem to be more reliable in the prediction of an early stage of atherosclerosis than BMI (Elkiran et al., 2013).

Secondary objectives

(i) Detect the most reliable association of Waist Circumference (WC) with CIMT, using two different techniques of measuring WC, and comparing the result with central adiposity assessed by Dual-energy X-ray Absorptiometry (DXA). Therefore, we pretend to achieve the most reliable WC measure as an indicator of CIMT development, since WC is more practical to implement and inexpensive than DXA.

Trial design

Since this study will be part of a dissertation thesis, therefore with a limited time to be concluded and presented, we aimed to perform a cross sectional study. We expect with this design to avoid delays in data collection and analysis.

METHODS

Participants and outcomes

Study Setting

This clinical study will take place in the Faculty of Physical Education and Sports of the Universidade Lusófona de Humanidades e Tecnologias (ULHT), Lisbon, Portugal, in association with the Department of Pediatrics of the HSM, Lisbon, Portugal, and with the Faculty of Physical Education and Sports of the Universidade de Évora (UE), Évora, Portugal.

Eligibility Criteria

Inclusion Criteria

To be included all the participants have to be between 13 and 17 years-old and a BMI $\geq 95^{th}$ percentile, which represents an obesity state (Barlow & Committee, 2007). All participants have also to agree with the commitment.

Exclusion Criteria

Will be excluded all adolescents: (i) with major diseases or inability to perform regular PA, (ii) involved in other weight loss program, or (iii) with smoking habits.

Adolescents with major diseases or inability to perform regular PA will be excluded in order to avoid an adverse development of the clinical condition and to avoid outcomes bias, especially in time spent in Sedentary Behavior, PA (Williams et al., 2002) and CRF, which may influence all the others variables addressed in this study. Also to avoid bias in the outcomes, we will exclude all those with smoking habits, since over time this may induce structural changes in the endothelium, what may be observed trough CIMT (Raitakari et al., 2003), and may act as confounding variable.

Study Variables

All participants will hold a variety of anthropometric and clinical assessments, in order to evaluate the associations between primary outcomes. Secondary outcomes will be also evaluated.

Primary variables

(i) BMI (kg/m^2);
(ii) Mean and Maximum CIMT (mm);
(iii) CRF (VO2 max) (ml/kg/min);
(iv) PA (min/day);
(v) Sedentary Behavior (min/day);
(vi) Trunk Fat (g);
(vii) WC (cm).

Secondary variables

(i) Height (m);
(ii) Trunk Peripheral Fat (g);
(iii) Weight (cm);
(iv) Whole Body Fat (g);
(v) Whole Body Peripheral Fat (g).

Participant timeline

Participants will be assigned into three cohorts to be evaluated at different times. This division will allow that all the participants allocated in the same group can be evaluated at the same time, and recruit more participants for the subsequent groups. We expect to conduct the battery assessments with a six months interval between cohorts. The investigation team will meet each cohort every Saturday mornings, till all assessments are completed, which may represent a time frame of one month.

(i) Moment 1 - Height, Weight, BMI, and WC. At this first moment we will distribute the accelerometers for all subjects to use during one week;
(ii) Moment 2 – CIMT;
(iii) Moment 3 - Body composition (Trunk Fat, Trunk Peripheral Fat, Whole Body Fat, and Whole Body Peripheral Fat) and CRF. In order to spare time, half group will performed CRF evaluation, while the other half will perform DXA exam.

Sample size

Accordingly with Meyer and colleagues (2006), it is possible to find statistical significant associations between CIMT and CRF, with $p < 0.05$, in a sample size of 33 participants, using both Mean and Maximum values of CIMT.

Recruitment

All the participants will be recruited from the pediatric outpatient obesity clinic of the HSM and from schools in Lisbon area.

HSM - The study will be presented by the pediatrician to the adolescents who meet the inclusion criteria, and went the consultation for the first time. Then, the participants will be taken to the exercise physiologist, who may better explain the study and collect more personal information. We will call parents participants who do not visit the exercise physiologist in this first moment, in order to explain the study and ask their collaboration.

Schools – Initially the study will be presented to the Physical Education teachers, of all the schools we can, with the permission of the school administration. In addition, to facilitate the recruitment in schools, we will provide flyers and a promotional video to be distributed and played, respectively, by the Physical Education teachers.

Website – We will also build a website page with pertinent study information, contacts, and online recruitment.

All the participants recruited online or from schools will be addressed to the Pediatric Department of the HSM.

Data collection, management, and analysis

Data collection methods

As written previously, the participants will be divided into three cohorts, to be evaluated at different times. Measurements and instruments are summarized in Table 1.

Anthropometry

The anthropometric assessments will be conducted in the Faculties of Physical Education and Sports, of the ULHT, and in the UE. All the assessments will be assigned to trained investigators. To the exception of body composition, all the other anthropometric assessments will be performed twice as a confirmation procedure. The Technical Error of Measurement (TEM) will be calculated ($TEM=\sqrt{[\sum Dif^2/2n]}$) (Withers, Laforgia, & Heymsfield, 1999).

(i) Body Weight (bioimpedance Scale OMRON BF-511, Taiwan, Japan), measured to the nearest 0.1 kg, in the anthropometric position (with

the palms turned into thighs), with the subjects wearing fewer clothes as possible, and without shoes. ULHT;

(ii) Height (height stadiometer, SECA 217, Hamburg, Germany), accessed in the anthropometric position, without shoes, with the participant backs to the stadiometer, and after an expiratory phase. Height will be registered to the nearest 0.1 cm. ULHT;

(iii) BMI will be calculated as body weight in kilograms divided for the square of height in meters [BMI= weight (kg)/height^2 (m)] (Cole et al., 2000). ULHT;

(iv) Body composition, using Dual-energy X-ray Absorptiometry (DXA) (DXA, HOLOGIC QDR 1500, Waltham, Massachusetts, USA), performed in accordance with the American College of Sports Medicine (ACSM) protocol (American College of Sports Medicine, 2005). To assess Trunk Fat upper limbs will be kept way from the trunk in order to include all trunk but not arm tissue in the analysis. UE;

(v) WC (circumference measuring tape, SECA 203, Hamburg, Germany), measured 1 cm above the iliac crest, and WC measured at the half distance between the edge of the lower ribs and the edge of the supra-iliac crest. Both measures will be taken in the end of a common expiration, with the subject standing. ULHT.

Sedentary Behavior, Physical Activity and Cardiorespiratory Fitness

Data from Sedentary Behavior, PA and CRF assessments will be treated and/or collected, as well, in the Faculties of Physical Education and Sports of the ULHT and the UE.

(i) Sedentary Behavior time and PA will be assessed with accelerometers (ACTIGRAPH GT3X, Pensacola, Florida, USA). All the subjects will use one accelerometer above the right hip, near to the iliac crest, during at least one weekend day and two week days, except during sleep, bath or swimming. The accelerometer will be programmed to use a 5-second cycle. Only days with more than 480 m (8h) counted,

will be considered into the analysis. Moreover, periods of 60 m with "zero activity" will be interpreted as un-using equipment. The data upload will be performed with the ActiLife software, version 6.8.0. The cut-points of Sedentary Behavior, Light PA, Moderate PA, and Vigorous PA were controlled for Age (Trost et al., 2002) (Table 1);. ULHT;

(ii) CRF will be assessed with the Shuttle-Run test (SR) (FITNESSGRAM® battery test, version 8.0, Cooper institute for aerobic research, Dallas, Texas, USA), conducted as the FITNESSGRAM® test battery guidelines (Cooper Institute for Aerobic Research, 1994), also in the Faculty of Physical Education and Sports facilities, Évora. Briefly, the SR test consists in running a 20-meters distance between two points marked on the floor. The running speed is defined by audio signals from a pre-recorded CD. The test begins with a running speed of 8.0 km/h (first stage), changing to 9.0 km/h in the second stage, and increasing 0.5 km/h each minute from thereafter. The test ends when the participants fail for the second time the marks on the floor at the time determined for the audio CD, which may represent the incapacity to maintain the rhythm required. Our choice in assess CRF with the SR test is based on three main reasons: 1) the SR test is an ecological and cost-effective test, allowing multiple participants to perform the test simultaneously; 2) All the participants are familiarized with the SR test, once FITNESSGRAM® test battery is part of the Portuguese physical education curriculum, since the fifth grade; 3) It is possible and reliable estimate the maximal oxygen uptake (VO_2 max) from the SR test, using an equation [VO_2 max= 43.313+4.567*sex–0.560*BMI+2.785*stage] validated for Portuguese youth (Silva et al., 2012). UE.

Carotid Intima-Media Thickness

CIMT will be measured in the Vascular Surgery Unit of the HSM, Lisbon. In our study, CIMT will be measured with an ultrasound imager using a 12-5 MHz linear

transducer (Philips ultrasound imager HD15, Andover, Massachusetts, USA). In practical CIMT may be defined as the distance between the lumen-intima and the media-adventitia interfaces. The measurement will be performed in the longitudinal plane on both right and left common carotid arteries, about 2-3 cm proximal to the carotid bifurcation (Touboul et al., 2007). Three different places on the far wall will be measured using a semiautomatic technique (automatic plus manual correction). The participants will be examined in the supine position with the neck slight extended and head tuned slight to the side. All the measurements will be made for just one observer eliminating inter-observer variability. We will use both Mean and Maximum values of CIMT for statistical purposes since the first may be more reproducible (Stein et al., 2008) and the second may represent an advanced stage of thickness (Touboul et al., 2007), which implies a higher risk of atherosclerosis.

Data management

Data will be registered in the participant individual paper file by the investigator responsible for the assessment. Then, an investigator responsible for the central database will attribute a identification code to the participant, and register the assessment result. Central database will be storage at the computer of the investigation center of the Faculty of Physical Education and Sports of the ULHT. To guarantee the security of the central database, this will be coded with a password. Only the main responsible for the original project (TOP) and for the central database will have knowledge of the password. To access data from the central database, all the interested investigators will have to fulfill a request, which will be then evaluated by the Principal Investigators and core researchers. A specific database will be produced only with the data requested by the proposal.

Statistical Methods

Data will be analyzed using the IBM SPSS statistics (IBM SPSS statistics, version 21.0, IBM, New York, USA).

In order to evaluate the associations between Sedentary Behavior time, PA, levels of CRF (VO_2 max) and CIMT we expect to use partial correlations, controlling for Sex and Age. Is possible however include other statistical analysis, since statistical analysis are dependent on the number of participants included.

Monitoring

Data monitoring

Data Monitoring Committee (DMC) would not be formed, since we only will collect baseline data.

Harms

In the case of detected uneasiness on an assessment, the participant will have the choice of not perform the assessment. If any adverse or unintended event is reported, the psychologist of the Department of Pediatrics of the HSM will evaluate the impact of that event, and will act accordingly. All the adverse or unintended events will be discussed in a weekly reunion (see forward *Protocol amendments*). If relevant, those cases will be reported in the study.

Table 5. Accelerometry cut- points determined by age.

Age	Intensity	Counts per minute
12-13	Sedentary time	0-149
	Light PA	150-2392
	Moderate PA	2393-5374
	Vigorous PA	>5375
14	Sedentary time	0-149
	Light PA	150-2579
	Moderate PA	2580-5878
	Vigorous PA	> 5879
15	Sedentary time	0-149
	Light PA	150-2780
	Moderate PA	2781-6006
	Vigorous PA	> 6007
16	Sedentary time	0-149
	Light PA	150-2999
	Moderate PA	3000-6362
	Vigorous PA	> 6363
17	Sedentary time	0-149
	Light PA	150-3238
	Moderate PA	3239-6751
	Vigorous PA	> 6752

PA- Physical Activity

Table 6. Summary of measurements and instruments.

	Measure	Units	Instrument	Formula
	Weight	kg	Bioimpedance Scale OMRON BF-511	
	Height	cm	Height stadiometer, SECA 217	
FEFD	BMI	kg/m^2		[BMI= weight (kg)/height^2 (m)]
ULHT	WC	cm	Circumference measuring tape, SECA 203	
	PA	min/day	Accelerometer ACTIGRAPH GT3X	
	Sedentary Behavior	min/day	Accelerometer ACTIGRAPH GT3X	
	Trunk Fat	g	DXA, HOLOGIC QDR 1500	
	Trunk Peripheral Fat	g	DXA, HOLOGIC QDR 1500	
FEFD	Whole Body Fat	g	DXA, HOLOGIC QDR 1500	
UE	Whole Body Peripheral Fat	g	DXA, HOLOGIC QDR 1500	
	CRF (VO_2 max)	ml/kg/min	Shuttle-Run test, FITNESSGRAM®	[VO_2 max= 43.313+4.567*sex–0.560*BMI+2.785*stage]
HSM	CIMT	mm	Philips Ultrasound Imager HD15	

BMI - Body Mass Index; CIMT - Carotid Intima-Media Thickness; CRF - Cardiorespiratory Fitness; DXA - Dual-energy X-ray Absorptiometry; FPES UE - Faculty of Physical Education and Sports of the Universidade de Évora; FPES ULHT - Faculty of Physical Education and Sports of the Universidade Lusófona de Humanidades e Tecnologias; HSM - Hospital de Santa Maria; PA - Physical Activity; Fitness; WC - Waist Circumference.

ETHICS AND DISSEMINATION

Research ethics approval

This study was already approved by the research ethics committee (REC) of the Faculty of Medicine of the Universidade de Lisboa, Lisbon, Portugal. The approval was registered with the number 292/2013.

Protocol amendments

Any protocol modification will be communicated in a weekly reunion. This reunion will also allow clarifying any doubt about the study protocol and discuss any adverse or unintended event. All the contributing investigators will have to attend the weekly reunions.

Consent

Informed consent will be obtained from both participants and their parents, in the exercise physiologist office, in the HSM. To be included, all the participants have to agree with the commitment.

Confidentiality

Confidentiality will be kept by coding participant identification and database. (See *Data management*).

Declaration of interests

All the authors declare no conflict of interests.

Access to data

All the interested investigators who pretend to access the central database will have to fulfill a request. Then, the relevance of the request will be evaluated by the Principal Investigators and core researchers, who will decide if the investigator would or would not access the database.

Dissemination policy

Primarily, we pretend to communicate the results of this study in a conference dedicated to the participants and their parents. The communication will be leaded by one of the authors. We also aimed to submit the study to international peer-reviewed journals, related to the subject, such as Pediatrics (Official Journal of the American Academy of Pediatrics), BMC Pediatrics (BioMed Central), Circulation (Journal of the American Heart Association), Journal of the American College of Cardiology (American College of Cardiology Foundation), between others. The authorship of all the studies published resulting of this study protocol must have as first author one of the study protocol authors.

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