The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Sara Isabel Sampaio Pereira

Porto, 2019
The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Dissertation written in order to achieve the PhD degree in Sports Science included in the doctoral course in Sports Science designed by the Center of Research, Education, Innovation, and Intervention in Sport CIFI2D, Faculty of Sport, University of Porto (Decree-Law n.º 74/2006, of March 24th), supervised by Professor Dr. José António Ribeiro Maia and co-supervised by Professor Dr. Peter Katzmarzyk and Professor Dr. Donald Hedeker

Sara Isabel Sampaio Pereira
Porto, 2019

KEYWORDS: SIBLINGS, SOMATOTYPE, PHYSICAL ACTIVITY, HEALTH MARKERS, METABOLIC SYNDROME
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The mind that opens to a new idea never returns to its original size.

(Albert Einstein)

Is not the end; it is the beginning.
DEDICATION

To my Family.
To my Friends.
To my Professors.
To my Colleagues.

By your Love.
By your Light.
By your Lessons.
By your Care.
ACKNOWLEDGMENTS

O doutoramento foi um processo longo, desafiante, repleto de erros e imperfeições. Contudo, a minha bagagem também vai carregada de belas histórias, vitórias e conquistas. Tudo isto devo às pessoas incríveis que estiveram ao meu lado em todo o caminho. Muito obrigada por todo o apoio e incentivo!

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RESUMO

Esta tese pretendeu descrever e interpretar a teia relacional entre fatores individuais, familiares e ambientais na forma do corpo, composição corporal, aptidão física, síndrome metabólica e estilos de vida em pares de irmãos. Amostraram-se 1583 pares de irmãos com idades compreendidas entre 9 e 20 anos; 202 pares foram seguidos durante três anos consecutivos. As variáveis utilizadas foram colhidas com protocolos estandardizados e a informação obtida foi hierarquicamente distribuída por três domínios: individual, familiar e ambiental. Os procedimentos estatísticos recorreram aos softwares SPSS, Somatotype calculation, Mplus, MIXREGLS, Supermix e STATA. Os resultados mostraram que: (1) a semelhança fraterna é maior nos irmãos do mesmo sexo comparativamente com os irmãos do sexo oposto; (2) um índice familiar de magnitude moderado foi observado no somatótipo e na agregação de marcadores de saúde (MS); (3) os fatores individuais e familiares influenciaram, em diferentes magnitudes, as características em análise. Porém, a idade cronológica, a maturação biológica e a aptidão física (AptF) associaram-se com todos os fenótipos; (4) as características ambientais relacionaram-se com a variação nos fenótipos; (5) emergiram dois perfis de MS sendo o mais prevalente (86.7%) caracterizado por menores valores de percentagem de gordura, maiores níveis de AptF e maiores scores de dieta não saudável; (6) apenas a profissão e habilitações literárias do pai associaram-se à pertença aos perfis; (7) os valores de tracking individual são mais elevados nos marcadores de obesidade comparativamente com as componentes de AptF; (8) todos os tipos de pares tendem a ser consistentes nas suas trajetórias dos marcadores de obesidade, mas nas componentes de AptF são as irmãs que se mostraram mais consistentes; (9) os estilos de vida e as características ambientais influenciam os marcadores de obesidade. Contudo, nas componentes de AptF são as características biológicas que mais afetam as suas trajetórias. Esta tese destacou um conjunto de questões e sugestões que devem ser consideradas aquando da elaboração de programas que visem a promoção de saúde em crianças e adolescentes.

PALAVRAS-CHAVE: IRMÃOS, SOMATÓTIPO, ATIVIDADE FÍSICA, MARCADORES DE SAÚDE, SINDROME METABÓLICA
ABSTRACT

This thesis aimed to describe and interpret the network of relations between individual, familial and environmental factors in their body shape and composition, physical fitness, metabolic syndrome and lifestyle behaviours in sibling pairs. We sampled 1583 sibling pairs aged 9 to 20 years; 202 pairs were consecutively followed for three years. All data was collected using standardized protocols and the information was distributed over three nested domains: individual, family and environmental. Statistical procedures were done in SPSS, Somatotype calculation, Mplus, MIXREGLS, Supermix and STATA software. Results showed that: (1) fraternal similarity is greater in same-sex siblings compared to the opposite sex siblings; (2) A familial index of moderate magnitude was observed in the somatotype and in the clustering of health markers (HM); (3) individual and family factors differently associated with traits under scrutiny with different effect-sizes. However, chronological age, biological maturation and physical fitness (PF) associated with all phenotypes; (4) environmental characteristics were related with phenotype’s variation; (5) two HM profiles emerged: the most prevalent (86.7%) was characterized by lower values of percentage body fat, higher PF levels and higher unhealthy diet scores; (6) only father’s occupation and educational qualifications were associated with membership in these two profiles; (7) individual tracking was higher in obesity markers compared to PF components; (8) all types of pairs tend to be consistent in their trajectories of obesity markers; however, in PF components sisters were more consistent; (9) lifestyle behaviours and environmental characteristics influenced obesity markers; however, in the PF components, were the biological characteristics that most affected sibling’s trajectories. This thesis highlighted a set of issues and suggestions that need to be considered when designing health promotion programs in children and adolescents.

KEYWORDS: SIBLINGS, SOMATOTYPE, PHYSICAL ACTIVITY, HEALTH MARKERS, METABOLIC SYNDROME
# List of Abbreviations and Symbols

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<tr>
<td>%BF</td>
<td>Body fat percentage</td>
</tr>
<tr>
<td>1-MR</td>
<td>1-mile run/walk</td>
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<tr>
<td>50YD</td>
<td>50-yard dash</td>
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<tr>
<td>95% CI</td>
<td>95% confidence intervals</td>
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<tr>
<td>AIC</td>
<td>Akaike information criteria</td>
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<tr>
<td>BB</td>
<td>Brother-brother</td>
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<tr>
<td>BIC</td>
<td>Bayesian information criteria</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BS</td>
<td>Brother-sister</td>
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<td>Cardiorespiratory fitness</td>
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<td>Food frequency questionnaire</td>
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<td>Fasting glucose</td>
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<td>GS</td>
<td>Grip strength</td>
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<td>GWAS</td>
<td>Genome-wide association study</td>
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<td>HDL-C</td>
<td>High density lipoprotein cholesterol</td>
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<td>HWR</td>
<td>Height-weight ratio</td>
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<td>ICC</td>
<td>Intraclass correlation coefficients</td>
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<td>ICC_{itrk}</td>
<td>Individual sibling tracking</td>
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<td>Kgf</td>
<td>Kilogram-force</td>
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<td>LTPA</td>
<td>Leisure time physical activity</td>
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<td>MS</td>
<td>Metabolic syndrome</td>
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<td>MZ</td>
<td>Monozygotic twins</td>
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<td>PA</td>
<td>Physical activity</td>
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<td>PF</td>
<td>Physical fitness</td>
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<td>PHV</td>
<td>Peak height velocity</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SES</td>
<td>Socioeconomic status</td>
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<td>SHR</td>
<td>Shuttle-run (SR – Shuttle-run)</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SLJ</td>
<td>Standing long jump</td>
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<td>SNPs</td>
<td>Single nucleotide polymorphisms</td>
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<td>SPI</td>
<td>Sports participation index</td>
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<td>SS</td>
<td>Sister-sister</td>
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<td>TPAI</td>
<td>Total physical activity index (TPA – Total physical activity)</td>
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<td>Triglycerides</td>
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<td>TV</td>
<td>Television</td>
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<td>WC</td>
<td>Waist circumference</td>
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<td>Z-MS</td>
<td>Total metabolic syndrome score</td>
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<td>Z-Muscular fit</td>
<td>Muscular fitness z-score</td>
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<tr>
<td>β</td>
<td>Beta (regression coefficient)</td>
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<td>p</td>
<td>p-value</td>
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<td>ρ</td>
<td>Rho (intraclass correlation)</td>
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<td>σ²</td>
<td>Variance</td>
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<td>χ²</td>
<td>Chi-square</td>
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<td>More or less</td>
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<td>~</td>
<td>Approximately</td>
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CHAPTER I

GENERAL INTRODUCTION, PURPOSES AND FORMAL STRUCTURE OF THE THESIS
GENERAL INTRODUCTION

1. Child and adolescent growth, maturation and development

Infancy and adolescence comprise two critical periods of life remarkably characterized by accelerated physical growth and development (Ellison & Reiches, 2012). A myriad of changes take place during these periods including biological maturation (Malina, Bouchard & Bar-Or, 2004), increases in height and weight (Tanner, 1970), skeletal growth coupled with increases in skeletal mass as well as changes in various compartments of the body composition (Malina et al., 2004). The sequence of these changes is apparently consistent among children and adolescents (Tanner, 1971). However, their timing and tempo vary considerably not only among individuals but also between the sexes (Marshall & Tanner, 1969, 1970; Tanner, 1971). At the time of childhood and adolescence subjects learn and develop their fundamental and culturally referenced motor skills, several attributes and behaviours, as well as distinct cognitive functions that will be important in their later life as adults, especially in terms of their health (Beunen & Malina, 1988). Hence, physical growth, biological maturation and motor development as well as their links to active and healthy lifestyles are intertwined processes (Robinson, 2018; Werneck et al., 2016).

In very general terms, physical growth refers to increases in body size (Malina, 2016), and height and weight are the two most important body dimensions extensively used in research to examine children and adolescents’ physical growth. These body dimensions increase gradually during childhood and a period of rapid growth (called a growth spurt) is observed at the beginning of adolescence (9-10 years in girls and 11-12 years in boys approximately) until it reaches a peak – peak height velocity (PHV) (Malina et al., 2004). Thereafter, the rate of growth has a propensity to decrease and growth in height may eventually stop (Malina, 2016). The growth spurt in body weight begins slightly after PHV and its expression is far more complex than height as it results from a composite measure of many body tissues, although it is often viewed in terms of its fat-free mass and fat mass. Fat mass increases more gradually during
childhood and adolescence (Malina, 2016). Although individuals undergo numerous changes in their biological and cognitive functions, they all vary in their growth spurt, PHV as well as in the “termination” of their growth (Roche & Sun, 2003; Tanner, 1962).

There are many candidate factors to explain the inter-individual variation observed in physical growth (Rogol, Clark & Roemmich, 2000). Biological maturation is by far an important factor that also varies among individuals (Beunen, Rogol & Malina, 2006). Generally speaking, biological maturation refers to progress towards maturity or the biologically mature state and their status and progress of children and adolescents are typically viewed according to two main biological systems: skeletal and sexual (Tanner, Whitehouse, Marshall, Healy & Goldstein, 1975). Moreover, biological maturation is a dynamic process marked by two of its important facets – timing and tempo. Timing refers to when specific maturational events occur (e.g., the age at the appearance of pubic hair in boys and girls) and tempo refers to the rate at which maturation progresses (e.g., how quickly or slowly boys and girls passed through the adolescent growth spurt) (Malina et al., 2004).

A widely used marker of maturity status is the age-at-PHV. Although it requires longitudinal data and a suitable mathematical model to estimate its value, a relatively recent and widely used method to identify such an event is the maturity offset (Mirwald, Baxter-Jones, Bailey & Beunen, 2002). In brief, this method estimates the distance, in decimal years, each subject is from age-at-PHV. A positive (+) maturity offset represents the number of years a given subject is beyond age-at-PHV, whereas a negative (−) maturity offset represents the number of years he/she is before age-at-PHV. While this occurs, human development proceeds simultaneously in several domains – cognitive, social, emotional, moral and motor (Berger, 2008).

Recent research in Motor Development has tried to link motor skills and motor performance (especially physical fitness) with health outcomes across the lifespan (Stodden et al., 2008). Very briefly, motor development refers to a continuous process of changes in motor behaviour across the lifespan (Cech &
Martin, 2012). These changes emerge from a set of interacting biological, behavioural and environmental factors that altogether induce changes in motor competence (Newell, 1986). This can be easily apprehended in descriptive or normative changes in motor behaviour.

Considering the complex network underlying motor behaviour there has been an urge to describe and interpret it and a few heuristic models have been proposed (Newell, 1986; Stodden et al., 2008). For example, Karl Newell (1986) proposed a model highlighting the effects induced by the individual, task and environmental constraints in promoting changes in human movement across time, where patterns of these interactions lead to significant changes in motor development. More recently, Stodden et al. (2008) proposed a conceptual model hypothesizing the relationships among physical activity, motor competence, perceived motor competence, health-related physical fitness, and obesity. Basically, these authors emphasized the key role of motor competence to promote positive or negative trajectories in physical activity and weight status, with physical fitness and perceived motor competence functioning as putative mediators.

2. A complex network of growth, fitness, lifestyle and health underlying human development

Although anchored within the lifespan perspective, human development does not limit itself only to physical growth and development of motor and cognitive skills. There are many other important features that children and adolescents need to acquire and develop throughout their life (Bornstein, 2014). As such, development can be seen as an evolving multidimensional dynamical system, operating on several interrelated factors and characteristics, i.e., biological, behavioural and environmental (Jelenkovic et al., 2016; Johnson, Llewellyn, van Jaarsveld, Cole & Wardle 2011; Patwardhan, Mutalik & Tillu, 2015; Sallis & Nader, 1998). Furthermore, these factors and characteristics change at different time scales and may show continuities as well as discontinuities revealing their consistency and change across the lifespan (Bornstein, Putnick & Esposito, 2017).
Despite the existence of different ways of approaching the study of human development, the following goals have prevailed across studies: 1) to describe and identify changes across the lifespan; 2) to explain and predict these changes and; 3) to identify putative factors and characteristics that also contribute to healthy or unhealthy trajectories of the human condition, as well as their consistencies and changes over time (Salkind, 2004). In sum, human development studies focus on how different individuals are from one another. However, addressing these issues is not an easy task as it may seem. Instead, this is a very challenging endeavor as the complexity and multidimensionality that characterize human development requires the adoption of more comprehensive and systematic approaches linking the network of biological, behavioural and environmental characteristics on physical growth and development.

Such complexity was foreseen by Urie Bronfenbrenner (1994) when he proposed his integrated heuristic approach - the bioecological model of development. This model consists of an extension of Bronfenbrenner's original theoretical framework known as ecological systems theory. The theoretical framework of the bioecological model highlights the relevance of understanding the bidirectional influences between children and adolescents' development and the environments in which they live (Bronfenbrenner, 1994). Such a model stressed the need to understand individuals' development within their environments (Bronfenbrenner, 1994). Therefore, it conceptualizes the environment in terms of five ecological systems, namely: microsystem (interpersonal interactions and relationships established with immediate environments), mesosystem (interconnections between several nuances of the microsystem), exosystem (indirect environment), macrosystem (social and cultural values that affect an individuals’ environment) and chronosystem (how both individuals and their surrounding environments continually evolve over time) (Bronfenbrenner & Ceci, 1994). Bronfenbrenner and Morris (1998) further postulated an additional model: The process-person-context-time model. Hence the foundation for the bioecological model, viewing human development as a set of processes through which the person and the environment reciprocally interact.
together to produce consistency and change in individuals in their lifespan (Bronfenbrenner, 1994). This model includes four intertwined and fundamental concepts in human development: the person, the process, the context and time. Particularly, it allows the analysis of variation in the process and product of development combining the influence of personal and environmental characteristics (Bronfenbrenner & Morris, 1998). Although these models have been originally established within developmental psychology, they have been extended to other domains of the human life. For example, Ruy Krebs (1997) was the first to extrapolate these ideas to the field of Physical Education and Sports Sciences in the Portuguese speaking countries. He emphasized the usefulness of the ecological perspective in the study of physical growth, motor development, and physical fitness as well as the influence of environmental characteristics (Krebs, 1997).

Similarly, Sallis et al. (2006) applied the theoretical rationale of the ecological perspective to physical activity epidemiology trying to identify putative environmental and policy influences on four domains of an active life: recreation, transport, occupation, and household. Moreover, Sallis, Owen & Fisher (2008) scrutinized the applicability of the ecological model in examining health behaviours. More recently, Katzmarzyk et al. (2013) embraced these ideas and developed a multinational study involving twelve countries - The international Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE). This study was conceived to help in answering major questions concerning the input of distinct factors from multiple levels of the socio-ecological model to childhood obesity and related behaviours (Katzmarzyk et al., 2019).

The last decades have witnessed a significant increase in research conducted within schools, sports and familial contexts aiming to better understand the complex and dynamic relationships between physical growth, physical fitness, motor development and the environment (Antunes et al., 2018; Barnett, Hinkley, Okley, Salmon, 2013; Chaves et al., 2015; Christiansen, Mora & Herrera, 1975; Ferreira, Godinez, Gabbard & Vieira 2018; Flôres, Rodrigues, Copetti, Lopes & Cordovil, 2019; Gomes, dos Santos, Zhu, Eisenmann & Maia, 2014; Kahan & McKenzie, 2017; Lim, Donovan, Harper & Naylor, 2017; Souza
et al., 2017; Sudfeld et al., 2015; Thomas & Strauss, 1992; Zaqout et al., 2016). Moreover, there has also been an “explosion” of research linking health/unhealthy behaviours and outcomes to obesogenic environments. These environments have significantly contributed to the development of non-communicable diseases (e.g., cardiovascular diseases, cancers, chronic respiratory diseases, and diabetes) (Ford, Patel & Narayan, 2017; Frumkin & Haines, 2019; Papas et al., 2007). Behaviours like physical inactivity, sedentariness, and unhealthy dietary intake are risk factors connected with increases in non-communicable diseases (World Health Organization, 2011) besides entailing enormous economic burdens (Chen, Kuhn, Prettner & Bloom, 2018; Muka et al., 2015). On the contrary, the adoption of health-related behaviours like regular involvement in moderate-to-vigorous physical activities, use of healthy diets, combined with adequate physical fitness (Smith et al., 2014), and healthy body fat levels (May, Kuklina & Yoon 2012) are likely to reduce the risk of development of non-communicable diseases.

Lifestyle behaviours acquired during infancy and adolescence are central to health and physical growth (Wiium, Breivik & Wold, 2015). Its importance goes far beyond its role in achieving, for example, energy balance to prevent and treat obesity and overweight, as well as adequate daily physical activity levels, which improves cardiovascular and metabolic health, brain/mental health, and musculoskeletal health. In fact, these lifestyle behaviours tend to track from childhood to adulthood, indicating that early and ongoing opportunities for the development of healthy markers are needed to benefit maximum adult health and to prevent diseases (Haire-Joshu & Tabak, 2016; Warburton, Nicol & Bredin, 2006). The interconnectedness of such a constellation of markers is influenced by individual, familial and environmental characteristics (Sallis et al., 2008). Therefore, we claim the need to adopt a holistic model (i.e., facets of the bioecological model) to unravel aspects of human development and health trajectories considering a combination of factors that exert dissimilar influences between them.
3. Siblings: a particular subsystem and its link to multilevel modeling

Under this rationale, a central tenet of research is to identify potential factors accounting for the variation in body composition, physical fitness, metabolic risk and lifestyle behaviours. Genetic and environment variability are the most expected factors to explain human variability (Jelenkovic, Hur, et al., 2016; Jelenkovic, Sund, et al., 2016). Members of a particular subsystem - the family - share a substantial part of their lives, namely: genes, family environment, built environment, school, friends, etc. Therefore, it is expected that individuals within the same family will be more similar to one another than individuals from different families in their physical growth and fitness, lifestyle and health. However, this does not always occur and the variation between related subjects is also remarkable (Plomin & Daniels, 2011). Hence, a fundamental question that may emerge is the following: Why are individuals in the same family more different than alike?

Researchers interested in this phenomenon have been using related subjects’ designs, i.e., extended or nuclear families, siblings and twin pairs, to examine the degree of familial resemblance based on two different statistics, namely: heritability and correlation (Bochud, 2017; Elston, Olson & Palmer, 2002; Visscher, Hill & Wray, 2008). Briefly, heritability estimates how much of the total variation in a phenotype is due to variation in genetic factors, thereby expressing aspects of familial aggregation. Familial correlation expresses the degree of homogeneity among family members in any given trait, i.e., the degree of their resemblance. Previous studies have clearly shown that an extended plethora of characteristics tend to aggregate within families to varying degrees. For example, Rebato, Jelenkovi and Salces (2007) using nuclear families showed a low-to-moderate familial aggregation in somatotype components (heritability estimates of 0.55, 0.52 and 0.46 for endomorphy, mesomorphy and ectomorphy, respectively). Additionally, they also reported low familial correlations across all types of family members. Similarly, Katzmarzyk et al. (2000) in a nuclear family study but using body composition markers also identified low-to-moderate familial aggregation (heritability estimated ranging from 0.29 in waist circumference to 0.60 in body mass index), as well as low
familial correlations (between 0.00 for waist circumference to 0.30 for body mass index). Furthermore, other studies indicated that familial resemblance is likely due to genetic and common environmental factors. For example, de Chaves et al. (2014) using Portuguese nuclear family data estimated familial resemblance in body fat, blood pressure and total physical activity, as well as the magnitude of genetic and environmental influences and showed that genetic and common environmental factors explained 30% to 44% of body fat and blood pressure, and 24% of total physical activity.

Despite the relevance of shared factors within families in explaining part of the total variation in several phenotypes at the population level, most of the available research has also highlighted the importance of non-shared influences (i.e., biological, behavioural or environmental characteristics), as relevant facets that may act differently in each family member (Plomin & Daniels, 2011; Turkheimer & Waldron, 2000). This feature suggests the unique or uncorrelated exposure of family members, and non-related subjects, to shape many of their traits and characteristics. Furthermore, available data provided evidence regarding the reciprocal interaction between shared and non-shared factors which may contribute for the variation in the expression of many phenotypes (Robinson, Wray & Visscher, 2014). Indeed, there is a powerful dichotomy, albeit unnecessary, between those traits that are inherited (genetic inheritance) and those which are acquired as a product of external factors (i.e., exposure or experience and learning of an individual).

This brings us to the concepts of nature versus nurture and their interaction. Galton in 1874 clearly defined these concepts: “Nature is all that a man brings with himself into the world; nurture is every influence that affects him after his birth” (Galton, 1874). At the beginning, there was the belief that nature and nurture factors were opposite influencers, i.e., both contributed independently to our traits, but we now undoubtedly know that nature and nurture mutually cooperate to make us what we are (Moore, 2002).

A very fertile ground of research has attempted to estimate the relative contribution of genes, along with shared and non-shared environmental factors.
to explain variation in diverse phenotypes describing physical growth, physical fitness, lifestyle and health. For example, Maia, Thomis and Beunen (2002) tried to address these issues in sports participation index (SPI) and leisure time physical activity (LTPA) using Portuguese twins. The authors found that genetic factors explained 68.4% and 63% of the variation in SPI and LTPA in males, respectively. Moreover, common environment contribution was 20% for SPI and not significant for LTPA, while unique environment explained 11.6% and 31.8% of SPI and LTPA, respectively. In females, genetic factors explained 63% and 32% of SPI and LTPA, respectively, whilst common environment explained 28.4% and 38% and unique environment 31.8% and 30% of the variation in SPI and LTPA, respectively. Also, previous studies conducted in families showed that the intra-generational resemblances tend to be higher than inter-generational, reflecting the potential of using siblings and twins’ samples to explore these questions (Aarnio, Winter, Kujala & Kaprio, 1997; An et al., 1999; Hu et al., 2013; Johnson et al., 2012; Katzmarzyk et al., 2000; Perusse et al., 2000).

It is well-known that full biological siblings share a substantial part of their genes identical-by-descent, on average 50%, and develop under relatively similar circumstances. Moreover, they also share common family environments as well as neighborhood histories and school contexts. However, they differ in their chronological age, sex and health behaviours, as well as in their physical growth, biological maturation, and motor development trajectories (Frisell, Oberg, Kuja-Halkola & Sjolander, 2012; Keyes, Smith & Susser, 2013). Altogether, these factors may be linked to differences in their similarity and/or dissimilarity trajectories of their life histories.

However, we were not able to find studies that have attempted to seriously examine sibling resemblance in terms of their physical growth, physical fitness, lifestyles and health, while simultaneously investigating the association of biological, behavioural and environmental characteristics on these relationships. It is worth mentioning that such an approach for studying sibling resemblance requires examining the dependence of observations. Indeed, this is very important because siblings within a family share common environments and
genes, since the family orbit is characterized by a nested, hierarchical structure. Consequently, the observations obtained from siblings within families are not independent. Rather, they tend to be dependent and/or correlated. In fact, Minuchin (1985) stated that “If the individual is part of an organized family system, he or she is never truly independent and can only be understood in context”. However, the majority of traditional methods commonly used to address these issues have struggled to cope with the nested structure that characterizes family data. Hence, a major question here is: What sort of methods and/or statistical tools may be well suited for measuring and analyzing the clustered data?

Multilevel modeling encompasses a set of methods and statistical tools that allow us to adequately analyze non-independent or clustered data such as children nested in families or children nested within their classrooms. Under this multilevel framework, the total variance is partitioned into different levels coherent with the nested structure that characterize the data (Hox, 2010). For example, when analyzing sibling pairs, we can estimate the between- and within-sibling variances. The within-sibling variance represents the extent to which siblings within the family differ from one another with higher values indicating more dissimilarity between them. The between-sibling variance indicates the extent to which sibling pairs differ from each other relative to the overall mean of the response variable. Moreover, this procedure is extremely flexible as it allows researchers to embed in the same equation (at the sibling level) putative covariates (i.e., confounders or predictors) as well as their interactions. In sum, using sibling’s data in conjunction with multilevel modeling help us to better understand the ecology of the family, and thus allows us to answer the question: Why are siblings more different than alike?
GENERAL INTRODUCTION, PURPOSES AND FORMAL STRUCTURE OF THE THESIS

The present thesis was conceived and methodologically designed with the following aim always in mind: describe and interpret the complex intertwined relationship between individual, familial and environmental characteristics in body shape and composition, physical fitness, metabolic syndrome, and health behaviours in sibling pairs (see Figure 1). This endeavor, framed within Bronfenbrenner’s ecological dynamical approach, required the systematic use of the multilevel modeling statistical framework with data arising from cross-sectional and longitudinal studies to search for an in-depth understanding regarding these phenotypes within the family orbit.

Figure 1. An illustration of the Portuguese siblings’ study model

Given the complexity of the issues tackled by this thesis (see Figure 2), a set of specific purposes were outlined based first on cross-sectional data: (1) investigate sibling resemblance in body shape, physical fitness, physical activity and metabolic syndrome adjusted for individual, familial and environmental characteristics; (2) examine the joint effects of familial and environmental characteristics in multivariate profiles of health-related markers.
Secondly, we relied on longitudinal data (see Figure 3) to: (1) analyze the tracking of individuals within their sib-ship in obesity markers and physical fitness components during 2 years of follow-up; (2) probe the consistency in sibling resemblance in these markers and components; (3) analyze the combined influence of individual and familial characteristics on trajectories of these phenotypes. To meet these purposes siblings were followed during three consecutive years (2011-2013).
FORMAL STRUCTURE OF THE THESIS

This thesis encompasses a compilation of seven original papers that were published in peer-reviewed journals. Chapter I comprises the general introduction, the purposes and the outline of the thesis; chapter II presents the general methodology; chapter III contains all the research based on cross-sectional data, whereas chapter IV is dedicated to the longitudinal research. Chapter V presents a general discussion, conclusions, and limitations of the thesis together with some indications for future research.

Table 1. Thesis formal outline

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<thead>
<tr>
<th>Chapter I</th>
<th>General introduction, purposes and formal structure of the thesis</th>
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<tr>
<td>Chapter II</td>
<td>General methodology which include study sample and procedures</td>
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<tr>
<td>Chapter III</td>
<td>Research based on cross-sectional data</td>
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**Paper I**

Multilevel modelling of somatotype components: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Aims: to estimate siblings’ resemblance in their general somatotype; and to investigate the effects of biological, behavioural and familial with siblings’ somatotype components as well as the resemblance in somatotype components.

*Published in Annals of Human Biology* (2016),
doi:10.1080/03014460.2016.1243727

Authors: Sara Pereira, Peter T. Katzmarzyk, Thayse Natacha Gomes, Michele Souza, Raquel N. Chaves, Fernanda K. dos Santos, Daniel Santos, Donald Hedeker, José Maia

**Paper II**

Resemblance in physical activity levels: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

15 Sara Pereira
Aims: to investigate the relationships of biological, behavioural, familial, and environmental characteristics with siblings' physical activity (PA) levels as well as the intrapair resemblance in PA.

Published in American Journal of Human Biology (2017), doi: 10.1002/ajhb.23061

Authors: Sara Pereira, Peter T. Katzmarzyk, Thayse Natacha Gomes, Michele Souza, Raquel N. Chaves, Fernanda K. dos Santos, Daniel Santos, Donald Hedeker, José Maia

**Paper III**

A multilevel analysis of health-related physical fitness. The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Aims: to investigate biological, behavioural and sociodemographic correlates of intrapair similarities, and estimates sibling resemblance in health-related physical fitness.

Published in PloS One (2017), doi:10.1371/journal.pone.0172013

Authors: Sara Pereira, Peter Todd Katzmarzyk, Thayse Natacha Gomes, Michele Souza, Raquel Nichelle Chaves, Fernanda Karina dos Santos, Daniel Santos, Donald Hedeker, José Maia

**Paper IV**

Sibling Similarity in Metabolic Syndrome: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Aims: to estimate sibling resemblance in metabolic syndrome (MS) markers, and to investigate the associations of biological and behavioural characteristics with MS.


Authors: Sara Pereira, Peter T. Katzmarzyk, Thayse Natacha Gomes, Rojapon Buranarugsa, Marcos A. Moura-Dos-Santos, Donald Hedeker, José Maia
Paper V

Profile Resemblance in Health-Related Markers: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Aims: to identify multivariate profiles of health-related markers, analyze their associations with biological, sociodemographic, and built environment characteristics, and to estimate sibling resemblance in these profiles.

Published in International Journal of Environmental Research and Public Health (2018), doi: 10.3390/ijerph15122799

Authors: Sara Pereira, Peter T. Katzmarzyk, Donald Hedeker, José Maia

Chapter IV

Research based on longitudinal data

Paper VI

Change and stability in sibling resemblance in obesity markers: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

Aims to: (1) describe mean changes in obesity markers across sib-ships; (2) analyze tracking of individuals within their sib-ship in these markers during 2 years of follow-up; (3) probe consistency in sibling resemblance in these markers, and (4) analyze the joint influence of individual and familial characteristics in these markers.

Published in Journal of obesity, doi: 10.1155/2019/2432131

Authors: Sara Pereira, Peter T. Katzmarzyk, Donald Hedeker, José Maia

Paper VII

Change and stability in sibling resemblance in physical fitness: The Portuguese Sibling Study

Aims to: (1) describe mean changes in muscular and motor fitness components in sib-ships over 2-years; (2) analyze individual tracking within sib-ships in these components; (3) investigate sibling resemblance over time,
and (4) examine the joint influence of biological, behavioural and familial characteristics in their fitness.

*Accepted for publication in Medicine & Science in Sports & Exercise*

Authors: Sara Pereira, Peter T. Katzmarzyk, Donald Hedeker, José Maia

| Chapter V | General overview, limitations and conclusions of the thesis as well as future research. |
REFERENCES


CHAPTER II

GENERAL METHODOLOGY
GENERAL METHODOLOGY

1. The Portuguese sibling study on growth, fitness, lifestyle and health

Under the umbrella of the Portuguese Healthy Family Study we designed and conducted seven sub-studies aiming to increase our understanding of familial resemblance in body shape and composition, metabolic syndrome, physical activity, and physical fitness (S. Pereira et al., 2019). The present thesis stems from one such sub-study and is entitled: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health. This study aims to describe and interpret the complex intertwined relationship between individual, familial and environmental characteristics in body shape and composition, physical fitness, metabolic syndrome, and health behaviours in sibling pairs.

The data collection was conducted between 2011 and 2013 during the school year (i.e., from September to June) under the supervision of Professor José Maia of the KineLab from the Faculty of Sport, University of Porto. The research team comprised 9 members. A total of 30 schools from mainland (north and center) Portugal and from the Azores islands were enrolled in the study. The following operational steps were followed: 1) selected schools were contacted and the project was presented to the coordinator of the Physical Education Department, school Principal and Pedagogical Department as well as to the Parent Council; 2) after agreement a consent form (invitation to participate in the study) was sent to all students who had their siblings studying in the same school.

The Portuguese sibling study on growth, fitness, lifestyle and health was approved by the Ethics Committee of the University of Porto as well as by all school authorities.

2. Study Sample

The sample comprised 1583 sibling pairs aged 9-20 years. Moreover, 202 sibling pairs were followed for three consecutive years (2011-2013). Participants were sampled from schools in mainland (north and central) Portugal and from
the Azores islands. This was a convenience sample as is common in all family studies. Additionally, it is also part of the Portuguese Healthy Family Study (D. M. V. Santos, 2013). Children and adolescents who had their siblings studying in the same school were invited to take part in the study, and the response rate was ~80%. Further, children and adolescents with chronic diseases, physical handicaps or psychological disorders were excluded. Parents or legal guardians provided written informed consent.

3. Procedures

3.1. Individual characteristics

**Anthropometry**

Anthropometric measures [height, weight, skinfolds (triceps, subscapular, suprailiac and calf), girths (flexed upper arm, and calf), and breadths (bicipitellar humerus and femur)] were assessed using standardized protocols established by The International Society for the Advancement of Kinanthropometry (Ross & Ward, 1986). Height was measured with a portable stadiometer (Holtain, UK), weight with a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan), skinfolds with a Holtain caliper, girths with a non-elastic tape (Sanny, American Medical of Brazil, Brazil), and breadths with a Holtain compass.

**Biological maturation**

Biological maturation was estimated with the maturity offset procedure, proposed by Mirwald, Baxter-Jones, Bailey and Beunen (2002), which estimates the distance, in decimal years, each subject is from age at peak height velocity (PHV). A positive (+) maturity offset represents the number of years the participant is beyond PHV, whereas a negative (−) maturity offset represents the number of years the participant is before PHV.

**Body fat**

Body fat percentage was estimated with youth in light clothing and using a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body
Composition Analyzer Tanita Corporation, Tokyo, Japan). This method has been shown to be reliable and valid (Kabiri, Hernandez & Mitchell 2015).

Body mass index

Body mass index was computed using the standard formula: Weight (kg)/Height² (m)

Body shape

Somatotype components were calculated according to the Health-Carter method (Heath & Carter, 1967) which provides for an anthropometric derivation of a three-component - endomorphy, mesomorphy and ectomorphy - rating showing their relative dominance. Endomorphy reflects the relative fatness and was obtained by the following formula: endomorphy = - 0.7182 + 0.1451 (X) - 0.00068 (X²) + 0.0000014 (X³), where X = (sum of triceps, subscapular and supraspinale skinfolds) multiplied by (170.18/height in cm). Mesomorphy represents the relative musculoskeletal robustness and was estimated by: mesomorphy = 0.858 x humerus breadth + 0.601 x femur breadth + 0.188 x corrected arm girth + 0.161 x corrected calf girth – height 0.131 + 4.5. Ectomorphy expresses the relative linearity of body physique. For this component three different equations were available according to the height-weight ratio (HWR): If HWR ≤ 38.25 then ectomorphy = 0.1; HWR between 38.25 and 40.75 then ectomorphy = 0.463 HWR - 17.63; HWR ≥ 40.75 then ectomorphy = 0.732 HWR - 28.58.

Health-related physical fitness

Using the Bouchard and Shephard (1994) health-related physical fitness model encompassing morphological (waist circumference and body fat percentage), muscular (strength and power), motor (speed and agility) and cardiorespiratory (aerobic capacity) components, all siblings were assessed with the following tests:

a) Morphological component: Body fat percentage (%BF) was estimated using a portable bioelectrical impedance scale (Tanita BC-418 MA segmental body composition analyser, Tanita Corporation, Japan). Waist
circumference (WC), anatomically identified as the smallest circumference between the lowest rib and the superior border of the iliac crest, was measured, in centimeters (cm), with a non-elastic tape (Sanny, American Medical of Brazil, Sao Paulo, Brazil).

b) Muscular component: Muscular strength, namely static strength, was assessed with the grip strength (GS) test using a hand dynamometer (Takei Digital Grip Strength Dynamometer, Model T.K.K.5401, Tokyo, Japan), and the result was recorded in Kilogram-force (kgf). Muscular power was obtained with the standing long jump test (SLJ), and the results were recorded in cm.

c) Motor component: Speed was assessed with the 50 yard dash (50yd), and agility was evaluated through the shuttle-run (SHR) test; time was recorded in seconds (s).

Metabolic Syndrome

Metabolic syndrome markers included WC, high-density lipoprotein cholesterol (HDL-C), triglycerides (TRI), fasting glucose (GLU), and systolic blood pressure (SBP). SBP was measured with an automatic digital Omron sphygmomanometer (Omron M6, hem 7001-E, Omron Healthcare). All participants rested for at least 5 min before the first measurement. Three consecutive measures were obtained, with a 2 or 3-min interval between them, and the mean value was used for analysis. If the difference between the three measurements was greater than 10 mm Hg, a fourth measurement was taken and the measurement with higher differences was removed; HDLC, TRI, and GLU were obtained after a 10–12 hours overnight fast from a finger stick blood sample, and analyzed by the Cholestech LDX (Cholestech Corporation, Hayward, CA, USA), a previously validated instrument (Cholestech LDX, 2003). Daily checks of the equipment were made according to the manufacturer’s instructions.

Physical activity

Physical activity (PA) was estimated with the Baecke questionnaire (Baecke, Burema & Fritjers, 1982), a reliable (Miller, Freedson & Kline, 1994; M. A. Pereira et al., 1997); and valid instrument (Helmerhorst, Brage, Warren,
Besson & Ekelund, 2012; Philippaerts, Westerterp & Lefevre, 1999); that describes three basic PA domains: work/school PA (questions related to sitting, standing, walking, lifting and sweating during work/school), leisure-time PA (questions associated with mode of transportation to school and time spent watching TV, walking and cycling), and sport participation (frequency of practice and sweating during sports’ practices). The Portuguese version of this questionnaire has been widely used in children and youth (Antunes et al., 2015; Seabra, Mendonça, Thomis, Malina & Maia, 2007), twin studies (J. A. Maia et al., 2002), as well as in family studies (J. Maia et al., 2014; D. M. Santos et al., 2014). The total physical activity index (TPAI) was obtained from the unweighted sum of the three domain scores. For each domain, the score ranges from 1 to 5, such that the TPAI varies between 3 and 15. All participants answered the questionnaire during their physical education classes under the supervision of their physical education teacher and a trained member of the research group.

Screen time

Information about screen time was obtained using the U.S. Youth Risk Behaviour Surveillance Survey (U.S. Centers for Disease Control and Prevention, 2013) questionnaire by self-administered questions: “How long do you watch TV per day?” and “How long do you use your computer or play non-active video games per day?” and the response options for both questions, were: (1) <30 minutes; (2) 30 minutes-1 hour; (3) 1 hour-1:30 hours; (4) 1:30 hours-2 hours; (5) >2 hours). Importantly, available literature provides evidence of several techniques used to assess sedentary behaviour. Yet, the use of screen time as a marker of sedentary behaviour in children is by far the most commonly used. Self-reported information about sedentary behaviour has also been generally used in studies from different countries and/or cultures (Arango et al., 2014; Rey-Lopez et al., 2012; Rey-Lopez et al., 2011) as well as with Portuguese samples (S. Pereira et al., 2015; Vasconcelos & Maia, 2001).

Dietary intake

Diet intake data were obtained from a food frequency questionnaire (FFQ) adapted and modified from the Health Behaviour in School-aged Children
Survey (HBSC) (Currie et al., 2008) using typical Portuguese food items. This questionnaire was systematically used in multi-country studies (Katzmarzyk et al., 2013). Youth were asked about various types of food consumed in a typical week. For each item, the reported answers were converted into weekly portions as follows: “never” = 0; “less than once per week” = 0.5; “once per week” = 1; “2–4 days per week” = 3; “5–6 days per week” = 5.5; “once a day, every day” = 7; and “more than once a day” = 10, as previously advocated (Mikkila et al., 2015). Food items related to healthy diet were as follows: fruits, vegetables, dark-green vegetables, orange vegetables, fruit juice, skim milk, low-fat milk, whole milk, cheese, other milk products, bread or whole grains, beans, lentils, bean curd, eggs, fish. Food items related to unhealthy diet were as follows: sweets, sugar-sweetened sodas, cakes, pastries, donuts, diet sodas, ice cream, potato chips, French fries, fast foods, sports drinks, energy drinks, fried food.

3.2. Familial characteristics

Parental occupation

Parents’ occupation was categorized into ten groups (from 0–9) according to the Portuguese National Classification of Occupations (2010), where Group 0 is the highest socioeconomic status (SES) and Group 9 is the lowest. Categories are as follows: (0) armed forces; (1) central administration/politicians and executive directors; (2) specialists of intellectual and scientific activities; (3) technicians and intermediate-level jobs; (4) back-office jobs; (5) security, seller, and individual services; (6) farmer and qualified workers of farm, fish, and forest; (7) qualified industry and building jobs: (8) machine and equipment operators; and (9) nonqualified jobs.

Parental education

Parents’ education was obtained according to the following categories: (1) <Grade 12; (2) Grade 12/diploma for technical qualification (equivalent to high school); (3) university level.

Parental support for physical activity
Participants answered a questionnaire concerning the perceived support for PA received from their parents. This valid questionnaire was based on Sallis, Grossman, Pinski, Patterson and Nader (1987), and includes a list of items relating to parental encouragement for children’s PA practice (Kitzman-Ulrich, Wilson, Van Horn & Lawman, 2010; Sallis, Alcaraz, McKenzie & Hovell, 1999; Sallis et al., 1988). The response options for all questions range from 1 (never) to 5 (very often), and the sum of the responses was computed to obtain a score for parental support.

3.3. Environmental characteristics

*Built Environment*

Environmental characteristics were inferred by built environment, obtained via questionnaire. We applied the Portuguese version of the Environmental module (environmental perception of the residential area) of the International Physical Activity Study, a reliable and valid instrument (Craig et al., 2003), previously used in the Portuguese population (Delgado, 2005). This questionnaire includes questions about the traffic system, accessibility to public transportation and shops/markets, housing density, perceived safety of the neighborhood, the presence of sidewalks and bike paths, and recreational facilities. The response item options were as follows: completely disagree, partially disagree, partially agree, or completely agree. For this study, options were dichotomized into two categories: 0 = disagree (completely disagree, partially disagree); 1 = agree (partially agree or completely agree).

4. Data quality and control

To ensure data quality, the following procedures were conducted: (1) training of all team members by experienced researchers of the KineLab of the Sports Faculty, University of Porto, Portugal; (2) Random retests were conducted on each assessment day, and the usual statistical techniques were used to estimate reliability - the technical error of measurement and the ANOVA-based intraclass correlation coefficient; (3) systematic checks of all data entry, and identification/correction of putative punching errors.
5. **Statistical analysis**

Basic exploratory - data checks for outliers and normality tests – as well as descriptive statistics (mean±standard-deviations) were computed. Student’s t-test and Analysis of Variance (ANOVA) to test for mean differences were done. A z-score transformation was used to standardized variables whenever necessary. These analyses were conducted in IBM-SPSS v25.

Somatotype calculations and their graphical displays (somatocharts) were done with a somatotype calculation and analysis software (MER Goulding Software Development).

Identification of the best fitting number of latent profiles in health behaviours was done in *Mplus* software 7 using an iterative maximum likelihood estimation technique.

Multilevel models were also used. Specifically, separate within- and between sib-ship variances, and therefore different intraclass correlations ($\rho$), with corresponding 95% confidence intervals (95% CI) were estimated following an approach described by Hedeker, Mermelstein and Demirtas (2012). All calculations were done in STATA (version 14), Supermix (version 2.1) (Hedeker, Gibbons, du Toit & Cheng, 2008) and in MIXREGLS software (Hedeker & Nordgren, 2013).
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CHAPTER III

RESEARCH BASED ON CROSS-SECTIONAL DATA
Multilevel modelling of somatotype components: The Portuguese sibling study on growth, fitness, lifestyle and health

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Multilevel modelling of somatotype components

ABSTRACT

Somatotype is a complex trait influenced by different genetic and environmental factors as well as by other covariates whose effects are still unclear. Our aims were: (1) estimate siblings’ resemblance in their general somatotype; (2) identify sib-pair (brother-brother (BB), sister-sister (SS), brother-sister (BS)) similarities in individual somatotype components; (3) examine the degree to which between and within variances differ among sib-ships; (4) investigate the effects of physical activity (PA) and family socioeconomic status (SES) on these relationships. The sample comprises 1058 Portuguese siblings (538 females) aged 9-20 years. Somatotype was calculated using the Health-Carter method, while PA and SES information was obtained by questionnaire. Multilevel modelling was done in SuperMix software. Older subjects showed the lowest values for endomorphy and mesomorphy, but the highest values for ectomorphy; and more physically active subjects showed the highest values for mesomorphy. In general, the familiality of somatotype was moderate ($\rho=0.35$). Same-sex siblings had the strongest resemblance (endomorphy: $\rho_{SS}>\rho_{BB}>\rho_{BS}$; mesomorphy: $\rho_{BB}=\rho_{SS}>\rho_{BS}$; ectomorphy: $\rho_{BB}>\rho_{SS}>\rho_{BS}$). For the ectomorphy and mesomorphy components, BS pairs showed the highest between sib-ship variance, but the lowest within sib-ship variance; while for endomorphy BS showed the lowest between and within sib-ship variances. These results highlight the significant familial effects on somatotype and the complexity of the role of familial resemblance in explaining variance in somatotypes.
INTRODUCTION

Somatotype is a gestalt, i.e., an overall description of body shape or physique independent of its size (Sheldon, 1954). It is represented by a series of three numbers quantifying its basic components: endomorphy (relative fatness), mesomorphy (musculoskeletal robustness), and ectomorphy (physique linearity), and the anthropometric methodology developed by Heath and Carter (1967) objectively describes it. This methodology has allowed for a plethora of research in aesthetics (W. D. Ross, Hebellinck, & Wilson, 1973), physical anthropology (Ventrella et al., 2008), physical exercise/nutrition (Bolonchuk, Siders, Lykken, & Lukaski, 2000; Koleva, Nacheva, & Boev, 2000; Mendonca, Sospedra, Sanchis, Manes, & Soriano, 2012), and sport sciences (Gutnik et al., 2015; Vila, Abraldes, Rodriguez, & Ferragut, 2015). In youth the somatotype has been associated with health-related factors such as blood pressure (Makgae, Monyeki, Brits, Kemper, & Mashita, 2007), coronary heart disease risk (Singh, 2007), echocardiographic dimensions (Katzmarzyk, Malina, Song, Theriault, & Bouchard, 1998), metabolic syndrome indicators (Katzmarzyk, Malina, Song, & Bouchard, 1998) as well as motor/sports performance (Marta, Marinho, Costa, Barbosa, & Marques, 2011; Purenovic-Ivanovic & Popovic, 2014).

Furthermore, sex and age are usually associated with individual somatotype components (Ventrella et al., 2008). For example, Mladenova, Nikolova, Andreenko and Boyadjiev (2010) investigated age- and sex-related longitudinal changes in somatotype characteristics of Bulgarian children and adolescents and showed that, during their growth, girls are significantly more endomorphic than boys, while boys tend to be more mesomorphic and ectomorphic. This general trend has been previously summarized by Carter and Heath (1990). Additionally, Mladenova et al. (2010) also reported increases in girls’ endomorphy across the study period (four years); among boys this increase was observed until the age of 11, after which it decreased. The mesomorphy and ectomorphy components also increased with age in both sexes, but with different trajectories – mesomorphy increased with age, but ectomorphy increased only until puberty, followed by a decrease which was more pronounced in girls. Moreover, somatotype has also been linked to behavioural factors, although physical activity is scarcely reported. For example,
Longkumer (2014) showed that endomorphy was significantly higher among inactive pre- and pubertal boys as compared to active ones; ectomorphy, on the other hand, was significantly higher in active pubertal boys.

Based on data from nuclear/extended families as well as from twins, it has been suggested that somatotype is influenced by the additive effects of genetic factors (Bouchard, Malina, & Pérusee, 1997). Katzmarzyk et al. (2000) examined Canadian nuclear families’ somatotypes and found that the heritability \((h^2)\) estimates, i.e., the proportion of total variation that is due to genetic factors, were of moderate effect size (56%, 68% and 56% for endomorphy, mesomorphy and ectomorphy, respectively). Rebato, Jelenkovic, and Salces (2007) reported somewhat lower values: 55% for endomorphy, 52% for mesomorphy and 46% for ectomorphy, in a similar study of Basque families. On the other hand, Peeters et al. (2003), using Belgian twins aged 10 to 18 years, showed age and sex variation in \(h^2\) estimates of the three components: in boys the \(h^2\) 95% confidence intervals ranged from 21% to 88% for endomorphy, 46% to 76% for mesomorphy and 16% to 73% for ectomorphy; for girls the range was smaller, 76% to 89% for endomorphy, 36% to 57% for mesomorphy and 57% to 76% for ectomorphy.

Somatotype data collected among siblings is apparently scarce. We were able to identify only two previous studies (Rebato, Salces, Rosique, San Martin, & Susanne, 2000; Vasques, Lopes, Seabra, da Silva, & Maia, 2006). Rebato et al. (2000), in the Basque region, showed that the highest correlation between sib-ship was for mesomorphy in same sex sibs: \(\rho=0.66\) and \(\rho=0.42\) for sister-sister and brother-brother, respectively. For opposite sex sibs (brother-sister) the correlation was the same for the three somatotype components \((\rho=0.19)\). Vasques et al. (2006), in mainland Portugal, showed different and lower correlations. For example, for sister-sister sib-ship the highest was for endomorphy \((\rho=0.46)\), and the lowest for ectomorphy \((\rho=0.42\) and \(\rho=0.27)\), within brother-brother and brother-sister sib-ship, respectively.

Data from family, twins, and siblings have a hierarchical structure where individuals are nested within families, or within sib-ship, and multilevel models provide a very flexible analytical framework for handling this type of clustered data allowing both fixed and random effects to be functions of a series of covariates (Atkins, 2005). Furthermore when using sibling data, and since sibs
share on average 50% of their genes identical-by-descent, as well as common familial environments (Lynch & Walsh, 1998), the intraclass correlation coefficient (\( p \)) computed from the variance components’ part of these models provides a suitable statistic to describe the degree to which sib-pairs are similar in their traits, even when adjusting for a specified set of covariates. Given the complex nature of the somatotype as a multi-dimensional phenotype and the possible links with physical activity levels and family socioeconomic status, it is of interest to renew the investigation on sibling resemblance in physique using the multilevel model as a framework. The key of this study is to investigate more than one child in each family to better understand how environmental, behavioural or genetic factors affect siblings’ differences and/or similarities in their somatotype. Therefore, the present study aims to: (1) estimate siblings’ resemblance in their somatotypes taken as a whole, i.e., as a multivariate representation of their physique; (2) identify different sib-pair (brother-brother, sister-sister, brother-sister) similarities in individual somatotype components; (3) examine the degree to which between and within variances differ among sibships, and (4) investigate the effects of physical activity and family socioeconomic status on these relationships.

METHODS

Study participants

The sample of the present study is part of the Portuguese sibling research project on their physical growth, body composition, physical fitness, physical activity, metabolic syndrome and health behaviours. Children and adolescents aged 9 to 20 years were recruited in schools from the north and central regions of mainland Portugal, and were invited to participate in the project with their siblings and parents, since they were also previously called to be part of the Portuguese Healthy Family Study (Santos et al., 2013, Santos et al., 2014). The siblings’ response rate was ~80%. Parents or legal guardians provided written informed consent. The project was approved by the Ethics Committee of the University of Porto as well as by school authorities. Following their approval, all identified siblings were invited to participate in the study. A total of 1058 biological siblings (538 females and 520 males) from 520 nuclear families
(96.3% two sibs; 3.7% three or more siblings), aged between 9 to 20 years, were sampled (Table 1).

Table 1. Distribution of sample size per age and gender

<table>
<thead>
<tr>
<th>Age group</th>
<th>Boys</th>
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</thead>
<tbody>
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<td>8</td>
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</tr>
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<td>19</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>520</td>
<td>538</td>
<td>1058</td>
</tr>
</tbody>
</table>

**Anthropometry**

Height, weight, skinfolds (triceps, subscapular, suprailliac and calf), girths (flexed upper arm, and calf), and breadths (biepicondilar humerus and femur) were measured by five researchers from the Kinanthropometry Lab, Faculty of Sport, University of Porto, using standardized protocols established by the International Society for the Advancement of Kinanthropometry (D. Ross & Ward, 1986). Height was measured with a portable stadiometer (Holtain, UK), weight with a portable bioelectrical impedance scale (TANITA BC- 418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan), skinfolds with a Holtain caliper, girths with a non-elastic tape (Sanny, American Medical of Brazil, Brazil), and breadths with a Holtain compass.

**Somatotype**

The somatotype components were calculated according to the Health and Carter method (1967) which provides for an anthropometric derivation of a three-
component (endomorphy, mesomorphy and ectomorphy) rating showing their relative dominance. Details concerning somatotype formulas can be found in Heath and Carter (1967).

Physical activity

Total physical activity (TPA) was estimated with the Baecke questionnaire (Baecke, Burema, & Frijters, 1982), a reliable and valid instrument (Miller, Freedson, & Kline, 1994; Pereira et al., 1997; Philippaerts, Westerterp, & Lefevre, 1999) that describes three basic PA domains: school PA (questions related to sitting, standing, walking, lifting and sweating during school); leisure-time PA (questions associated with mode of transportation to school and time spent watching TV, walking and cycling) and sport participation (frequency of practice and sweating during sport practice). The results of the questionnaire are based on 16 questions answered with a Likert-type scale wherein higher scores indicate higher PA levels. The Portuguese version of this questionnaire is widely used in children and youth (Antunes et al., 2015; Seabra, Mendonca, Thomis, Malina, & Maia, 2007) as well as in family studies (de Chaves et al., 2014; J. A. Maia, Thomis, & Beunen, 2002; Santos et al., 2014). The TPA score is obtained from the unweighted sum of the three domains. For each domain each score ranges from 1 (minimal) to 5 (maximal) and consequently the TPA score varies between 3 and 15. All participants answered the questionnaires during their physical education classes under the supervision of their physical education teacher as well as by a trained research team member.

Socioeconomic status

Socioeconomic status (SES) was assessed by asking participants about their parents’ occupations. The occupation was categorized into nine groups (from 0–9) according to the Portuguese National Classification of Occupations (2010), where group 0 is the highest SES and group 9 is the lowest. Categories are as follows: (0) armed forces; (1) central administration/politicians and executive directors; (2) specialists of intellectual and scientific activities; (3) technicians and intermediate level jobs; (4) back-office jobs; (5) security, seller and individual services; (6) farmer and qualified workers of farm, fish and forest; (7) industry and building qualified jobs; (8) machine and equipment operators and (9) nonqualified jobs.
Data quality control

To ensure data quality, the following procedures were conducted: (1) training of all team members by experienced researchers of the Kinanthropometry Laboratory of the Sports Faculty, University of Porto, Portugal; (2) conducting random retests on each assessment day to compute reliability estimates using the technical error of the measurement (TEM) - TEM=0.1 cm for height, 0.2 mm for triceps skinfold, 0.1 mm for subscapular skinfold, 0.3 mm suprailiac skinfold, 0.1 mm calf skinfold, 0.1 cm for biepicondylar breadth of the humerus and the femur, and 0.1 Kg for weight.

Statistical analysis

Basic descriptive statistics (mean; standard-deviation) were calculated in IBM-SPSS 21 after a preliminary data check for outliers and normality. Further, somatotype calculations and their graphical displays in the somatochart were done with the somatotype calculation and analysis software (MER Goulding Software Development).

Multilevel modelling of sibling data was done in a series of steps. First, we fitted a series of models, one for each somatotype component (data not shown), using the following set of covariates: age, age^2, age^3, TPA, age-by-TPA interaction age^2-by-TPA interaction, age^3-by-TPA interaction, and SES in the fixed part of the model. Covariates were centered at their respective means as advocated (Hox, 2010). Since age^3, all age-by-TPA interactions, and SES were not statistically significant in any of the models, they were dropped from subsequent analysis for matters of parsimony. Second, and following the multilevel specifications for the multivariate case suggested by Snijders and Bosker (2004), we used a three-level model where the three somatotype components are at level-1, individuals are at level-2, and sib-ships at level-3 to estimate siblings’ resemblance in their somatotype taken as an overall impression of their physique. This model treats the three somatotype components as being nested and correlated within individuals, while simultaneously treating the individuals as being nested and correlated within sib-ships. Thus, this multilevel model adequately accounts for the correlation in the data due to nesting of somatotype components within individuals, and individuals within sib-ships.
This analysis was done in SuperMix (Hedeker, Gibbons, Du Toit, & Cheng, 2008) Third, a follow-up analysis was then conducted for each of the somatotype components using a statistical approach developed by Hedeker, Mermelstein, and Demirtas (2012), where the within sib-ship and between sib-ship variances are modeled in terms of covariates; this allows us to estimate separate variances, and therefore separate intraclass correlations, for the three types of sib-ship pairs. Estimation was done with the MIXREGLS software (Hedeker & Nordgren, 2013). In all models, the brother-brother (BB) pair served as the reference category; all parameters were simultaneously estimated using maximum likelihood, with explicit details described elsewhere (Goldstein, 2003; Hedeker et al., 2008; Snijders & Bosker, 2004).

RESULTS

Descriptive statistics for anthropometry, somatotype components, SES and TPA are presented in Table 2. On average, the BB pairs are taller, heavier, with greater biepicondilar breadths, and girths, but with lower fat folds as compared to brother-sister (BS) and sister-sister (SS) pairs. SS pairs had the highest mean skinfolds. A higher endomorphy was found in SS pairs, while BB pairs showed the highest mesomorphy; the ectomorphy component was similar among the three sib-pairs. Additionally, SS pairs had lower TPA levels as well as SES when compared to BB and to BS pairs.
Table 2. Descriptive statistics (mean and standard deviations) per sib-ship

<table>
<thead>
<tr>
<th>Variables</th>
<th>Brother-brother (n=156)</th>
<th>Sister-sister (n=134)</th>
<th>Brother-sister (n=250)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>2.2</td>
<td>13.0</td>
</tr>
<tr>
<td>Basic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.2</td>
<td>13.3</td>
<td>153.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>51.8</td>
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<td>48.7</td>
</tr>
<tr>
<td>Biepicondilar breadths (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humerus</td>
<td>6.2</td>
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<td>5.7</td>
</tr>
<tr>
<td>Femur</td>
<td>8.9</td>
<td>0.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Girths (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>25.8</td>
<td>3.8</td>
<td>24.5</td>
</tr>
<tr>
<td>Calf</td>
<td>33.4</td>
<td>3.9</td>
<td>32.6</td>
</tr>
<tr>
<td>Skinfolds (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>11.7</td>
<td>5.4</td>
<td>15.7</td>
</tr>
<tr>
<td>Subscapular</td>
<td>9.6</td>
<td>5.7</td>
<td>12.2</td>
</tr>
<tr>
<td>Suprailiac</td>
<td>15.9</td>
<td>9.3</td>
<td>21.1</td>
</tr>
<tr>
<td>Calf</td>
<td>12.4</td>
<td>6.8</td>
<td>16.2</td>
</tr>
<tr>
<td>Somatotype components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endomorphy</td>
<td>3.9</td>
<td>1.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Mesomorphy</td>
<td>4.2</td>
<td>1.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Ectomorphy</td>
<td>2.9</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Demographic indicator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>6.7</td>
<td>2.8</td>
<td>7.1</td>
</tr>
<tr>
<td>Behaviour indicator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>8.2</td>
<td>1.7</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Figure 1 shows the somatocharts for each of the sib-ship pairs. The BB pairs were classified as mesomorph-endomorph (3.9-4.3-2.9), while SS and BS pairs were both classified as mesomorphic-endomorph (5.2-3.5-2.6 and 4.7-3.9-2.8, SS and BS, respectively).
Based on the variance components derived from the multivariate multilevel model which jointly analyzed the three somatotype components with simultaneous adjustments made (age, age2 and TPA) in the fixed part (data not shown here), the obtained correlation matrices are presented in Table 3. The subject-level correlations indicate a very strong negative association between ectomorphy and the other two components (endomorphy and mesomorphy), and a moderately high positive association between endomorphy and mesomorphy. At the sib-pair level, the correlations are still quite strong, though diminished relative to the subject-level correlations. Interestingly, the correlation of endomorphy and ectomorphy are similar at both levels. The overall familiality index (overall $\rho=\text{SUM}($Level-3 variances and covariances)/[$\text{SUM}($Level-3 variances and covariances)+SUM($Level-2 variances and covariances)$]) of the somatotype taken as a whole among sibs was $\rho=0.35$.

Table 3. Somatotype components’ correlation matrices at the individual and sib-ship levels

<table>
<thead>
<tr>
<th></th>
<th>Individual-level</th>
<th>Sib-ship level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endo</td>
<td>Meso</td>
</tr>
<tr>
<td>Endomorphy</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Mesomorphy</td>
<td>0.59</td>
<td>1.00</td>
</tr>
<tr>
<td>Ectomorphy</td>
<td>-0.81</td>
<td>-0.91</td>
</tr>
</tbody>
</table>

Results of the analyses of each somatotype component are included in Table 4. The endomorphy mean of the BB sibship group is 3.9, with age lowering this mean ($\beta=-0.75\pm0.259$), but TPA did not significantly ($p>0.05$) affect this physique component. SS pairs have significantly higher values ($\beta=1.23\pm0.188$), as do BS pairs ($\beta=0.78\pm0.153$). Between-pairs variance ($\sigma^2_{B}$) is more variable for BB sib-pairs ($\sigma^2_{B_{BB}}=1.21$), and the lowest for BS ($\sigma^2_{B_{BS}}=0.57$), while the lowest within-pairs variance ($\sigma^2_{W}$) is for BS ($\sigma^2_{W_{BS}}=1.33$), and the highest for SS ($\sigma^2_{W_{SS}}=2.57$). SS pairs had the highest endomorphic resemblance ($\rho=0.42$), followed by BB ($\rho=0.38$) and BS were the least similar ($\rho=0.16$).

The mean mesomorphy for the BB pairs was 4.2, and significantly declines with age ($\beta=-0.88\pm0.168$). The higher the TPA, the higher the mesomorphy ($\beta=0.06\pm0.024$); SS pairs have lower values ($\beta=0.76\pm0.116$), and the same occurs for BS ($\beta=0.29\pm0.101$). Between sib-ships variance is greater
for BS ($\sigma^2_{B_{BS}}=0.82$), and the lowest is for SS ($\sigma^2_{B_{SS}}=0.54$). Further, within
sibships dissimilarity is greater for BB ($\sigma^2_{W_{BB}}=0.66$) and is lowest for BS
($\sigma^2_{W_{BS}}=0.42$). Brother-brother, and SS have the same mesomorphy
resemblance (their correlation is the same, $\rho=0.47$), whereas BS are less alike
($\rho=0.29$).

The mean ectomorphy for the BB pairs was 2.9, with age increasing this
mean ($\beta=0.84\pm0.202$); TPA did not significantly affect this component ($p>0.05$).
On average, SS pairs have lower values ($\beta=-0.30\pm0.135$). BS pairs are more
heterogeneous ($\sigma^2_{B_{BS}}=1.31$), than BB ($\sigma^2_{B_{BB}}=0.84$) and SS ($\sigma^2_{B_{SS}}=1.07$). Yet,
within sib-ships ($\sigma^2_{W}$) the opposite occurs, since unlikeness is greater for BB
($\sigma^2_{W_{BB}}=1.06$) than for SS ($\sigma^2_{W_{SS}}=0.97$) and BS ($\sigma^2_{W_{BS}}=0.78$). Same sex
sibships are more correlated ($\rho=0.44$ and $\rho=0.37$ for BB and SS, respectively)
than BS pairs ($\rho=0.27$).
Table 4. Parameter estimates and intraclass correlation (95%CI) obtained from the MIXREGLS

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Endomorphy</th>
<th>Mesomorphy</th>
<th>Ectomorphy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (BB)</td>
<td>3.91 0.122***</td>
<td>4.24 0.079***</td>
<td>2.92 0.096***</td>
</tr>
<tr>
<td>Age</td>
<td>-0.75 0.259**</td>
<td>-0.88 0.168***</td>
<td>0.84 0.202***</td>
</tr>
<tr>
<td>Age²</td>
<td>0.03 0.005**</td>
<td>0.03 0.006***</td>
<td>-0.03 0.007***</td>
</tr>
<tr>
<td>TPA</td>
<td>-0.05 0.036ns</td>
<td>0.06 0.024**</td>
<td>-0.02 0.028ns</td>
</tr>
<tr>
<td>SS</td>
<td>1.23 0.188***</td>
<td>-0.76 0.166***</td>
<td>-0.30 0.135*</td>
</tr>
<tr>
<td>BS</td>
<td>0.78 0.153***</td>
<td>-0.29 0.101***</td>
<td>-0.18 0.121ns</td>
</tr>
</tbody>
</table>

Variance components (σ²)

<table>
<thead>
<tr>
<th></th>
<th>Between Sib-ships (σ²B)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>SS</td>
<td>BS</td>
</tr>
<tr>
<td></td>
<td>1.21 0.58</td>
<td>1.09 0.54</td>
<td>0.57 0.82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Within Sib-ships (σ²W)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>SS</td>
<td>BS</td>
</tr>
<tr>
<td></td>
<td>1.96 0.66</td>
<td>2.57 0.60</td>
<td>1.33 0.42</td>
</tr>
</tbody>
</table>

Intraclass correlation (ρ)

<table>
<thead>
<tr>
<th></th>
<th>95% CI</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>SS</td>
<td>BS</td>
</tr>
<tr>
<td></td>
<td>0.38 0.27-0.55</td>
<td>0.42 0.30-0.58</td>
<td>0.16 0.08-0.34</td>
</tr>
<tr>
<td></td>
<td>0.47 0.36-0.61</td>
<td>0.47 0.35-0.62</td>
<td>0.29 0.19-0.43</td>
</tr>
<tr>
<td></td>
<td>0.44 0.33-0.59</td>
<td>0.37 0.25-0.54</td>
<td>0.27 0.18-0.42</td>
</tr>
</tbody>
</table>

BB=brother-brother; SS=sister-sister; BS=brother-sister; ns=non-significant; *p<0.05; ** p<0.01; ***p<0.001
DISCUSSION

Data from extended or nuclear families or twins or siblings have, inherently, a nested structure that requires adequate statistical models to suitably investigate their nature to unravel the presence of familiality in any quantitative or discrete trait. The results of this study showed that SS and BS pairs had higher endomorphy mean values, as well as lower mean values in mesomorphy and ectomorphy components, when compared to BB pairs. Vasques et al. (2006) reported similar values in a northeast mainland Portuguese sample. Available data (Heath & Carter, 1967) consistently reports sex differences in somatotype components - boys are systematically more mesomorphic and less endomorphic than girls. Our sibling data confirms this condition in which SS and BS pairs are more endomorphic and lower in mesomorphy. In BS siblings’ higher values are found than in BB siblings, but lower values than in SS pairs and this may be caused by sisters’ higher values. In addition, the sexual dimorphism in physique has likely contributed to our finding of greater same-sex sibling similarity than opposite-sex sibling similarity. We speculate that these results may be due to similarities in sets of behaviours as well as activities involving same-sex sibs which may be influenced by cultural or social factors (Richerson & Boyd, 2013). For example, a recent review by Deaner, Balish, and Lombardo (2016) about differences between sexes concerning their interest and motivation to be involved in sports, showed that boys and men generally have different sport-related experiences than girls and women; further, they demonstrated that exposure to a potential socializer is associated with differences in sports interest. It is also possible that the similarity among same sex-sibs may be connected to physiological factors, because of differences in biochemistry and in the structure of peripheral organs, since sex-differences are observed in the number of motoneurons controlling the striated perineal muscles and muscles themselves (de Vries & Forger, 2015).

Although somatotype components are independent of body size, older subjects tend to display lower mean endomorphic and mesomorphic values, but higher ectomorphy means (Malina, Bar-Or, & Bouchard, 2004). This physique plasticity has been shown in Belgian boys and girls followed longitudinally from
6 to 17 years though variation exists in their migratory distances which may express the moderate tracking coefficients in somatotype components (Duquet et al., 1993). This heterogeneity in somatotype plasticity during growth has also been reported by Ventrella et al. (2008) in Estonian and Italian children with decreases in endomorphy and mesomorphy components and increases in ectomorphy, except for the Italian boys where endomorphy increases with age. Additionally, Hebbelinck, Duquet, Borms, and Carter (1995) using longitudinal data from Belgian children and adolescents followed from 6 to 17 years, reported similar results for boys (decrease in endomorphy from 2.1 to 1.8, and in mesomorphy from 4.2 to 3.8; and increase in ectomorphy from 2.6 to 3.9). Yet, in girls only the mesomorphic component decreased with age.

Our sibling data showed that those who were more physically active tended to display a higher mesomorphy component score. Apparently, no studies have investigated the association between PA and somatotype in siblings or in family-independent individuals which leads to some difficulty in comparing our results with previous research. We were able to find only one study in youth, but not sibs. Longkumer (2014) studied a small sample (n=289) of Indian boys aged 8 to 15 years and showed mean endomorphy was significantly higher and ectomorphy significantly lower, respectively, in inactive boys compared to active boys. However, mesomorphy did not differ among the active and inactive boys. On the contrary, in our sibling data, differences were only found in the mesomorphy component in which more active individuals had higher mesomorphy. This may reflect greater muscular development, and therefore more muscular strength in active children (Marta et al., 2011). This relationship cascade is in line with available research that links higher physical activity levels to higher physical fitness (Martinez-Vizcaino & Sanchez-Lopez, 2008). Correlation matrices (see Table 3) showed strong associations between somatotype components at the individual level as well as at the sib-pair level confirming that it is a gestalt and should be analyzed as such (Cressie, Withers, & Craig, 1986). This gestalt sib similarity was $\rho=0.35$. Physique similarity in Northern Ontario families was investigated by Katzmarzyk et al. (2000). They used principal component analysis on individual somatotype components to
identify this *gestalt* and extracted, as expected, only one component which was expressed with a scalar, i.e., a single numerical somatotype descriptor as a whole; a subsequent correlational analysis on this scalar showed $\rho=0.32$, independently of generation. Using a similar analysis strategy with Biscay families, Rebato et al. (2007) reported a higher resemblance between siblings ($p=0.34$) than within parents ($p=0.21$). Yet, these studies did not include SES or physical activity adjustments. In sum, this general familial trend for somatotype similarity seems to point to a genetic basis (Bouchard et al., 1997). Yet, we are not aware of any study that identified genes from genome-wide linkage analysis, case-control associations or genome-wide association studies (GWAS) involving somatotype as a whole, or any of its components.

Nevertheless, since subjects from the same generation tend to be more similar in their physiques than those from different generations (parents-siblings), this increased dyadic resemblance may also be associated with their shared environmental as suggested by Peeters et al. (2003) in their twin study. Between and within sibships variances for endomorphy (relative fatness) was higher among same sex pairs than BS pairs. Our intraclass correlation results showed higher resemblance in endomorphy for same sex sibs than opposite sex sibs ($p_{SS}>p_{BB}>p_{BS}$). Similarly, Bouchard, Demirjian, and Malina (1980) using French-Canadian families showed that same sex sibs were more similar than opposite sex sibs; however in this sample BB pairs had higher correlations ($p_{BB}=0.60>p_{SS}=0.40>p_{BS}=0.22$). In the same way, Vasques et al. (2006) in a previous study with Portuguese sibs, found similar results, but SS pairs ($p=0.46$) had greater values than BB pairs ($p=0.14$) and BS pairs ($p=0.07$). In addition, Rebato et al. (2000), studying Spanish sibs, also reported higher resemblance for same sex sibs, but in this case the BB pairs showed the highest values ($p=0.31$) when compared to SS and BS ($p=0.21$ and $p=0.19$, respectively).

Furthermore, twin studies have described higher resemblance for endomorphy in monozygotic twins (MZ) than dizygotic twins (DZ) of both sexes. For example, Song, Perusse, Malina, and Bouchard (1994) sampled 102 twin pairs and reported significant resemblance in endomorphy for MZ pairs from both sexes; yet MZ female pairs tend to have higher correlations than MZ males.
Additionally, DZ pairs showed lower correlations than their MZ peers. Taken together, these results suggest that endomorphy is also influenced by genetic factors although no Genome-wide Association Studies (GWAS) results are available. Nevertheless, there is evidence of genes affecting phenotypes which are related to endomorphy. For example, GWAS data from den Hoed et al. (2010) identified 17 SNPs significantly associated with the sum of skinfolds (triceps, biceps, sub-scapular and supra-iliac), which explained 1.1% of the total variance. On the other hand, Fox et al. (2012) using GWAS results on abdominal adipose depots quantified by computed tomography, identified nominal associations at 7 loci. Furthermore, they uncovered a new locus for visceral adipose tissue in THNSL2 gene in women but not in men. Between sib-ship pairs variance in mesomorphy was higher among BS pairs than same sex pairs. However, within sib-ship variances showed a different trend, with higher values being observed in BB and SS pairs. Song et al. (1994) reported a lower variance within DZ male pairs than MZ’s, while among female pairs the opposite was observed (DZ female had higher variance than MZ peers). This means that, despite genetic influences governing dissimilarities within sib and DZ pairs, environmental and/or behavioural factors can contribute significantly to the manifestation of this phenotype. Intraclass correlation results showed resemblance similarity in same sex sib-ships ($\rho=0.47$). Vasques et al. (2006) and Bouchard et al. (1980) also reported higher values within same sex siblings. On the contrary, Rebato et al. (2000) found different results with higher resemblance among SS pairs ($\rho=0.60$), followed by BB pairs ($\rho=0.42$), and BS pairs ($\rho=0.16$). Song et al. (1994) found higher resemblance for MZ twins than DZ twins, and higher resemblance values were found among females when compared to males, independent of zygotic status.

To date, no data are available identifying candidate genes for mesomorphy. Yet, there is evidence of proteins associated with the development of muscle mass, namely the myostatin which is responsible for inhibiting the growth and regeneration of skeletal muscles (McPherron, Lawler, & Lee, 1997). The location of its gene is well known (chromosomal region 2q33.2) (Gonzalez-Cadavid et al., 1998). This protein differs from other proteins related to growth
differentiation factors, since it has been expressed almost exclusively in skeletal muscle (Lee, 2004).

For ectomorphy, higher between sib-ship variance was found in BS pairs when compared to BB and SS pairs. However, the within sib-ship variance has a similar trend to that found in the other two components, wherein BS pairs had lower variance than BB and SS pairs. The intraclass correlation revealed higher resemblance for same sex siblings when compared to opposite sex siblings ($\rho_{BB} > \rho_{SS} > \rho_{BS}$), an analogous trend also reported by Vasques et al. (2006) and Rebato et al. (2000). On the contrary, Bouchard et al. (1980) found different results ($\rho_{BB} = 0.40; \rho_{BS} = 0.38; \rho_{SS} = 0.35$). Although results are slightly different among studies, they are consistent in implying that genetic factors are also important in regulating the expression of this phenotype. We were not able to find any published GWAS results for ectomorphy. However, for BMI (which is a reciprocal index based on the same variables used to calculate ectomorphy, i.e. height and weight), GWAS data reported by Locke et al. (2015) suggested that the identified gene variants accounted for >20% of BMI variation at the population level.

Previous research with somatotype data mostly used statistical models/software implementations within the genetic epidemiology framework (Katzmarzyk et al., 2000; Rebato et al., 2007; Rebato et al., 2000; Song et al., 1994). In this set-up, analysis is done in a series of steps. Firstly, trait refinement i.e., data residualisation in each somatotype component, is achieved with multiple regressions where age, sex, age-by-sex interaction, age2 and age3 are used as covariates. Secondly, SES, parental education and sibling members may also be used as covariates. Thirdly, a more extended trait refinement uses somatotype components other than the one under analysis as covariates. The problem with this last refinement is that not only somatotype components are highly correlated causing multicollinearity, but the remaining variance at the trait level is usually very low, ~5%, which leaves very little to explain. An additional issue pertains to a possible undermining of the somatotype concept, a gestalt. At the final step, using regression residuals, the correlational analysis is done.
The novelty of this study is that, using a multilevel model, all analyses were done in one single step in the multivariate situation (all somatotype components analyzed simultaneously), as well as in the univariate one (each somatotype analyzed individually). So, the approach used is highly appealing in terms of modelling flexibility and extension, as well as fertile in terms of parameter estimates and their corresponding confidence intervals, especially in correlations. Our statistical approach and results can be used in two different ways. Firstly, they may provide new research opportunities to deeply analyze somatotype familiality in the presence of sets of covariates within cross-sectional settings, as well as how sibs’ somatotypes unfold over time; furthermore, multilevel modelling is highly flexible to accommodate univariate and multivariate designs, and available software is relatively easy to use.

Secondly, given consistent somatotype links with healthy/unhealthy traits and behaviours, care should be taken when designing intervention programs to 1) identify and intervene on behaviours that can influence physique and 2) involve the entire family.

Notwithstanding the relevance of these results, this study has limitations. Firstly, the sample is only from the north of mainland Portugal which limits the generalization of the results to all Portuguese children. Secondly, the use of a questionnaire to obtain information about physical activity is prone to errors even when it is collected under controlled conditions as in our study. However, the use of self-report instruments to obtain this information is consistently and frequently used in research (Ferreira, Marques, & Maia, 2002; Vasconcelos & Maia, 2001). Additionally, the Baecke questionnaire is regularly used in Portuguese studies with highly reliable results (Antunes et al., 2015; de Chaves et al., 2014; J. Maia, Gomes, Tregouet, & Katzmarzyk, 2014; Santos et al., 2014). Thirdly, SES was obtained based on the Portuguese National Classification of Occupations (2010) and we only considered the major nine categories as in previous studies (de Chaves et al., 2014; Gomes et al., 2014). However, in this classification each category also contains a range of subcategories that were not taken into account in the present study because it would scatter categories; as a result, some of
them would have few cases, and would create unnecessary problems in the data analysis.

This study also has several strengths: (1) the use of a large sample of siblings covering a very important window in youth growth and development; (2) the use of standard measurement protocols; (3) the highly reliable data; (4) the use of a novel approach linking multilevel methodology with location scale models allows for a thorough unraveling of somatotype familiality.

**CONCLUSION**

In conclusion, considering the complexity of sib-ship somatotype data, we provided a new methodological approach based on the multilevel model. Somatotype familiality, as a gestalt, is of moderate effect size. Same-sex siblings had stronger resemblance than opposite siblings in all somatotype components. Additionally, older subjects tend to be lower in both endomorphy and mesomorphy, but higher in ectomorphy compared to younger subjects. More physically active subjects tend to have higher values in mesomorphy. Socioeconomic status does not affect any somatotype component. Future research should consider not only families living in diverse environmental constraints, and longitudinal data, but also somatotype links with health-related physical fitness, sedentariness, and cardio-metabolic markers. This agenda may help researchers to better understand the interplay of nurture and nature in physique similarity within families, as well as its manifold expression, across the lifespan, in health and disease.
REFERENCES


Resemblance in physical activity levels: The Portuguese sibling study on growth, fitness, lifestyle and health

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ABSTRACT
To investigate the relationships of biological, behavioural, familial, and environmental characteristics with siblings physical activity (PA) levels as well as the intrapair resemblance in PA. The sample comprises 834 (390 females) biological siblings [brother-brother (BB), sister-sister (SS), brother-sister (BS)] aged 9 to 20 years. Total PA index (TPAI) was estimated by questionnaire. Information on potential behavioural, familial, and environmental correlates was obtained by self-report; body mass index (BMI), biological maturation, and physical fitness were measured. Multilevel models were used to analyze siblings’ clustered data, and sibling resemblance was estimated with the intraclass correlation (\( \rho \)). On average, younger sibs, those more physically fit, and those with more parental support had greater TPAI. Further, BB pairs had higher TPAI levels than SS or BS pairs, but also had greater within-pair variance. When adjusted for all covariates, SS pairs demonstrated greater resemblance in TPAI (\( \rho=0.53, \ 95\%\text{CI}=0.38-0.68 \)) than BS (\( \rho=0.26, \ 95\%\text{CI}=0.14-0.43 \)) or BB pairs (\( \rho=0.18, \ 95\%\text{CI}=0.06-0.44 \)). Age, physical fitness, and parental support were the best predictors of TPAI levels. A moderate level of resemblance in TPAI was observed in SS pairs, while lower resemblance was found for BS and BB pairs. These findings may be due to differences in the roles of shared genetic factors, familial, and environmental characteristics across different sibling types.
INTRODUCTION

The health-enhancing benefits of regular physical activity (PA) are well known, including its effects on weight control (Swift, Johannsen, Lavie, Earnest, & Church, 2014), cardiovascular disease risk factors, type-2 diabetes (Wahid et al., 2016), as well as reducing the negative effects of the metabolic syndrome (Oliveira & Guedes, 2016) in both youth and adults. However, a large proportion of children and adolescents worldwide do not comply with the suggested daily amounts of health-enhancing PA (Hallal et al., 2012).

As with most behaviours, the unfolding of the varied expressions of PA starts in the family because of shared environmental and genetic influences (Plomin, DeFries, McClearn, & McGuffin, 2008). Family studies of PA levels suggest that genetic factors are responsible for a substantial proportion of the variation in PA at the population level, although effect sizes vary (de Geus, Bartels, Kaprio, Lightfoot, & Thomis, 2014; de Vilhena e Santos, Katzmarzyk, Seabra, & Maia, 2012). Using questionnaire data, Aarnio, Winter, Kujala, and Kaprio (1997) showed that the intrapair correlation among same-sex sibs ($\rho=0.55$) was higher than opposite-sex sibs ($\rho=0.22$); however, Simonen et al. (2002), also using questionnaire data, found no differences in correlations among different sib-types (all $\rho=0.10$). More recently, and using objective PA data collected using pedometers, Jacobi et al. (2011) also reported similar correlations among all sib-type types (all $\rho=0.28$). These different results are most probably due to differences in sample origin, sample sizes, age ranges, diverse measures of PA, as well the use of different data analysis approaches.

Although important, previous research did not consider the influence of correlates of PA on sibling resemblance, because adjustments were made almost exclusively for age and/or sex. It is well known that PA is a complex behaviour conditioned by age (Sallis, Prochaska, & Taylor, 2000), sex (Crespo et al., 2013), biological maturation (Erlandson et al., 2011), screen time (Hands et al., 2011; Serrano-Sanchez et al., 2011), socioeconomic status, (Drenowatz et al., 2010; Veselska, Madarasova Geckova, Reijneveld, & van Dijk, 2011), parental support (Ornelas, Perreira, & Ayala, 2007; Zecevic, Tremblay, Lovsin, & Michel, 2010), and environmental factors (Davison & Lawson, 2006). Further,
siblings share, to a certain degree, the influences of the above mentioned correlates beyond the fact that they also share, on average, 50% of their genes identical-by-descent (Falconer & Mackay, 1996). Siblings also share, to varying degrees, their physical growth and physical fitness developmental trajectories (Malina, Bar-Or, & Bouchard, 2004).

The ecological model (Sallis & Owen, 2015) provides a transdisciplinary view of manifold PA correlates that operate at different levels, namely environmental, behavioural, and genetic, and it is important that these should be considered when examining their influence on siblings’ PA. Moreover, it is possible that new insights might be provided, which may be useful in the planning and development of intervention strategies to increase PA levels and promote health benefits for individuals and populations, especially when targeting families. Therefore, the present study aims (1) to investigate associations between biological, behavioural, familial, and environmental characteristics and siblings’ PA, and (2) to estimate sibling resemblance in PA.

METHODS

Study participants

A total of 834 biological siblings (390 females and 444 males) from 411 nuclear families (98.6% two siblings; 1.4% three or more siblings) aged between 9 to 20 years were sampled. This study is part of the “Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health.” Children and youth from the north and central regions of mainland Portugal, with siblings studying in the same school, were invited to take part in the project and the response rate was ~80%.

Written informed consent was obtained from their parents or legal guardians. The project was approved by the Ethics Committee of the University of Porto as well as by school authorities.
Physical activity

A total physical activity index (TPAI) was estimated using the Baecke questionnaire (Baecke, Burema, & Frijters, 1982), a reliable (Miller, Freedson, & Kline, 1994; M. A. Pereira et al., 1997) and valid instrument (Helmerhorst, Brage, Warren, Besson, & Ekelund, 2012; Philippaerts, Westerterp, & Lefevre, 1999) that describes three basic PA domains: work/school PA (questions related to sitting, standing, walking, lifting and sweating during work/school), leisure-time PA (questions associated with mode of transportation to school and time spent watching TV, walking and cycling), and sport participation (frequency of practice and sweating during sports’ practices). The Portuguese version of this questionnaire has been widely used in children and youth (Antunes et al., 2015; A. F. Seabra, Mendonca, Thomis, Malina, & Maia, 2007), twin studies (J. A. Maia, Thomis, & Beunen, 2002), as well as in family studies (J. Maia, Gomes, Tregouet, & Katzmarzyk, 2014; Santos et al., 2014). The TPAI is obtained from the unweighted sum of the three domain scores. For each domain, the score ranges from 1 to 5, such that the TPAI varies between 3 and 15. All participants answered the questionnaire during their physical education classes under the supervision of their physical education teacher and a trained member of the research group.

Biological characteristics

Height and weight were measured using standardized protocols established by the International Society for the Advancement of Kinanthropometry (Ross & Ward, 1986). Height was measured with a portable stadiometer (Holtain, UK) and weight with a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan). Body mass index (BMI) was computed using the standard formula: BMI=weight (kg)/height (m)².

Biological maturation was estimated with the maturity offset (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002), which estimates the distance, in decimal years, each subject is from age at peak height velocity (PHV). A positive maturity offset represents the number of years the participant is beyond PHV, whereas a
negative maturity offset represents the number of years the participant is before PHV.

*Physical fitness*

Physical fitness (PF) was assessed with the following tests taken from Eurofit (1993), Fitnessgram (1994), and AAHPERD (1976) test batteries: (1) handgrip strength (Takei Physical Fitness Test GRIP-D, Japan) with maximum isometric effort, which is maintained for about 5 to 10 seconds; (2) 1-mile run/walk – all participants ran/walked 1609 meters in the shortest time possible; (3) standing long jump - all participants attempted to jump as far as possible, landing on both feet without falling backwards; (4) shuttle-run – all participants ran as fast as possible to the other line, picked up a block and returned to place it behind the starting line, then returned to pick up the second block, and then repeated route; (5) 50 yard dash – all participants ran 50 yards in the shortest time possible. For the present study, an overall measure of physical fitness was used: first, individual test results were transformed into z-scores; then, an unweighted sum of all z-score was computed. Care was taken to reverse signs in 1-mile run/walk, shuttle-run and 50 yard dash performance.

*Behavioural characteristics*

Television (TV) time was estimated by questionnaire, where participants responded about time spent watching TV, and response options were: (1) <30 minutes; (2) 30 minutes-1 hour; (3) 1 hour-1:30 hours; (4) 1:30 hours-2 hours; (5) >2 hours). Self-reported information about sedentary behaviour is commonly used in studies from different countries and/or cultures (Greca, Silva, & Loch, 2016; Shang et al., 2015; Vallance, Buman, Stevinson, & Lynch, 2015), as well as in studies with Portuguese samples (S. Pereira et al., 2015; Vasconcelos & Maia, 2001).

*Sociodemographic characteristics*

Socioeconomic status (SES) was assessed by asking participants about their parents’ occupations. The occupation was categorized into ten groups (from 0–9) according to the Portuguese National Classification of Occupations (2010),
Resemblance in physical activity levels

where group 0 is the highest SES and group 9 is the lowest. Categories are the following: (0) armed forces (1) central administration/politicians and executive directors; (2) specialists of intellectual and scientific activities; (3) technicians and intermediate-level jobs; (4) back-office jobs; (5) security, seller and individual services; (6) farmer and qualified workers of farm, fish and forest; (7) industry and building qualified jobs: (8) machine and equipment operators; and (9) nonqualified jobs.

Parental support for physical activity

Participants answered a questionnaire concerning the perceived support for PA received from their parents. This valid questionnaire was based on Sallis, Grossman, Pinski, Patterson, and Nader (1987), and includes a list of items relating to parental encouragement for children’s PA practice (Kitzman-Ulrich, Wilson, Van Horn, & Lawman, 2010; Sallis, Alcaraz, McKenzie, & Hovell, 1999; Sallis, Patterson, Buono, Atkins, & Nader, 1988). The response options for all questions range from 1 (never) to 5 (very often), and the sum of the responses was computed to obtain a score for parental support.

Environmental characteristics

Neighborhood environmental support for PA was assessed by asking participants the following questions: “In my neighborhood, or near my neighborhood, there are facilities for cycling such as special tracks, separate paths or trails, shared use path for cyclists and pedestrians,” and “In my neighborhood there are several recreational and leisure areas, such as parks, cycle paths, recreation centers, public swimming pools, etc., free or with low prices.” The response options, for both questions, were: (1) completely disagree; (2) partially disagree; (3) partially agree; (4) completely agree. These questions are part of the Portuguese version of the environmental perception of the residential area survey (Delgado & Mota, 2005).

Statistical analysis

Basic exploratory and descriptive statistics [means and standard deviations (SD), counts and percentages (%)] were computed using IBM-SPSS 21. Sibling data are clustered by nature, i.e., individuals are nested within their
sib-ships. As such, multilevel models, as implemented in STATA 14 software, were used as a suitable statistical method to analyze the data. A sequential approach, with increasing complexity, was used in modeling siblings’ PA resemblance: the first model (M0), with no covariates, was the baseline; the next model (M1) included biological covariates, namely age, age2, BMI, maturity offset, as well as physical fitness z-scores and behavioural characteristics; the final model (M2) added familial and environmental characteristics. All covariates were centered as advocated (Hox, 2010). Following a statistical approach developed by Hedeker, Mermelstein, and Demirtas (2012) which expands beyond the classical multilevel model, we estimated separate within and between siblings’ variances, and therefore separate intraclass correlations (q); 95% confidence intervals (95% CI) for the three sibling types [brother-brother (BB), sister-sister (SS) and brother-sister (BS)] were also estimated. Unadjusted, partially adjusted (for M1 covariates), and fully adjusted (for all covariates) intraclass correlations were estimated. In the fixed part of all models, the brother-brother pair is the reference category. All parameters were simultaneously estimated using maximum likelihood (Goldstein, 2003).

RESULTS

Descriptive statistics for the biological, physical fitness, behavioural, familial, and environmental characteristics are presented in Table 1. On average, the different sibling pair types had similar chronological ages, BMI, TV time, socioeconomic status and parental support; further, SS pairs were more mature than BB and BS pairs, and BB pairs were more physically fit and active than BS and SS pairs.

Regarding environmental characteristics, namely neighborhood facilities for PA, the highest percentages of responses were observed for “partially agree” in all sib types (34.2%, 30.8 and 31.8%, for BB, BS and SS pairs, respectively). For recreational and leisure areas, few siblings of all types responded “completely agree” (13.5%, 17.7% and 13.9%, BB, BS and SS pairs, respectively).
Table 1. Descriptive statistics [means and standard deviations (SD); Count and percentages (%)] for siblings pairs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Brother-Brother (n=260)</th>
<th>Brother-Sister (n=373)</th>
<th>Sister-Sister (n=201)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological characteristics</strong></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>13.3 ± 2.0</td>
<td>12.9 ± 1.7</td>
<td>13.0 ± 2.0</td>
<td>2.455 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Age difference (years)</td>
<td>2.4 ± 2.0</td>
<td>2.7 ± 1.8</td>
<td>2.6 ± 1.9</td>
<td>1.260 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.7 ± 3.8</td>
<td>20.2 ± 3.6</td>
<td>20.5 ± 3.6</td>
<td>0.534 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Maturity offset (years)</td>
<td>-0.2 ± 1.8</td>
<td>-0.2 ± 1.4</td>
<td>-0.01 ± 1.3</td>
<td>4.663 &lt;i&gt;*&lt;/i&gt;</td>
</tr>
<tr>
<td><strong>Physical fitness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handgrip strength (Kgf)</td>
<td>27.1 ± 9.4</td>
<td>24.0 ± 7.1</td>
<td>22.3 ± 5.3</td>
<td>3.597 &lt;i&gt;**&lt;/i&gt;</td>
</tr>
<tr>
<td>1-mile run/walk (min)</td>
<td>8.8 ± 2.0</td>
<td>9.4 ± 2.1</td>
<td>10.7 ± 2.3</td>
<td>63.348 &lt;i&gt;***&lt;/i&gt;</td>
</tr>
<tr>
<td>Standing long Jump (cm)</td>
<td>164.9 ± 32.6</td>
<td>150.8 ± 31.2</td>
<td>132.4 ± 24.3</td>
<td>86.417 &lt;i&gt;***&lt;/i&gt;</td>
</tr>
<tr>
<td>Shuttle-run (s)</td>
<td>11.3 ± 1.8</td>
<td>11.4 ± 1.8</td>
<td>12.3 ± 1.9</td>
<td>22.354 &lt;i&gt;***&lt;/i&gt;</td>
</tr>
<tr>
<td>50 yard dash (s)</td>
<td>7.9 ± 1.1</td>
<td>8.3 ± 1.1</td>
<td>8.7 ± 1.0</td>
<td>42.332 &lt;i&gt;***&lt;/i&gt;</td>
</tr>
<tr>
<td>z-score total fitness</td>
<td>1.5 ± 3.8</td>
<td>0.1 ± 3.7</td>
<td>-2.1 ± 3.1</td>
<td>77.125 &lt;i&gt;***&lt;/i&gt;</td>
</tr>
<tr>
<td><strong>Behavioural characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television time</td>
<td>2.8 ± 1.4</td>
<td>2.7 ± 1.2</td>
<td>2.6 ± 1.2</td>
<td>0.104 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Total physical activity</td>
<td>8.4 ± 1.5</td>
<td>8.0 ± 1.5</td>
<td>7.6 ± 1.3</td>
<td>32.145 &lt;i&gt;***&lt;/i&gt;</td>
</tr>
<tr>
<td><strong>Familial characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>5.4 ± 2.3</td>
<td>5.2 ± 2.4</td>
<td>6.0 ± 2.1</td>
<td>2.690 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Parental support for PA</td>
<td>19.4 ± 6.8</td>
<td>19.3 ± 6.4</td>
<td>18.2 ± 5.9</td>
<td>2.690 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td><strong>Environmental characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighborhood Facilities for PA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completely disagree</td>
<td>65 ± 25.0</td>
<td>110 ± 29.5</td>
<td>62 ± 30.8</td>
<td>8.395 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Partially disagree</td>
<td>62 ± 23.8</td>
<td>73 ± 19.6</td>
<td>37 ± 18.4</td>
<td>8.395 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Partially agree</td>
<td>89 ± 34.2</td>
<td>115 ± 30.8</td>
<td>64 ± 31.8</td>
<td></td>
</tr>
<tr>
<td>Completely agree</td>
<td>44 ± 16.9</td>
<td>75 ± 20.1</td>
<td>38 ± 18.9</td>
<td></td>
</tr>
<tr>
<td>Neighborhood recreational and leisure areas</td>
<td>69 ± 26.5</td>
<td>93 ± 24.9</td>
<td>68 ± 33.0</td>
<td>1.04</td>
</tr>
<tr>
<td>Partially disagree</td>
<td>78 ± 30.0</td>
<td>105 ± 28.2</td>
<td>43 ± 21.4</td>
<td>3.720 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Partially agree</td>
<td>78 ± 30.0</td>
<td>109 ± 29.2</td>
<td>62 ± 30.8</td>
<td>9.720 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
<tr>
<td>Completely agree</td>
<td>35 ± 13.5</td>
<td>66 ± 17.7</td>
<td>28 ± 13.9</td>
<td>3.720 &lt;i&gt;ns&lt;/i&gt;</td>
</tr>
</tbody>
</table>

*p<0.001; **p<0.01; *p<0.05; <i>ns</i>=non-significant

Multilevel analysis results are presented in Table 2. Results for M0 showed that mean TPAI in BB pairs is 8.39±0.10, while SS (β=-0.86±0.15) and BS (β=-0.41±0.14) pairs are less active. Model 1, including individual-level covariates, fits the data significantly better than M0 [Deviance of M0=2895.86 and Deviance of M1=2845.50; Δ=50.36 with 6 df, p<0.05]. Consistent with M0, model 1 showed that, on average, BB pairs are more physically active than BS and SS pairs; further, older subjects are less active (β=-0.11±0.05), and being more fit (β=0.10±0.02) and spending less time viewing TV (β=-0.08±0.04) were associated with higher TPAI levels. Finally, M2 including familial and
environmental-level covariates, fits the data significantly better than M1
[Deviance of M1=2845.50 and Deviance of M2=2655.25; Δ=190.24 with 4 df, p<0.05]. Sib-pair differences in TPAI as well as individual-level covariates remain similar as in M1, except for TV time (not statistically significant in this model). Of the four familial and environmental covariates, only the parental support for PA was statistically significant (p<0.05), showing that siblings with more support tend to be more physically active (β=0.10±0.01).

Intraclass correlation results between siblings revealed that, independent of the series of adjustments, SS pairs resembled each other more in PA levels than BS or BB pairs. Further, the resemblance in SS pairs was the same, even after the inclusion of all covariates (ρ=0.53); BB pairs decreased their PA likeness to some extent when individual-level covariates were included in the model (from ρ=0.24 to ρ=0.19), but remained similar when familial and environmental covariates were added (ρ=0.18). BS pairs’ resemblance was relatively stable when individual-level covariates were included in the model (from ρ=0.35 to ρ=0.37), and their resemblance decreased when familial and environmental covariates were added (ρ=0.26).
Table 2. Parameter estimates variance components and intraclass correlation coefficients for total physical activity index

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Model 0 Estimates±SE</th>
<th>Model 1 Estimates±SE</th>
<th>Model 2 Estimates±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (BB)</td>
<td>8.39±0.10***</td>
<td>8.37±0.15***</td>
<td>8.18±0.14***</td>
</tr>
<tr>
<td>SS</td>
<td>-0.86±0.15***</td>
<td>-0.56±0.15***</td>
<td>-0.47±0.14&quot;</td>
</tr>
<tr>
<td>BS</td>
<td>-0.41±0.14***</td>
<td>-0.32±0.13'</td>
<td>-0.34±0.11&quot;</td>
</tr>
<tr>
<td><strong>Biological characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.11±0.05'</td>
<td>-0.09±0.04'</td>
<td></td>
</tr>
<tr>
<td>Age²</td>
<td>-0.00±0.01ns</td>
<td>-0.00±0.01ns</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.01±0.01ns</td>
<td>-0.00±0.01ns</td>
<td></td>
</tr>
<tr>
<td>Maturity Offset</td>
<td>0.01±0.06ns</td>
<td>0.02±0.05ns</td>
<td></td>
</tr>
<tr>
<td><strong>Physical fitness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z-score total fitness</td>
<td>0.10±0.02***</td>
<td>0.08±0.01***</td>
<td></td>
</tr>
<tr>
<td><strong>Behavioural characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television time</td>
<td>-0.08±0.04'</td>
<td>-0.04±0.03ns</td>
<td></td>
</tr>
<tr>
<td><strong>Familial characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>-0.03±0.02ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental support for PA</td>
<td>0.10±0.01***</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Environmental characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE facilities for PA</td>
<td>0.08±0.05ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE recreational areas for PA</td>
<td>-0.04±0.05ns</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Variance components (σ²)** |                      |                      |                      |
| Between siblings'           |                      |                      |                      |
| BB                         | 0.50±0.20            | 0.41±0.21            | 0.28±0.15            |
| SS                         | 0.91±0.20            | 0.76±0.18            | 0.83±0.18            |
| BS                         | 0.73±0.17            | 0.72±0.16            | 0.37±0.12            |
| Within siblings'           |                      |                      |                      |
| BB                         | 1.61±0.21            | 1.71±0.23            | 1.27±0.17            |
| SS                         | 0.81±0.12            | 0.75±0.12            | 0.73±0.11            |
| BS                         | 1.37±0.15            | 1.25±0.13            | 1.05±0.11            |

| **Intraclass correlation (ρ)** |                      |                      |                      |
| BB                         | 0.24 (0.10-0.45)     | 0.19 (0.07-0.43)     | 0.18 (0.06-0.44)     |
| SS                         | 0.53 (0.38-0.67)     | 0.50 (0.35-0.66)     | 0.53 (0.38-0.68)     |
| BS                         | 0.35 (0.23-0.49)     | 0.37 (0.25-0.50)     | 0.26 (0.14-0.43)     |
| Deviance                   | 2895.86              | 2845.50              | 2655.25              |

***p<0.001; **p<0.01; *p<0.05; ns=non-significant
DISCUSSION

Based on the ecological model and multilevel statistical models, this study investigated the associations of biological, behavioural, familial, and environmental characteristics with siblings’ TPAI; further, it also estimated sibling resemblance in this complex behaviour. In the final model (M2), only chronological age, physical fitness, and parental support were significantly related to TPAI. Intraclass correlations showed that SS pairs were more alike in their TPAI than BS and BB pairs.

Available data concerning correlates of PA at the population level is extensive, but effect sizes and signs are often divergent, which is probably due to sampling differences as well as to the multitude of methods used to assess PA (Rice & Howell, 2000). Consistent with our sibling data, chronological age has been negatively associated with PA (Baptista et al., 2012; Corder et al., 2016; Nader, Bradley & Houts, 2009; Sherar, Esliger, Baxter-Jones & Tremblay, 2007; Telama & Yang, 2000), but the magnitude of this decline differs among studies. In our study, for each year increase in sibling age, TPAI was 0.09 points lower. Telama and Yang (2000), based on self-reported PA, also showed a small decline expressed in percentages (males aged 15 to 18 years = 2.3%; females age 12 to 15 years = 1.5%). Using accelerometry data collected among Portuguese children, Baptista et al. (2012) demonstrated a decline in total PA with age (10.4 and 29 minutes from 10 to 11 years to 12 to 13 years in boys and girls, respectively). Contrary to this trend, A. F. Seabra et al. (2007), in a sample of Portuguese adolescents aged 10 to 18 years, reported that TPAI was lower in females only after 16 years of age, while male mean values were higher at older ages.

Physical fitness was found to be positively associated with PA, although in previous reports the direction and magnitude of this relationship differed. For example, Huang and Malina (2002) studying Taiwanese adolescents of both sexes showed that only those in the highest quartile of their estimated energy expenditure were significantly more physically fit. On the other hand, Blaes, Baquet, Fabre, Van Praagh, and Berthoin (2011) using objectively measured PA did not find a significant relationship with physical fitness. However, Larsen,
Kristensen, Junge, Rexen, and Wedderkopp (2015) using longitudinal data from children aged 6 to 12 years, showed a positive association of health-related fitness and performance-related fitness with moderate-to-vigorous PA (MVPA). Similarly, Bai et al. (2016), based on self-reported PA, showed that being physically active was positively associated with cardiorespiratory fitness in 1114 children and adolescents 6 to 15 years of age.

In the present study, parental support for PA was positively associated with TPAI which was consistent with previous research (Yao & Rhodes, 2015). For example, Wenthe, Janz, and Levy (2009) using adolescent data, investigated putative associations between a set of factors (self-efficacy to overcome barriers, enjoyment of physical activity, family support, peer support, perceived school climate, neighborhood safety, and access to PA) with MVPA, and reported that only family support emerged as the most significant and consistent factor associated with MVPA in both sexes. Likewise, Schaben, Welk, Joens-Matre, and Hensley (2006) studying 12 to 19 year old youth indicated that parental influences accounted for 15% of total variance in PA, which is consistent with multi-country data (Harrington et al., 2016).

Sibling resemblance in TPAI showed an unexpected pattern. Despite small variations in intraclass correlation values, with increasing levels of covariate adjustments, SS pairs showed stronger resemblance than BS and BB pairs. Using Portuguese nuclear family data, and the same PA assessment tool, A. F. Seabra, Mendonca, Goring, Thomis, and Maia (2008) reported a similar trend but with different ρ values (ρSS=0.26; ρBS=0.24; ρBB=0.21), although adjustments were only made for age, sex, and family socioeconomic status. Likewise, Maia et al. (2014), also using Portuguese family data, reported comparable correlations between sib-types - BSρ=0.37, BBρ=0.34, and SSρ=0.32. Additionally, Aarnio et al. (1997) based on self-reported PA, with no covariate adjustments, reported that SS and BB pairs (all ρ=0.55) demonstrated stronger resemblance that BS sibs (ρ=0.22). These findings highlight the complexities of the interaction between genetic factors, other biological indicators, and the manifold environmental characteristics. For example, De Moor et al. (2011) using twin and parental data reported that from the total
variance in exercise participation, additive genetic factors explained 42% in male twins and 36% in female twins; furthermore, shared environmental variance also differed between the sexes - 41% and 52% in males and females, respectively. The unique environment was responsible for 14% (in males) and 12% (in females) of their exercise participation, and 3% of this variance was related to vertical cultural transmission in male twins.

Several reports have studied a few candidate genes and their influence on PA (de Vilhena e Santos et al., 2012; Lightfoot, 2011; Wolfarth et al., 2014). However, the available results are ambiguous, most probably because of specificities in the production of genome maps in genome-wide linkage studies, uses of different methods to estimate PA, different sample sizes as well as demographics. For example, in a review by Lightfoot (2011), only 2 candidate genes showed consistent associations in the regulation of PA: dopamine receptor 1 (Drd1) and helixloop helix 2 (Nhlh2). In another review, de Vilhena e Santos et al. (2012) reported genome-wide linkage with markers near different activity related genes - EDNRB, MC4R, UCP1, FABP2, CASR, and SLC9A9; however, no marker was present in more than one study. On the other hand, male and female cellular environments are different, given known differences in the hormonal milieu and gene expression (Pardue & Wizemann, 2001) that probably affect PA in different ways. However, to our knowledge, few studies have examined evidence for sex-specific genetic architecture in PA, and the available results remain unclear (Diego et al., 2015; Rawlik, Canela-Xandri, & Tenesa, 2016; Vink et al., 2012).

Our results demonstrated greater resemblance between BS pairs than BB pairs, and this result was unexpected. One possible explanation may be linked to different genetic and unique environmental factors. Yet, it is important to note that, when sib results were adjusted for familial and environmental characteristics, BB and SS variance remained similar, but the BS pair variance decreased suggesting that the magnitude of genetic factors may more strongly affect same-sex siblings than opposite-sex siblings. In addition, the SS pairs showed lower within-pair variation than BB or BS, which means that these combined factors may be more influential in SS pairs. The present results also
suggest that the biological and contextual mechanisms leading to different sibling resemblance are complex, potentially population-specific, and/or context-specific. For example, based on analyses in a Portuguese sample, A. C. Seabra et al. (2013) demonstrated that the amount of time engaged in PA is associated, in boys, with enjoyment and perceived physical competence whereas in girls PA levels are positively related to perceived acceptance by peers in games and sports as well as by parental encouragement. Other studies reported that boys’ motivators tend to be more intrinsic, namely improving their health, enhancing body shape, and being competitive; in females a combination of extrinsic and intrinsic factors, such as emotional support, social aspects, sense of well-being, and positive body image appear to be linked to engagement in PA (Cole & Maeda, 2015; Jonason, 2007; Sirard, Pfeiffer & Pate, 2006; Yli-Piipari, Leskinen, Jaakkola & Liukkonen, 2012). Also, Lightfoot (2008) showed that sex differences in PA may also be due to variation in sex hormones, namely higher estrogen and lower testosterone concentration in females. Furthermore, we did not consider possible factors related to neural (Grabowska, 2017) and physiological mechanisms that appear to influence participation in PA, reinforcing the need to develop testable models as well as their putative mechanisms on how biological, psychological, genetic, familial, and environmental factors influence PA levels.

This study is not without limitations. First, the sample is not from all Portuguese regions which limits the generalization of our results. Yet, the same applies to almost all published family, sibling, and twin studies. Second, the use of questionnaires to obtain information about PA and TV viewing is susceptible to errors. Yet, self-report instruments are very often used (Greca et al., 2016; Shang et al., 2015; Vallance et al., 2015). Additionally, the Baecke questionnaire is frequently used in Portuguese and Brazilian studies with valid and reliable results (Santos et al., 2014; A. F. Seabra et al., 2007; Silva et al., 2016). Third, the indirect estimation of maturity is prone to errors, notwithstanding its widespread use in research (Coelho et al., 2013; Cossio-Bolanos et al., 2015; Werneck et al., 2017). Moreover, other available methods to estimate biological maturity have ethical problems and intrusiveness (x-rays and secondary sexual characteristics). Fourth, the 1-mile run/walk test only provides an indirect
estimation of cardiorespiratory fitness; yet, it is considered a valid test (Cureton, Plowman & Mahar, 2014). Fifth, SES was obtained based on the Portuguese National Classification of Occupations (2010) and we only considered the nine major categories as in previous studies (de Chaves et al., 2014; Gomes et al., 2014). However, in this classification each category also contains a range of subcategories that were not considered in the present study because some of them would have few cases and would create unnecessary problems in the data analysis. Sixth, although possible, we did not consider a 3-level model with sibships nested within their schools.

In conclusion, SS pairs demonstrated greater resemblance in TPAI than BS and BB pairs. Additionally, younger sibs, those who had greater fitness, and those with more parental support, had higher TPAI levels. Given the complexity of factors influencing individual differences in their TPAI levels, it is not surprising that the same interventions for all individuals do not cause similar changes in this complex behaviour. Therefore, we suggest that sibling type, age, physical fitness levels and parental support, as well as genetic and environmental factors need to be identified and considered in order to develop more personalized intervention programs to bring about positive changes in PA.
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Resemblance in physical activity levels


Resemblance in physical activity levels


A multilevel analysis of health-related physical fitness. The Portuguese sibling study on growth, fitness, lifestyle and health

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ABSTRACT

This study investigates biological, behavioural and sociodemographic correlates of intrapair similarities, and estimates sibling resemblance in health-related physical fitness (PF). The sample comprises 1101 biological siblings (525 females) aged 9±20 years. PF components and markers were: morphological [waist circumference (WC) and %body fat (%BF)], muscular [handgrip strength (GS) and standing long jump (SLJ)], motor [50-yard dash (50YD) and shuttle run (SR)], and cardiorespiratory (1-mile run). Biological maturation was assessed; physical activity (PA), TV viewing and socioeconomic status (SES) information was obtained. On average, older and more mature subjects are better performers in all PF components; PA was negatively associated with SR, while SES was negatively associated with SLJ and SR. A pattern was observed in the intraclass correlations (ρ) wherein same sex siblings demonstrate greater resemblance for most PF components (sister-sister: 0.35≤ρ≤0.55; brother-brother: 0.25≤ρ≤0.60) than brother-sister pairs (BS) (0≤ρ≤0.15), except for %BF (ρBB>ρSS>ρBS), and the 1-mile run (ρSS>ρBS>ρBB). In conclusion, behavioural and sociodemographic correlates play different roles in siblings PF expression. Further, a significant familial PF resemblance was observed with different trends in different sibling types, probably due to variations in shared genetic factors and sociodemographic conditions.
INTRODUCTION

Physical fitness (PF) is a complex construct, broadly described as an individual attribute expressing the efficiency of a varied set of bodily systems and functions to perform work in a wide-ranging set of contexts (Bouchard & Shephard, 1994). It is widely accepted that the multivariate structure of PF varies in terms of its configuration, namely in the expression of its basic components and indicators (Safrit, 1999). Bouchard and Shephard (1994) presented a comprehensive definition of health-related PF, composed of several components (morphological, muscular, motor, cardiorespiratory, and metabolic) which has been used in several studies (Boreham et al., 2001; Erikssen, 2001; Matton et al., 2007). Further, it has been shown to be a consistent and reliable health marker in childhood and adolescence (Ortega, Ruiz, Castillo, & Sjostrom, 2008). Age and sex differences in PF are well known. For example, Ortega et al. (2011) studied adolescent boys and girls (12-17 years old) from 10 European countries and showed greater PF levels in boys than in girls (except for flexibility), as well as increased mean fitness with increasing age. Additionally, Bohr, Brown, Laurson, Smith, and Bass (2013) investigated the role of socioeconomic status (SES) on PF among US adolescents, and reported that girls in the low SES group were less fit than girls with higher SES; whereas for boys this relationship was not evident. Furthermore, in Swedish adolescents aged 14-15 years, Ekelund et al. (2001) found positive associations between cardiorespiratory fitness and physical activity and biological maturation and a negative association with body fat. Despite the expectation of sociodemographic influences on PF expression, we only found one study (Matejek & Starc, 2013) that investigated how children's PF development across time was related to age and sex, as well as school grade, place of residence, paternal education and maternal education, i.e., sociodemographic factors. Data showed that most of the observed differences in PF were mainly accounted for by age and sex; additionally, children from the suburbs were more physically fit than those from urban areas. The available research with twins and nuclear/extended families has indicated that genetic factors are responsible for a substantial portion of the total variation in PF at the population level. For example, Maes et al. (1996) investigating 10-yr-old twin pairs and their parents from the Leuven Longitudinal Twin Study.
found that, in general, MZ twins were more similar, as described by intraclass correlations (ρ), in their motor performance than DZ twins (MZ, ρ=0.46-0.83; DZ, ρ=0.28-0.54). Recently, Schutte, Nederend, Hudziak, de Geus, and Bartels (2016) also reported higher correlations in MZ twins (ρ=0.34-0.79) than DZ twins (ρ=0.28-0.54) in vertical jump, handgrip strength, balance and flexibility. In addition, Perusse et al. (1987) examined familial aggregation in aerobic power, muscular endurance, and strength and showed that in all PF components, siblings' correlations were higher than spousal and parent-child correlations. Furthermore, Lortie et al. (1982) also investigated the familial similarity in maximal aerobic power and reported higher spousal and sibling resemblance (ρ=0.34 and ρ=0.33, respectively) than parent-child resemblance (ρ=0.19).

Available data on PF among siblings is somewhat scarce, and we were able to find only eight papers on the topic, and the results were not consistent. For example, R. M. Malina and Mueller (1981) used US data to show that brother-brother pairs (ρ=0.46) were more similar than brother-sister (ρ = 0.24) and sister-sister pairs (ρ=0.19) in their muscular strength. On the contrary, Pawlak (1984) reported higher correlations in Polish adolescent sister-sister pairs (ρ=0.44) than brother-brother (ρ=0.25) or brother-sister pairs (ρ=0.21) for grip strength.

Siblings share, on average, 50% of their genes identical-by-descent, and also have a common familial environment (Falconer & Mackay, 1996). However, taking into account differences in their age and sex, as well as in their physical growth and motor development trajectories may provide more precise estimates of common and unique environmental variation in the expression of their health-related PF (R. Malina & Bouchard, 2004). Investigating sibling resemblance in the expression of this complex construct, i.e., studying more than one child per family, may provide important clues regarding the separate and joint effects of sociodemographic, behavioural, or genetic factors on youth differences and/or similarities in their PF. Additionally, they may provide relevant indications when designing optimal physical activity intervention programs to combat the rise of obesity incidence (Fogelholm, 2010) and decline in health-related PF levels (Tomkinson & Olds, 2007).

Using the multilevel model as a statistical framework (Kenny, Kashy, & Cook, 2006) as well as the Bouchard and Shephard PF template (Bouchard &
Shephard, 1994), the present study aims: to (1) investigate the separate and joint effects of biological, behavioural and sociodemographic characteristics in intra pair sibling similarities in PF, (2) and estimate sibling resemblance in health-related PF components. The following hypotheses were posited: (1) biological, behavioural and sociodemographic characteristics are significantly associated with siblings' health-related PF, and (2) sibling resemblance is higher in same-sex sibs than in opposite sex siblings for all PF components.

METHODS

Study participants

Data are from the Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health, which aims to investigate the physical growth, body composition, PF, physical activity, metabolic syndrome, and health behaviours of a large cohort of siblings. Children and adolescents, aged 9 to 20 years, were recruited in schools from the north and central regions of mainland Portugal, and were invited to freely participate in the project with their siblings and parents, since they were also previously called to be part of the Portuguese Healthy Family Study (Santos et al., 2014). The project was approved by the Ethics Committee of the University of Porto, as well as by school authorities. Following their approval, all identified siblings from the ~8000 students enrolled in selected schools were invited to participate in the study. From 1376 identified/invited siblings, about 80% (response rate) agreed to take part in the study. Thus, a total of 1101 biological siblings (525 females and 576 males) from 540 nuclear families (519 pairs and 21 triplets) were sampled. Parents or legal guardians provided written informed consent.

Anthropometry

Height and weight were measured using standardized protocols established by the International Society for the Advancement of Kinanthropometry (Ross & Ward, 1986). Height was measured with a portable stadiometer (Holtain, UK) and weight with a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan). Body mass index (BMI) was computed using the standard formula (BMI = weight (kg)/(height (m))^2).
Maturity offset

Biological maturation was estimated with the maturity offset procedure, proposed by Mirwald, Baxter-Jones, Bailey, and Beunen (2002), which estimates the distance, in decimal years, each subject is from age at peak height velocity (PHV). A positive (+) maturity offset represents the number of years the participant is beyond PHV, whereas a negative (−) maturity offset represents the number of years the participant is before PHV.

Health-related physical fitness

Using the Bouchard and Shephard (1994) health-related PF model comprising morphological (waist circumference and body fat percentage), muscular (strength and power), motor (speed and agility) and cardiorespiratory (aerobic capacity) components, all siblings underwent a systematic assessment comprised of the following tests:

Morphological component: Body fat percentage (%BF) was estimated using a portable bioelectrical bio impedance scale (Tanita BC-418 MA segmental body composition analyser, Tanita Corporation, Japan). Waist circumference (WC), anatomically identified as the smallest circumference between the lowest rib and the superior border of the iliac crest, was measured, in cm, with a non-elastic tape (Sanny, American Medical of Brazil, Sao Paulo, Brazil).

Muscular component: Muscular strength, namely static strength, was assessed with the grip strength (GS) test using a hand dynamometer (Takei Digital Grip Strength Dynamometer, Model T.K.K.5401, Tokyo, Japan), and the result was recorded in kg. Muscular power was obtained with the standing long jump test (SLJ), and results were recorded in centimetres (cm).

Motor component: Speed was assessed with the 50 yard dash (50yd), and agility was marked with the shuttle-run (SHR); time was recorded in seconds (s).

Cardiorespiratory component: Aerobic capacity was estimated with the 1-mile run/walk test (1-MR), and the result was recorded in minutes (min).
Physical activity

Total physical activity (TPA) was estimated with the Baecke questionnaire (Baecke, Burema, & Frijters, 1982), a reliable and valid instrument (Philippaerts, Westerterp, & Lefevre, 1999) that describes three basic PA domains: school PA (questions related to sitting, standing, walking, lifting and sweating during school), leisure-time PA (questions associated with mode of transportation to school and time spent watching TV, walking and cycling) and sport participation (frequency of practice and sweating during sport practice). The Portuguese version of this questionnaire is widely used in children and youth (Seabra, Mendonca, Thomis, Malina, & Maia, 2007) as well as in family studies (Santos et al., 2014). The TPA score is obtained from the unweighted sum of the three domains. For each domain, each score ranges from 1 (minimal) to 5 (maximal), such that the TPA score varies between 3 and 15. All participants answered the questionnaires during their physical education classes, under the supervision of their physical education teacher as well as by a trained research team member.

Screen time

Participants answered questions related to time spent watching television (TV), the response options for both questions, were: (1) <30 minutes; (2) 30 minutes-1 hour; (3) 1 hour-1:30 hours; (4) 1:30 hours-2 hours; (5) >2 hours). It is important to note that techniques to assess sedentary behaviours vary marked in the literature, and the use of screen time as a marker of sedentary behaviour in children is by far the most common. Self-reported information about sedentary behaviour has been commonly used in studies from different countries and/or cultures (Greca, Silva, & Loch, 2016; Herman et al., 2014; Shang et al., 2015; Vallance, Buman, Stevinson, & Lynch, 2015) as well as in studies with Portuguese samples (Pereira et al., 2015; Vasconcelos & Maia, 2001).

Socioeconomic status

Socioeconomic status (SES) was assessed by asking participants about their parents' occupations. The occupation was categorized into ten groups (from 0±9) according to the Portuguese National Classification of Occupations (2010), where group 0 is the highest SES and group 9 is the lowest. Categories are as follows: (0) armed forces (1) central administration/politicians and executive
directors; (2) specialists of intellectual and scientifically activities; (3) technicians and intermediate level jobs; (4) back-office jobs; (5) security, seller and individual services; (6) farmer and qualified workers of farm, fish and forest; (7) industry and building qualified jobs: (8) machine and equipment operators; and (9) nonqualified jobs.

Data quality control

To ensure data quality, the following procedures were used: (1) training of all team members by experienced researchers of the Kinanthropometry Laboratory of the Sports Faculty, University of Porto, Portugal; (2) conducting random retests on each assessment day. The technical error of measurement (TEM) for anthropometric measurements and body composition variables were 0.1 cm for height and WC, 0.1 Kg for weight, and 0.4% for %BF. Reliability calculations via ANOVA-based intraclass correlation coefficients (R) for all physical fitness tests were as follows: 1-MR, R = 0.97; SLJ, R = 0.95; SHR, R = 0.93; 50yd, R = 0.95; GS, R = 0.94.

Statistical analysis

Basic exploratory (data check for outliers and normality) and descriptive statistics (mean±standard-deviations) were computed in IBM-SPSS 21. Given the skewed distribution in GS, a log transformation was used to normalize it. A multilevel model implemented in STATA 14 software was used given the data clustering-individuals nested within siblings pairs. In the analyses, models were estimated for each individual marker of PF using a set of covariates: age, age2, maturity offset, TPA, TV, SES, TPA-by-SS interaction, TPA-by-BS interaction, Age-by-SS interaction, and Age-by-BS interaction. Covariates were centered at their respective means as advocated (Hox, 2010). Using a statistical approach developed by Hedeker, Mermelstein, and Demirtas (2012) that expands beyond the classical multilevel model, we estimated separate within and between siblings' variances, and therefore separate intraclass correlations (ρ) with corresponding 95% confidence intervals (95%CI), for the three sibling types [brother-brother (BB), sister-sister (SS) and brother-sister (BS)]. Unadjusted, partially adjusted (age and age2), and fully adjusted (for all covariates mentioned above) intraclass correlations were computed. In all models, the BB pair served
as the reference category. All parameters were simultaneously estimated using maximum likelihood (Goldstein, 2003).

RESULTS

Descriptive statistics for all PF components, demographic and behaviour indicators are provided in Table 1. On average, sibling pairs have similar chronological ages, but their mean age differences differ somewhat. Further, BB pairs are the tallest as well as the heaviest, have the highest WC on average, but lower %BF than BS and SS pairs. For the remaining PF components, BB pairs are stronger (GS and SLJ) and they performed better in the motor and cardiorespiratory components (50yd, SHR, and 1-MR) than BS and SS pairs. Mean SES and time spent watching TV is similar across sibling-types, but BB pairs are slightly more physically active.

Multilevel analysis results are presented in Table 2. For the morphological component, averages for BB pairs are 18.40±0.76% and 68.58±0.95 cm for %BF and WC (cm), respectively.

For both %BF and WC, significantly lower values were observed with higher age (β=-2.33±0.23; β=-0.84±0.32), and with greater biological maturity (β=1.52±0.20; β=2.68±0.25). TPA, SES and TV did not significantly associate with these components. Sister-sister and BS pairs have significantly higher %BF (β=6.49±0.68; β=2.97±0.60) and lower WC (β=-4.48±0.92; β=-3.02±0.73) than the BB pairs. Total physical activity did not significantly interact with siblings’ in terms of %BF or WC, however age-by-siblings’ did for %BF; for SS and BS pairs higher age was associated with higher mean %BF levels as compared to BB pairs.

For the muscular component, the mean performance of BB pairs is 3.28±0.03 for GS (logunits) and 166.74±2.78 for SLJ (cm). Older subjects are stronger (β=0.08±0.01; β=6.34±1.00) in both tests, as are more mature children (β= 0.06±0.01; β=3.7±0.80). Higher %BF is linked with poorer performance (β=-2.08±0.12) in SLJ, but not in GS; TPA and TV do not significantly associate with muscular performance. SS (β=-0.17±0.03; β=-19.06±2.55) and BS (β=-0.09±0.02; β=-8.19±2.08) have lower values than BB pairs on both tests. SES was significantly related with lower values in SLJ (β=-0.78±0.36), but not in GS. Total physical activity did not significantly interact with siblings’ for both strength...
tests, but age-by-siblings did, such that in SS and BS pairs, higher age showed lower mean GS and SLJ than in BB pairs.

Table 1. Descriptive statistics (means and standard deviations (SD)) for siblings pairs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Brother-brother (n=317) Mean</th>
<th>SD</th>
<th>Sister-sister (n=269) Mean</th>
<th>SD</th>
<th>Brother-sister (n=515) Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.5</td>
<td>2.0</td>
<td>13.3</td>
<td>2.1</td>
<td>13.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Age difference (years)</td>
<td>2.0</td>
<td>1.6</td>
<td>2.4</td>
<td>1.8</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.6</td>
<td>13.0</td>
<td>154.0</td>
<td>8.8</td>
<td>155.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.3</td>
<td>15.8</td>
<td>49.4</td>
<td>11.5</td>
<td>50.4</td>
<td>12.8</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>20.8</td>
<td>4.0</td>
<td>20.7</td>
<td>3.8</td>
<td>20.5</td>
<td>3.6</td>
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<tr>
<td><strong>Morphological component</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>70.4</td>
<td>9.9</td>
<td>66.0</td>
<td>8.0</td>
<td>67.7</td>
<td>8.1</td>
</tr>
<tr>
<td>%BF</td>
<td>19.9</td>
<td>6.5</td>
<td>26.5</td>
<td>5.7</td>
<td>23.3</td>
<td>6.9</td>
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<td><strong>Muscular component</strong></td>
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<tr>
<td>Hand grip strength (Kg)</td>
<td>28.4</td>
<td>9.7</td>
<td>22.6</td>
<td>5.4</td>
<td>25.1</td>
<td>8.1</td>
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<tr>
<td>Standing long jump (m)</td>
<td>168.0</td>
<td>33.6</td>
<td>136.8</td>
<td>24.2</td>
<td>151.4</td>
<td>31.8</td>
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<td><strong>Motor component</strong></td>
<td></td>
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<tr>
<td>50 yard dash (s)</td>
<td>7.9</td>
<td>1.1</td>
<td>8.6</td>
<td>0.9</td>
<td>8.3</td>
<td>1.1</td>
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<tr>
<td>Shuttle-run (s)</td>
<td>11.1</td>
<td>1.8</td>
<td>12.1</td>
<td>1.7</td>
<td>11.4</td>
<td>1.8</td>
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<td><strong>Cardiorespiratory component</strong></td>
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<td>1-mile run/walk (min)</td>
<td>8.6</td>
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<td>10.4</td>
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<td>5.9</td>
<td>2.2</td>
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<tr>
<td>Behavioural indicators</td>
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<td></td>
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<tr>
<td>Physical activity</td>
<td>8.0</td>
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<td>7.4</td>
<td>1.2</td>
<td>7.7</td>
<td>1.2</td>
</tr>
<tr>
<td>TV/day</td>
<td>2.8</td>
<td>1.4</td>
<td>2.7</td>
<td>1.2</td>
<td>2.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

n = total number of subjects by sibling type

The motor component averages for BB pairs are $\beta=7.96\pm0.11$ (50yd dash in s) and $\beta=10.84\pm0.20$ (SHR in s). More mature subjects performed better in both tests ($\beta=-0.17\pm0.03$ A multilevel analysis of health-related physical fitness and $\beta=-0.15\pm0.06$ for 50yd and SHR respectively). Those with higher %BF performed worse ($\beta=0.07\pm0.01$ and $\beta=0.08\pm0.01$ for 50yd and SHR, respectively). Age and TV did not significantly associate with the motor components.

TPA and SES showed no association with 50yd, but are significantly linked with lower SHR performance ($\beta=0.16\pm0.07$; $\beta=0.09\pm0.03$). Age-by-siblings' did not interact with the motor components. Yet, TPA-by-siblings' did, where in both SS and BS higher TPA was associated with higher means in both tests than BB pairs. SS pairs show lower performance levels than BB pairs in
both tests (β=0.23±0.10 and β=0.46±0.19 for 50yd and SHR, respectively). BS pairs does not differ from BB pairs (p>0.05).

Finally, in the cardiorespiratory component, BB pairs 1-Mile run/walk average is 9.07±0.21 (min): SS pairs need more time (β=0.65±0.22) to cover the distance than BB pairs, but not BS pairs (p>0.05); also, older subjects are better performers (β=-0.17±0.08). Of all covariates, only %BF is associated with the 1-MR; those with higher %BF require more time to cover the distance (β=0.16±0.01).
Table 2. Parameter estimates and variance components for each physical fitness test

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Body fat (%)</th>
<th>WC (cm)</th>
<th>HG (Log kg)</th>
<th>SLJ (cm)</th>
<th>50YD (s)</th>
<th>SR (s)</th>
<th>1-mile (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (BB)</td>
<td>18.40±0.76***</td>
<td>68.58±0.95***</td>
<td>3.28±0.03***</td>
<td>166.74±2.78***</td>
<td>7.95±0.11***</td>
<td>10.84±0.20***</td>
<td>9.07±0.21***</td>
</tr>
<tr>
<td>SS</td>
<td>6.49±0.68***</td>
<td>-4.48±0.92***</td>
<td>-0.17±0.03***</td>
<td>-19.06±2.55***</td>
<td>0.30±0.10*</td>
<td>0.46±0.19*</td>
<td>0.65±0.22**</td>
</tr>
<tr>
<td>BS</td>
<td>2.97±0.60***</td>
<td>-3.02±0.73***</td>
<td>-0.09±0.02***</td>
<td>-8.19±2.08***</td>
<td>0.11±0.08ns</td>
<td>0.07±0.15ns</td>
<td>0.08±0.15</td>
</tr>
<tr>
<td>Age</td>
<td>-2.33±0.60***</td>
<td>-0.84±0.32**</td>
<td>0.08±0.01***</td>
<td>6.34±1.00***</td>
<td>-0.07±0.04ns</td>
<td>-0.01±0.07ns</td>
<td>-0.17±0.08*</td>
</tr>
<tr>
<td>Age²</td>
<td>0.07±0.04ns</td>
<td>-0.02±0.05ns</td>
<td>-0.008±0.001***</td>
<td>-0.54±0.15***</td>
<td>0.02±0.01**</td>
<td>0.03±0.01*</td>
<td>0.01±0.01ns</td>
</tr>
<tr>
<td>Maturity offset</td>
<td>1.52±0.20***</td>
<td>2.68±0.25***</td>
<td>0.06±0.01***</td>
<td>3.78±0.80***</td>
<td>-0.17±0.03***</td>
<td>-0.15±0.06***</td>
<td>-0.01±0.06ns</td>
</tr>
<tr>
<td>TPA</td>
<td>0.09±0.23ns</td>
<td>0.28±0.35ns</td>
<td>0.01±0.01ns</td>
<td>-0.12±1.04ns</td>
<td>0.06±0.04ns</td>
<td>0.16±0.07*</td>
<td>-0.13±0.08ns</td>
</tr>
<tr>
<td>TV</td>
<td>0.29±0.16ns</td>
<td>0.05±0.20ns</td>
<td>0.003±0.006ns</td>
<td>0.05±0.61ns</td>
<td>-0.01±0.02ns</td>
<td>0.09±0.03**</td>
<td>0.00±0.028ns</td>
</tr>
<tr>
<td>SES</td>
<td>0.08±0.10ns</td>
<td>0.19±0.12ns</td>
<td>-0.000±0.003ns</td>
<td>-0.78±0.36</td>
<td>0.02±0.01ns</td>
<td>0.09±0.03</td>
<td>0.00±0.028ns</td>
</tr>
<tr>
<td>TPA*SS</td>
<td>-0.38±0.40ns</td>
<td>-0.77±0.56ns</td>
<td>-0.001±0.015ns</td>
<td>2.34±1.61ns</td>
<td>-0.17±0.07**</td>
<td>-0.36±0.12**</td>
<td>-0.17±0.14ns</td>
</tr>
<tr>
<td>TPA*BS</td>
<td>-0.55±0.36ns</td>
<td>-0.34±0.45ns</td>
<td>-0.01±0.01ns</td>
<td>2.50±1.39ns</td>
<td>-0.13±0.05*</td>
<td>-0.30±0.98**</td>
<td>-0.19±0.11ns</td>
</tr>
<tr>
<td>Age*SS</td>
<td>1.85±0.25***</td>
<td>0.21±0.34ns</td>
<td>-0.05±0.01***</td>
<td>-5.44±1.02***</td>
<td>0.01±0.04ns</td>
<td>0.02±0.08ns</td>
<td>0.10±0.09ns</td>
</tr>
<tr>
<td>Age*BS</td>
<td>0.99±0.25***</td>
<td>0.29±0.31ns</td>
<td>-0.02±0.01*</td>
<td>-1.93±0.97**</td>
<td>0.01±0.04ns</td>
<td>-0.01±0.07ns</td>
<td>0.05±0.07ns</td>
</tr>
<tr>
<td>%BF</td>
<td>----</td>
<td>-0.01±0.001ns</td>
<td>-2.08±0.12***</td>
<td>0.07±0.01***</td>
<td>0.08±0.01***</td>
<td>0.16±0.01***</td>
<td></td>
</tr>
</tbody>
</table>

Variance components (σ²)

<table>
<thead>
<tr>
<th></th>
<th>Body fat (%)</th>
<th>WC (cm)</th>
<th>HG (Log kg)</th>
<th>SLJ (cm)</th>
<th>50YD (s)</th>
<th>SR (s)</th>
<th>1-mile (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between siblings'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>21.700±3.736</td>
<td>27.345±6.389</td>
<td>0.003±0.001</td>
<td>127.527±45.925</td>
<td>0.176±0.065</td>
<td>0.823±0.237</td>
<td>0.306±0.254</td>
</tr>
<tr>
<td>SS</td>
<td>14.437±3.319</td>
<td>32.760±6.657</td>
<td>0.005±0.001</td>
<td>208.041±48.458</td>
<td>0.262±0.078</td>
<td>1.097±0.283</td>
<td>1.98±0.433</td>
</tr>
<tr>
<td>BS</td>
<td>0</td>
<td>7.038±3.551</td>
<td>0</td>
<td>46.365±38.218</td>
<td>0.091±0.053</td>
<td>0.310±0.204</td>
<td>0.421±0.208</td>
</tr>
<tr>
<td>Within siblings'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>14.619±1.812</td>
<td>38.337±4.840</td>
<td>0.006±0.001</td>
<td>387.042±47.825</td>
<td>0.542±0.067</td>
<td>1.749±0.218</td>
<td>2.570±0.319</td>
</tr>
<tr>
<td>SS</td>
<td>16.446±2.268</td>
<td>27.266±3.816</td>
<td>0.004±0.001</td>
<td>236.777±33.273</td>
<td>0.491±0.068</td>
<td>1.529±0.213</td>
<td>2.005±0.280</td>
</tr>
<tr>
<td>BS</td>
<td>44.557±3.180</td>
<td>41.329±4.216</td>
<td>0.008±0.001</td>
<td>479.695±49.310</td>
<td>0.647±0.066</td>
<td>2.405±0.249</td>
<td>2.472±0.250</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001; ns=non-significant
Unadjusted, partially adjusted and fully adjusted intraclass correlations are provided in Table 3. In most cases, the inclusion of covariates had only a minimal influence on the magnitude of the intrapair correlations. However, in some cases the correlations were affected by the inclusion of covariates. For example, the BB unadjusted correlation for 1MR was 0.35 which dropped to 0.11 after the inclusion of all covariates. In general, the correlations for BS pairs were much lower than for the same-sex pairs across the board, irrespective of the inclusion of additional covariates.
Table 3. Intraclass correlation coefficients (ρ) and (95% CI) for each physical fitness test

<table>
<thead>
<tr>
<th></th>
<th>Body fat (%)</th>
<th>Waist circumference (cm)</th>
<th>Hand grip (log kg(^f))</th>
<th>Standing long jump (cm)</th>
<th>50 yard dash (s)</th>
<th>Shuttle-run (s)</th>
<th>1-mile run/walk (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.54 (0.43-0.64)</td>
<td>0.41 (0.29-0.54)</td>
<td>0.21 (0.10-0.39)</td>
<td>0.29 (0.14-0.50)</td>
<td>0.43 (0.32-0.56)</td>
<td>0.44 (0.32-0.56)</td>
<td>0.35 (0.23-0.50)</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>0.54 (0.43-0.65)</td>
<td>0.43 (0.32-0.59)</td>
<td>0.27 (0.15-0.44)</td>
<td>0.33 (0.20-0.48)</td>
<td>0.39 (0.27-0.53)</td>
<td>0.42 (0.30-0.55)</td>
<td>0.37 (0.25-0.51)</td>
</tr>
<tr>
<td>Fully adjusted**</td>
<td>0.60 (0.48-0.70)</td>
<td>0.42 (0.28-0.56)</td>
<td>0.30 (0.16-0.48)</td>
<td>0.25 (0.12-0.44)</td>
<td>0.25 (0.12-0.44)</td>
<td>0.32 (0.19-0.43)</td>
<td>0.11 (0.02-0.42)</td>
</tr>
<tr>
<td>SS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.42 (0.29-0.56)</td>
<td>0.40 (0.27-0.55)</td>
<td>0.30 (0.17-0.47)</td>
<td>0.28 (0.15-0.46)</td>
<td>0.43 (0.30-0.57)</td>
<td>0.43 (0.30-0.57)</td>
<td>0.46 (0.33-0.59)</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>0.42 (0.29-0.56)</td>
<td>0.46 (0.34-0.60)</td>
<td>0.46 (0.33-0.59)</td>
<td>0.33 (0.20-0.50)</td>
<td>0.32 (0.19-0.49)</td>
<td>0.48 (0.35-0.61)</td>
<td>0.46 (0.33-0.59)</td>
</tr>
<tr>
<td>Fully Adjusted*</td>
<td>0.47 (0.33-0.61)</td>
<td>0.55 (0.41-0.67)</td>
<td>0.54 (0.40-0.69)</td>
<td>0.47 (0.32-0.62)</td>
<td>0.35 (0.20-0.53)</td>
<td>0.41 (0.27-0.58)</td>
<td>0.50 (0.36-0.64)</td>
</tr>
<tr>
<td>BS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.18 (0.09-0.32)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>0.28 (0.18-0.41)</td>
<td>0.08 (0.02-0.29)</td>
<td>0</td>
<td>0.06 (0.01-0.35)</td>
<td>0.03 (0.00-0.66)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fully Adjusted*</td>
<td>0.15 (0.05-0.34)</td>
<td>0</td>
<td>0.09 (0.02-0.36)</td>
<td>0.12 (0.04-0.34)</td>
<td>0.11 (0.03-0.35)</td>
<td>0.14 (0.05-0.34)</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for age and age\(^2\); **adjusted for all covariates
DISCUSSION

Biological, behavioural and sociodemographic characteristics are expected to be associated with PF components in different ways. For example, greater chronological age has consistently been associated with better PF during childhood and adolescence, although with varying degrees of magnitude (R. Malina & Bouchard, 2004). In the present study, the maturity offset was found to be positively associated with siblings muscular and motor components, negatively with morphological fitness, and had no significant effect on the cardiorespiratory component. Previous relationships between chronological and biological ages and PF have been reported, but it is not always easy to clearly separate their unique contributions as well as their links to changes in body size (R. Malina & Bouchard, 2004). For example, Welk, Saint-Maurice, and Csanyi (2015) using chronological age and Jones, Hitchen, and Stratton (2000) using biological age both showed positive associations with PF, while other studies based on chronological age reported the contrary (Guedes, Miranda Neto, Lopes, & Silva, 2012). Further research is required to better delineate the roles of chronological and biological age on PF levels in childhood.

As expected, %BF was negatively associated with PF in the present study, except for GS (not statistically significant). These results support previous reports using non-sibling data (R. Malina & Bouchard, 2004). For example, Moliner-Urdiales et al. (2011) showed that Spanish adolescents with lower %BF were more physically fit. In a different vein, behavioural characteristics (TV and TPA) were not strongly associated PF components in the present study. A previous study among 11 to 18 year old children showed that, among boys, high TV viewing time (≥2 h/day) was positively related with their WC, and that high total screen time (≥3 h/day) was positively associated with their WC and BMI; no such associations were found in girls (Arango et al., 2014). Moreover, Dencker et al. (2006) reported that cardiorespiratory fitness was positively correlated with vigorous physical activity but not with moderate physical activity in Swedish youth, and two studies in youth from the USA and Spain, Bai et al. (2016), Ara et al. (2004) showed positive associations between physical activity and PF. Overall, there is some evidence that physical activity is related to different PF
components in children; however more research is required to better determine the magnitude and direction of the associations.

In the present study, SES was negatively associated with SLJ and SR. Previous research on siblings PF did not consider the putative effect of SES. However, Mutunga et al. (2006) examined the relationship between SES and VO2max in a large sample of adolescents from Northern Ireland and reported that those with higher SES had higher cardiorespiratory fitness. Similarly, Jimenez Pavon et al. (2010) using data from different European countries showed that boys and girls with higher SES were also more physically fit in muscular, motor and cardiorespiratory components. In sum, it is important to consider SES as a covariate when interpreting familial aggregation of PF components. The interpretation of similarities among twins, siblings or nuclear family members in health-related PF is always conditioned by several issues, namely: (1) differences in sample size across studies, (2) population of origin/ethnicity, (3) differences in measurement protocols for PF assessments, (4) data reliability, (5) differences in statistical approaches, and (6) differences in covariates included in the models.

In general, same sex siblings’ morphological fitness (%BF and WC) were systematically more alike, whereas opposite sex siblings showed no resemblance in %BF, and only moderate similarity in WC. It is also possible that unique developmental histories of each member of the opposite sex in opposite-sex sibling pairs during his/her physical growth, biological maturation, exercise habits and food consumption may explain the absence of similarity in %BF, and low similarity in WC. This sexual dimorphism is pervasive in many biological traits (Palmer & Clegg, 2015; Rigby & Kulathinal, 2015; Taylor, Grant, Williams, & Goulding, 2010). Contrary to our findings, previous studies showed lower sibling similarity in morphological traits than we have shown. For example, Katzmarzyk et al. (2000) reported a intra-pair sibling correlation of 0.25 for WC and Rice et al. (1997) presented an intrapair sibling correlation of 0.32 for %BF in the HERITAGE Family Study. However, in these studies, siblings were not separated by type (BB, SS and BS pairs) and their analysis only adjusted for age and sex. Taken together, these positive correlations suggest that shared genetic
and sociodemographic factors play important roles in sibling similarity. To date, there are some specific genes that have been linked to morphological fitness traits. Rankinen et al. (2006) reviewed the available literature on the human obesity gene map and reported that WC was associated with biological markers in 10 genes. For example, Robitaille, Despres, Perusse, and Vohl (2003), K. S. Kim, Choi, Shin, Yang, and Yoon (2004) and Fornage et al. (2005) found associations with PPAR-ρ and WC; PPAR-ρ is a nuclear receptor that regulates adipocyte differentiation and possibly lipid metabolism and can therefore be a key regulator of fat storage.

Recently Lu et al. (2016) conducted a genome-wide association study (GWAS) for %BF in 100,716 individuals, and identified 12 loci, eight of which were previously associated with overall adiposity (BMI, %BF) and four were novel in their associations with %BF.

For muscular fitness (GS and SLJ), with increasing covariate adjustments, SS pairs were more alike in both tests than BB pairs, while BS similarity is very low or absent. These differences could partially be explained by sex-differences in physiological, biochemical and hormonal mechanisms associated with muscular strength (Lloyd et al., 2014; R. Malina & Bouchard, 2004), as well as by differences in body size since we did not scale their performance (see Asmussen (1973) Nevill, Ramsbottom, and Williams (1992). In any case, the higher resemblance in same-sex siblings may indicate biological, behaviour and sociodemographic characteristics, as well as genetic influences on these phenotypes (Kovar, 1976; Maes et al., 1996).

Mozambican (Saranga et al., 2010) and Polish (Pawlak, 1984) data also showed that SS pairs (ρ=0.19; ρ=0.44) were more similar than BB (ρ=0.09; ρ=0.25) and BS (ρ=0.02; ρ=0.21) in GS. For SLJ, correlations were different across studies: Saranga et al. (2010) reported the highest resemblance in SS (ρ = 0.44) followed by BS (ρ=0.39) and BB (ρ=0.23), while Pawlak (1984) showed the highest resemblance in BB pairs (ρ=0.29) but similar results in SS pairs (ρ=0.16) and BS pairs (ρ=0.16), although in these studies the analysis only adjusted for age and sex. The precise location of responsible genes for muscular fitness is still under scrutiny. GWAS data as well as association studies with
candidate genes are scarce and inconsistent, respectively. Two recent reviews examined candidate genes for muscular strength and categorized them according to either their involvement in the structural muscle function or their influencing role in muscle physiology (Hubal, Urso, & Clarkson, 2011; Thomis, 2011). For example, the MSTN K153R polymorphism is associated with the ability to produce peak power during muscle contractions, as assessed with jump tests, in young nonathletic men (Santiago et al., 2011). We only found one study that tried to identify single nucleotide polymorphisms (SNPs) associated with GS in middle-aged to older adults using GWAS (Chan et al., 2015). However, no genome-wide significant results were observed.

For the motor fitness component, BB and SS pairs demonstrated greater similarities than BS pairs. The unique environment, i.e., life history idiosyncrasies of each member of the sibling pair may help in the interpretation of dissimilarities between them. On the other hand, Saranga et al. (2010) reported greater familiality in Mozambican BB pairs (ρ=0.13) than SS (ρ=0.04) and BS (ρ=0.02) in the SR test. Differences between these two studies may be due to ethnicity as well as diverse sociodemographic conditions. Nonetheless, our results indicate that the motor performance component is likely influenced by genetic and sociodemographic characteristics, although we were not able to find a study that explicitly examined the role of any putative gene, or genes, in the phenotypic expression of this PF component. The ACTN3 gene has been associated with performance in sprint athletes. For example, Yang et al. (2003) studied athletes and non-athletes and reported that male and female elite athletes had significantly higher frequencies of the 577R allele than the non-athletes. Further, H. Kim, Song, and Kim (2014) examined the association between the distribution of ACTN3 genotypes and alleles in muscle power, speed, and strength-oriented athletics and showed that only the speed-oriented athletes had significant differences in the frequency distributions of the ACTN3 XX genotype from that of the controls.

For cardiorespiratory fitness, the unadjusted and adjusted ρ values showed that SS pairs display greater resemblance than BB and BS. These results may indicate that females are more prone to display genetic and/or
shared environmental putative effects than males. Previous reports with Mozambican (Saranga et al., 2010) and Portuguese (Vasques, Vitor, Seabra, Fermino, & Maia, 2007) siblings also showed greater familiality in SS pairs than in BB or BS pairs. However, the cardiorespiratory component is a highly complex phenotype in terms of its physiologic and biochemical mechanisms. It is thus rather difficult to signal the presence of relevant genes in the expression of this component, since there is no single gene to determine cardiorespiratory fitness (Wolfarth, 2011). Although we did not find any association studies with candidate genes in the phenotypic expression of the 1-mile run, or any other marker of cardiorespiratory endurance with children and adolescents, yet several studies based on other phenotypes were previously reviewed using adult samples (Loos et al., 2015), and more than 30 genes have been recently found, from more than five physiological areas (regulating hormones, muscle metabolism, lipid metabolism, growth factors, cellular mediators and others) (Wolfarth, 2011).

Although highly debatable (Bray et al., 2009), a polymorphism of the angiotensin I-converting enzyme (ACE) gene has been associated with metabolic efficiency, specifically the D-allele with an exaggerated response to training, and the I-allele with the lowest cardiac growth response. In light of the I-allele association with endurance performance, it seems likely that other regulatory mechanisms exist (Puthucheary et al., 2011).

Given the use of a large sample of siblings, a large age range covering childhood and adolescence, and extended PF assessment, this allows us to extend our conclusions to single children and adolescents enrolled in the same schools and living in similar environments. Since PF is an important marker of health in children and adolescents (Ortega et al., 2008) physical activity intervention program designers should consider targeting families given the importance of family members as role models which may have an influence on developing PF levels in children. Additionally, since different sex siblings are more dissimilar, programs to enhance PF levels in childhood should also consider sex-specific strategies. Although it is well acknowledged that individual responses to a physical activity intervention program is highly variable and may have a genetic basis (Bouchard & Rankinen, 2001) we anticipate that research
involving siblings will enhance our understanding of the interplay of nurture and nature in their PF similarity/dissimilarity during childhood and adolescence. This information is currently lacking.

Notwithstanding the relevance of these results, our study has limitations: (1) the sample does not cover all Portuguese regions, and this limits the generalizability of the results to all Portuguese children and adolescents; (2) the use of a questionnaire to obtain information about PA and TV viewing is prone to errors, even when it is collected under controlled conditions.

However, the use of self-report instruments to obtain this information is common (Ferreira, Marques, & Maia, 2002; Hallal et al., 2012; Vasconcelos & Maia, 2001). Additionally, the Baecke questionnaire is regularly used in Portuguese studies with highly reliable results (Antunes et al., 2015; de Chaves et al., 2014; Maia, Gomes, Tregouet, & Katzmarzyk, 2014; Santos et al., 2014). This study also has several strengths, including: (1) the use of a broad approach of health-related physical fitness with siblings, (2) the large sample size of siblings, (3) the examination of important time windows in children's growth and development, (4) the use of standard measurement protocols and highly reliable data, and (5) the use of a novel, multilevel statistical methodology.

CONCLUSIONS

In conclusion, our results revealed that siblings' PF similarity varies according to its components. Same-sex siblings demonstrate greater resemblance in all PF components. Additionally, older and more mature subjects are better performers. More physically active subjects do not perform better than those with lower physical activity levels, except in the shuttle run where the most active youth tend to have worse performance; time spent watching TV did not show any association with any physical fitness component, but higher SES was negatively associated with standing long jump and shuttle run performance. Taken together these results reinforce the idea that PF is not only the end result of genetic endowments, but also of shared and unique environmental factors which can act alone or in association with genes, modulating their expression.
REFERENCES


using mixed-effects location scale models. *Statistics in Medicine, 31*(27), 3328-3336.


A multilevel analysis of health-related physical fitness


A multilevel analysis of health-related physical fitness


Sibling similarity in metabolic syndrome: The Portuguese sibling study on growth, fitness, lifestyle and health

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**ABSTRACT**

This study aims to estimate sibling resemblance in metabolic syndrome (MS) markers, and to investigate the associations of biological and behavioural characteristics with MS. The sample comprises 679 biological siblings (363 females; 316 males) aged 9-20 years. MS markers included waist circumference (WC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TRI), fasting glucose (GLU) and systolic blood pressure (SBP). Body mass index (BMI), biological maturation, muscular, and cardiorespiratory fitness were also assessed. Behavioural characteristics, including dietary intake and physical activity, were self-reported by questionnaire. Multilevel models were used, and sibling resemblance was estimated using the intraclass correlation (ρ). In general, same-sex siblings showed higher resemblance in MS markers than opposite-sex siblings. However, variability in sibling resemblance in MS markers was evident with the inclusion of covariates. Biological characteristics including age, BMI and maturity offset influenced all MS markers except for TRI. Importantly, behavioural characteristics diversely influenced MS markers: fruit and vegetables only influenced SBP, whereas physical activity affected HDL-C. Additionally, muscular fitness impacted significantly on MS Z-score, WC, SBP and GLU, whilst cardiorespiratory fitness only affected WC. In conclusion, biological and behavioural characteristics influenced the expression of MS markers. These results confirmed the importance of considering individual characteristics when designing individualized programs for diminishing the adverse effects of specific MS markers.
INTRODUCTION

Metabolic syndrome (MS) represents a cluster of risk factors, including central adiposity, dyslipidemia, high blood pressure and insulin resistance (Alberti, Zimmet, & Shaw, 2006). The MS is often viewed as a precursor to several non-communicable diseases leading to an overall increase in morbidity and mortality (Eckel, Grundy, & Zimmet, 2005). Estimates of MS prevalence vary, depending on the population studied and the criteria used to define it. Moreover, with the recent increase in the prevalence of pediatric obesity, MS is becoming more evident in children and adolescents. Recently, a systematic review reported that the prevalence of MS in the youth population was approximately 3.3% (range 0%-19.2%), with higher prevalence in overweight (11.9%; range 2.8%-29.3%) and obese youth (29.2%; range 10%-66%) (Friend, Craig, & Turner, 2013). Furthermore, the clustering of risk factors that starts at childhood and adolescence tracks into adulthood and confers substantial risk for future non-communicable diseases (Agirbasli, Tanrikulu, & Berenson, 2016).

Epidemiological studies have shown that variation in the prevalence of MS at the population level is the result of the complex interactions of genes, behaviours and contextual factors (Eckel et al., 2005). However, much of this evidence has been derived from cohort studies of unrelated individuals, and the specific contributions from shared genetic, behavioural and environmental influences on MS are still not entirely clear. For example, the importance of healthy diets was highlighted as protective for MS development (Baxter, Coyne, & McClintock, 2006). Yet, data from a recent systematic review (Collese et al., 2017) showed that from the 11 studies passing the inclusion criteria, only one-third presented significant inverse associations of fruit and vegetable intake with MS components. Similarly, although low levels of physical activity have been associated with a higher risk of developing MS (McMurray, Bangdiwala, Harrell, & Amorim, 2008; Nguyen, Tang, Kelly, van der Ploeg, & Dibley, 2010; N. A. Stabelini, de Campos, Dos Santos, & Mazzardo, 2014), there is also evidence of no significant associations (Fam et al., 2013; Mehairi et al., 2013; N.A. Stabelini et al., 2011). Contrary to these opposite findings, physical fitness components (muscular and cardiorespiratory) have been recognized as putative
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protective factors in MS development (Artero et al., 2011; N.A. Stabelini et al., 2011; Steene-Johannessen, Anderssen, Kolle, & Andersen, 2009). Furthermore, and yet scarce, there is some evidence emphasizing the links between biological maturation and the prevalence of MS. For example, Widen et al. (2012) showed that earlier pubertal timing was positively associated with MS markers like blood pressure, HDL-cholesterol and fasting insulin. Similarly, and based on the age of attaining peak height velocity, Werneck et al. (2016) described that early maturing adolescents exhibited higher values for individual MS risk factors, as well as for MS scores. Still, it is important to recognize that these studies investigated the “effect” of each factor separately, i.e., the association between diet and MS or physical activity levels and MS, or physical fitness levels and MS, and that a more encompassing approach considering the additive effects of a set of predictors would probably reveal a more comprehensive picture.

Likewise, when the focus is on genetic and environmental effects there are also discrepancies among studies. For example, based on GWA studies, Arya et al. (2015) attempting to map susceptibility genes for MS, reported that more than ~50 different loci have been shown to be associated with MS and most were not replicated in more than one study. Similarly, family/twin data from several studies showed a wide range of heritability estimates for all MS markers, namely for waist circumference (0.38-0.51), HDL-cholesterol (0.40-0.63), triglycerides (0.16-0.28), glucose (0.15-0.47) and systolic blood pressure (0.16-0.43) (Bellia et al., 2009; Duan et al., 2011; Lin et al., 2005; Luo et al., 2010; van Dongen, Willemsen, Chen, de Geus, & Boomsma, 2013). Also, intra-pair correlations from twin/sibling studies revealed a high variation: waist circumference (0.24 to 0.72), HDL-C (0.24 to 0.71), triglycerides (0.09 to 0.54), glucose (0.10 to 0.64), and systolic blood pressure (0.17 to 0.66) (Miranda-Lora, Vilchis-Gil, Molina-Diaz, Flores-Huerta, & Klunder-Klunder, 2017; Sung, Lee, & Song, 2009; Zarkesh et al., 2014). Although important, these studies did not consider, in their design and analysis, the recognized influence of other correlates of MS in siblings’ and twins’ resemblance beyond age and/or sex.

It is well known that siblings share, on average, 50% of their genes identical-by-descent, and also have a common family environment (Malina, Bar-Or, & Bouchard, 2004), yet they differ in their chronological age, sex and health behaviours as well in their physical growth, biological maturation, and motor
development trajectories (Malina et al., 2004). These, of course, may be linked to MS in different ways. Using a multilevel framework (subjects nested within siblings) may help in elucidating how siblings resemble each other in their MS markers conditioned on the additive effects of their biological and behavioural traits, a research approach that apparently was never used. Hence this study aims to estimate sibling resemblance in MS markers, and to investigate the effects of biological (age, sex, biological maturation, physical fitness) and behavioural (physical activity, sugary drink and fruit and vegetable consumption) characteristics on these markers. We intend to answer the following questions: (1) what is the degree of sibling resemblance in MS markers as well as in the overall expression of MS? (2) Is this resemblance similar in effect size across all MS markers? (3) Is sibling resemblance sex-specific? (4) Does the similarity change when adjusted for the additive effects of biological and behavioural traits? (5) Across siblings, are all MS markers differently influenced by their biological and behavioural characteristics?

**METHODS**

*Study participants*

The study participants are from the Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health which investigates physical growth, body composition, physical fitness, physical activity, metabolic syndrome, and health behaviours in a cohort of siblings (S. Pereira et al., 2017; Pereira et al., 2018). Participants were recruited from schools in mainland (north and center) Portugal and from the Azores islands. This is a convenience sample as are most samples from family studies; further, it is also part of the Portuguese Healthy Family Study (Santos et al., 2014). Those children/adolescents who had their siblings studying in the same school were invited to take part in the study, and the response rate was ~80%. Written informed consent was obtained from legal guardians, and the project was approved by the Ethics Committee of the University of Porto and school authorities.

A total of 3285 siblings participated in the main study; however, the analytical sample for the present analysis comprised 679 biological siblings (363 females and 316 males) from 333 nuclear families (286 siblings and 50 twins)
with complete data on MS. However, we do not have information on the twins' zygosity as this usually requires genetic analysis from biological samples (blood for example). As is well-known, zygosity can be fairly assessed with reliable questionnaires instead of using biological samples (e.g., blood or cheek swabs). Yet, given limited time as well as operational constraints within each school setting to collect the data, we were not able to send twin mothers a putative cross-culturally validated questionnaire to classify their zygosity. It is important to note, nonetheless, that no statistically significant mean differences were observed between included and excluded subjects with respect to weight, BMI, physical fitness, and physical activity; only height showed a significant difference (excluded subjects were, on average, ≈1.5 cm taller).

**Anthropometry**

Height, weight and waist circumference (WC) were measured according to standardized protocols. Height was measured with a portable stadiometer (Holtain, UK) and WC with a non-elastic tape (Sanny, American Medical of Brazil, Brazil). Weight was measured with a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan), and body mass index (BMI) was computed using the standard formula; further, participants were categorized as normal weight and overweight/obese according to cut points from the World Health Organization (WHO) (WHO, 1995).

**Biological Maturation**

Biological maturation was assessed with the maturity offset (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002), which estimates, in decimal years, the distance from estimated age-at-peak height velocity (PHV). A positive maturity offset indicates the number of years the participant is beyond PHV, whereas a negative maturity offset represents the number of years the participant is before PHV.

**Metabolic syndrome markers**

MS markers included WC, high-density lipoprotein cholesterol (HDL-C), triglycerides (TRI), fasting glucose (GLU), and systolic blood pressure (SBP).
SBP was measured with an automatic digital Omron sphygmomanometer (Omron M6, hem 7001-E, Omron Healthcare). All participants rested for at least 5 minutes before the first measurement. Three consecutive measures were obtained, with a 2 or 3-minute interval between them, and the mean value was used for analysis. If the difference between the three measurements was greater than 10 mm Hg, a fourth measurement was taken and the measurement with higher differences was removed; HDL-C, TRI, and GLU were obtained after a 10-12 h overnight fast from a finger stick blood sample, and analyzed by the Cholestech LDX (Cholestech Corporation, Hayward, CA, USA), a previously validated instrument (Cholestech LDX, 2003). Daily checks of the equipment were made according to manufacturer’s instructions. A standardized z-score for each MS marker (WC, SBP, HDL-C, TRI, and GLU) was computed as advocated (J.C. Eisenmann, 2008). The z-score for HDL-C was multiplied by (-1), since lower values are an indicative of higher risk. The z-scores of the MS markers were summed to obtain the total MS score (Z-MS) – higher Z-MS values correspond to higher metabolic risk (J.C. Eisenmann, Laurson, DuBose, Smith, & Donnelly, 2010).

Fruits, vegetables and sugary drinks consumption

Dietary intake data were obtained from a food frequency questionnaire (FFQ), adapted from the Health Behaviour in School-aged Children Survey (HBSC) (Currie et al., 2008), and modified with the inclusion of a variety of typical Portuguese food items. Participants were asked about various types of food consumed in a typical week. For this report, only questions related to fruits, vegetables and sugary drinks were used. For each item, the answer options were: “never”; “less than once per week”; “once per week”; “2–4 days per week”; “5–6 days per week”; “once a day, every day”; and “more than once a day”. For fruit/vegetable consumption, responses were categorized into two groups: 0=“daily consumption” (children who consume this type of food every day of the week) and 1= “no daily consumption”. Answers for sugary drink consumption were also dichotomized: 0=“less than two times per week” and 1=“two or more times per week”. In all analyses the reference category was 0.
**Physical activity**

Physical activity (PA) was assessed with the Baecke questionnaire (Baecke, Burema, & Frijters, 1982), a reliable (Miller, Freedson, & Kline, 1994; M. A. Pereira et al., 1997) and valid instrument (Helmerhorst, Brage, Warren, Besson, & Ekelund, 2012; Philippaerts, Westerterp, & Lefevre, 1999) which includes a total of 16 questions, organized into three specific domains: work/school PA, leisure-time PA, and sports participation. The sum of these three specific domains provides an estimation of total PA (TPA). For each domain, each score ranges from 1 (minimal) to 5 (maximal), such that the TPA score varies between 3 and 15. The questionnaire was administered during physical education classes and all participants answered under the supervision of their teacher as well as by a trained research team member.

**Muscular and cardiorespiratory fitness**

Muscular fitness was assessed using two tests: handgrip (static strength) and standing long jump. Handgrip – using a hand dynamometer (Takei Digital Grip Strength Dynamometer, Model T.K.K.5401, Japan) – all participants gripped the dynamometer with maximum force for 5 to 10 seconds. A ratio handgrip/weight was calculated and used in analysis to adjust for body size as suggested (Cohen et al., 2014; Gomes, Dos Santos, Katzmarzyk, & Maia, 2017). Standing long jump test (muscle power) – all participants jumped as far as possible from a standing position; A standardized z-score for each muscular fitness test was computed. The z-scores were then summed to obtain a muscular fitness z-score (Z-Muscular fit) as advocated (Huang & Malina, 2007). Cardiorespiratory fitness (CF) was assessed using 1-mile run/walk test – all participants ran/walked the distance in the shortest time possible.

**Statistical analysis**

Descriptive statistics and tests (ANOVA at the sib-ship level) were computed in IBM-SPSS 24 to describe and examine differences between sib-ship pairs using standard statistical methods. Since TRI was highly skewed, it was log transformed. Given that individuals were nested within sib-ships, we used a multilevel model implemented in STATA 14. Specifically, we estimated separate within- and between-sib-ship variances, and therefore different
intraclass correlations ($\rho$), with corresponding 95% confidence intervals (95% CI), for the three sib-ship pairs [brother-brother (BB), sister-sister (SS) and brother-sister (BS)] following an approach described by Hedeker, Mermelstein, and Demirtas (2012). Models were estimated first for Z-MS and then for each MS marker using the following set of covariates: twin (dummy coded: 0=no twin sib-ships; 1=twin sib-ships), age, age$^2$, BMI (except for the waist circumference analysis), maturity offset, fruit and vegetable consumption, sugary drink consumption, TPA and Z-Muscular fitness and and Z-cardiorespiratory fitness; when needed, covariates were centered at their respective means. Unadjusted, and adjusted (for all covariates) intraclass correlations were computed and tested for equality across sib-ship pairs. This was done using a likelihood-ratio test comparing a model that constrained the ICC to be equal across sib-ship pairs to a model that varied the ICCs across sib-ship pairs. In all models, the BB pairs were the reference category. All models were estimated with and without the twin information, and differences were trivial in the variance components. This is the reason why we only report on twin effects on the fixed part of all models.

RESULTS

Descriptive statistics for MS markers, as well as for biological and behavioural indicators are presented in Table 1. On average, no statistically significant differences ($p>0.05$) were found among sib-ship pairs for chronological age, biological maturation, BMI, HDL-C, SBP, and Z-MS; BB pairs were more physically active, fitter (both in muscular fitness and cardiorespiratory fitness) and had the highest WC when compared to SS and BS pairs ($p<0.05$). Additionally, SS pairs had the highest mean TRI values ($p<0.05$), and were also those who consumed less sugary drinks; BS pairs had the highest GLU mean values, and were also those who consumed more fruits and vegetables ($p<0.05$).
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Table 1. Descriptive statistics [means and standard deviations (SD)] for sib-ship pairs

<table>
<thead>
<tr>
<th></th>
<th>Brother-Brother (n=170)</th>
<th>Brother-Sister (n=287)</th>
<th>Sister-Sister (n=222)</th>
<th>F</th>
<th>Post-hoc comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Chronological age (y)</td>
<td>13.6</td>
<td>2.3</td>
<td>13.4</td>
<td>2.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Maturity offset (y)</td>
<td>0.3</td>
<td>2.2</td>
<td>0.2</td>
<td>1.9</td>
<td>0.4</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>21.1</td>
<td>4.0</td>
<td>20.9</td>
<td>3.9</td>
<td>21.2</td>
</tr>
<tr>
<td>TPA (arbitrary units)</td>
<td>8.4</td>
<td>1.4</td>
<td>7.9</td>
<td>1.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Muscular fitness (z-scores)</td>
<td>1.0</td>
<td>1.7</td>
<td>-0.0</td>
<td>1.8</td>
<td>-0.7</td>
</tr>
<tr>
<td>Cardiorespiratory fitness (min)</td>
<td>8.4</td>
<td>2.1</td>
<td>9.3</td>
<td>2.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Standing long jump (cm)</td>
<td>169.6</td>
<td>31.9</td>
<td>149.9</td>
<td>33.0</td>
<td>138.7</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>71.6</td>
<td>10.7</td>
<td>69.1</td>
<td>9.5</td>
<td>67.4</td>
</tr>
<tr>
<td>HDL-C (mg·dL⁻¹)</td>
<td>52.0</td>
<td>16.0</td>
<td>50.2</td>
<td>14.3</td>
<td>53.4</td>
</tr>
<tr>
<td>TRI (mg·dL⁻¹)</td>
<td>70.4</td>
<td>43.7</td>
<td>71.4</td>
<td>40.7</td>
<td>86.1</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>112.8</td>
<td>14.6</td>
<td>112.5</td>
<td>13.9</td>
<td>110.8</td>
</tr>
<tr>
<td>GLU (mg·dL⁻¹)</td>
<td>86.5</td>
<td>8.1</td>
<td>88.5</td>
<td>8.8</td>
<td>86.3</td>
</tr>
<tr>
<td>Z-MS (z-score)</td>
<td>0.0</td>
<td>2.8</td>
<td>0.2</td>
<td>2.6</td>
<td>-0.3</td>
</tr>
<tr>
<td>Fruits/vegetables %</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>χ²:</td>
</tr>
<tr>
<td>All days</td>
<td>9.4</td>
<td>20.2</td>
<td>18.4</td>
<td></td>
<td>7.256*</td>
</tr>
<tr>
<td>&lt; All days</td>
<td>90.6</td>
<td>79.8</td>
<td>81.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugary drinks %</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>&lt; 2 days/week</td>
<td>45.7</td>
<td>51.1</td>
<td>61.1</td>
<td></td>
<td>8.284*</td>
</tr>
<tr>
<td>≥ 2 days/week</td>
<td>54.3</td>
<td>48.9</td>
<td>38.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unadjusted and adjusted intraclass correlations are presented in Table 2. Among the two conditions (equal variance components, and hence equal intraclass correlation coefficients, versus different variance components among sib-types), the model fit is statistically improved under the latter condition for all variables except for TRI. Further, results indicated that the degree and the magnitude of sibling resemblance varied depending on the MS marker and sib-ship. For example, based on unadjusted correlations BB pairs showed higher resemblance in Z-MS (0.52), WC (0.58) and HDL-C (0.47). However, SS pairs are more similar in WC (0.42), HDL-C (0.47) and GLU (0.36), but BS pairs in HDL-C (0.47), TRI (0.31), SBP (0.31) and GLU (0.36). Furthermore, the inclusion
of covariates changed the magnitude of the correlations and are sib-ship dependent. For example, after the inclusion of the covariates, SS pairs demonstrate higher resemblance in Z-MS. However, BB and BS pairs don’t change substantially. Further, for WC the BS unadjusted correlation was 0.18, but with the inclusion of covariates the value changed to 0; in addition, no differences were observed for BB correlations before and after the inclusion of covariates (0.58) and SS pairs decreased from 0.42 to 0.22. In general, same-sex siblings were more alike than opposite-sex sibling pairs for all MS markers, (except in TRI and BB pairs in GLU). Additionally, given that our sample includes twin pairs and, they tend to share more genetic factors than siblings (especially monozygotic twins), we added a supplementary table where ρ values and respective 95% CI’s were computed for each MS marker and Z-MS without inclusion of the twin pairs. These results show a similar trend as in Table 2. Yet, the results with twin data show relatively higher values in some MS markers comparatively to non-twin data, namely: Z-MS (in BB and BS), WC (in BB and SS), HDL and TRI (in all sibtypes) and GLU (in SS). However, ρ values from siblings with twins revealed to be lower than in siblings without twins in Z-MS (in SS), GLU (in BB and BS) and equal values in WC (in BS) and SBP (in BB and BS). Finally, all the 95% CI’s of reported ρ values from these two samples tend to overlap, suggesting a consistent sibling resemblance.
Table 2. Intraclass correlation coefficients (ρ) and their 95% confidence intervals for each MS marker and Z-MS: unadjusted and adjusted (partial correlations) values

<table>
<thead>
<tr>
<th>Z-MS (z-score)</th>
<th>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Unadjusted&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Adjusted&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Δ LL(χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-MS (z-score)</td>
<td>BB (95%CI)</td>
<td>SS (95%CI)</td>
<td>BS (95%CI)</td>
<td>Log likelihood (LL)</td>
<td>Δ LL(χ²)</td>
</tr>
<tr>
<td>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 (0.22-0.41)</td>
<td>0.31 (0.22-0.41)</td>
<td>0.31 (0.22-0.41)</td>
<td>-1532.43</td>
<td>10.45(20.90)&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Unadjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.52 (0.37-0.67)</td>
<td>0.31 (0.17-0.50)</td>
<td>0.15 (0.05-0.38)</td>
<td>-1521.98</td>
<td>7.03(14.06)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 (0.26-0.50)</td>
<td>0.37 (0.26-0.50)</td>
<td>0.37 (0.26-0.50)</td>
<td>-867.41</td>
<td></td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61 (0.43-0.77)</td>
<td>0.48 (0.29-0.67)</td>
<td>0.22 (0.07-0.51)</td>
<td>-860.38</td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 (0.29-0.47)</td>
<td>0.38 (0.29-0.47)</td>
<td>0.38 (0.29-0.47)</td>
<td>-2479.40</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>Unadjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58 (0.44-0.71)</td>
<td>0.42 (0.28-0.57)</td>
<td>0.18 (0.07-0.39)</td>
<td>-2471.78</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 (0.13-0.42)</td>
<td>0.25 (0.13-0.42)</td>
<td>0.25 (0.13-0.42)</td>
<td>-1476.85</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58 (0.40-0.74)</td>
<td>0.22 (0.04-0.63)</td>
<td>0</td>
<td>-1459.56</td>
</tr>
<tr>
<td>HDL-C (mg dL⁻¹)</td>
<td>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 (0.39-0.55)</td>
<td>0.47 (0.39-0.55)</td>
<td>0.47 (0.39-0.55)</td>
<td>-2735.18</td>
</tr>
<tr>
<td>HDL-C (mg dL⁻¹)</td>
<td>Unadjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.59 (0.45-0.72)</td>
<td>0.54 (0.41-0.66)</td>
<td>0.34 (0.21-0.49)</td>
<td>-2730.91</td>
</tr>
<tr>
<td>HDL-C (mg dL⁻¹)</td>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 (0.30-0.52)</td>
<td>0.40 (0.30-0.52)</td>
<td>0.40 (0.30-0.52)</td>
<td>-1790.59</td>
</tr>
<tr>
<td>HDL-C (mg dL⁻¹)</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 (0.26-0.66)</td>
<td>0.59 (0.43-0.74)</td>
<td>0.24 (0.09-0.50)</td>
<td>-1785.15</td>
</tr>
<tr>
<td>TRI transformed</td>
<td>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 (0.22-0.41)</td>
<td>0.31 (0.22-0.41)</td>
<td>0.31 (0.22-0.41)</td>
<td>-402.88</td>
</tr>
<tr>
<td>TRI transformed</td>
<td>Unadjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.39 (0.24-0.58)</td>
<td>0.29 (0.15-0.48)</td>
<td>0.27 (0.15-0.45)</td>
<td>-401.03</td>
</tr>
<tr>
<td>TRI transformed</td>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 (0.24-0.48)</td>
<td>0.35 (0.24-0.48)</td>
<td>0.35 (0.24-0.48)</td>
<td>-250.99</td>
</tr>
<tr>
<td>TRI transformed</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38 (0.18-0.63)</td>
<td>0.37 (0.18-0.61)</td>
<td>0.29 (0.14-0.52)</td>
<td>-246.30</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28 (0.20-0.39)</td>
<td>0.28 (0.20-0.39)</td>
<td>0.28 (0.20-0.39)</td>
<td>-2690.89</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Unadjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34 (0.18-0.54)</td>
<td>0.10 (0.01-0.47)</td>
<td>0.31 (0.19-0.48)</td>
<td>-2678.13</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 (0.24-0.48)</td>
<td>0.35 (0.24-0.48)</td>
<td>0.35 (0.24-0.48)</td>
<td>-1717.60</td>
</tr>
</tbody>
</table>
### Table 1: Sibling similarity in metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>GLU (mg·dL⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GLU</td>
<td>Unadjusteda</td>
<td>Unadjustedb</td>
<td>Adjusteda</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36 (0.27-0.45)</td>
<td>0.38 (0.23-0.57)</td>
<td>0.24 (0.13-0.41)</td>
</tr>
<tr>
<td></td>
<td>GLU</td>
<td>0.36 (0.27-0.45)</td>
<td>0.39 (0.25-0.55)</td>
<td>0.24 (0.13-0.41)</td>
</tr>
<tr>
<td></td>
<td>GLU</td>
<td>0.36 (0.27-0.45)</td>
<td>0.31 (0.18-0.47)</td>
<td>0.24 (0.13-0.41)</td>
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<tr>
<td>Adjusteda</td>
<td>GLU</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>GLU</td>
<td>0.24 (0.13-0.41)</td>
<td>0.23 (0.07-0.53)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLU</td>
<td>0.33 (0.15-0.58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLU</td>
<td>0.23 (0.07-0.53)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **BB** = brother-brother; **SS** = sister-sister; **BS** = brother-sister
- a = same intraclass correlation values; b = different intraclass correlation values
- **p**<0.001; **p**<0.01; **p**<0.05; ns = non-significant

**Note:** The table shows intraclass correlation coefficients (ICC) for various metabolic syndrome markers, including glucose (GLU) and other parameters, comparing different sibling pairs (brother-brother, sister-sister, brother-sister) and between unadjusted and adjusted models.
Results from the multilevel analyses are provided in Table 3. Older siblings have lower values of Z-MS, WC, and SBP (p<0.05) as well as higher HDL-C (p<0.05). Those with higher BMI had higher values (p<0.05) in almost all MS markers and Z-MS (except for TRI). The same occurs in those ahead in their biological maturation (except in TRI and GLU). Also, lower consumption of fruits and vegetables was associated with higher SBP (p<0.05). Further, those more physically active had higher HDL-C (p<0.05), and muscularly fitter sib-ship pairs had higher Z-MS, SBP and GLU (p<0.05) and lower WC (p<0.05). In addition, siblings with lower cardiorespiratory fitness had higher WC (p<0.05). BB pairs had, on average, higher WC than SS and BS pairs, lower TRI than SS pairs and lower GLU than BS pairs (p<0.05). Additionally, there was no statistically significant differences between siblings and twins in Z-MS and all markers (p>0.05), except for TRI where twin had less TRI than siblings (β=-0.14±0.06, p<0.05). Moreover, results from the multilevel analyses without twins (Supplementary Table 2) showed similar associations between biological and behavioural characteristics with twin data included (Table 3), except for WC which positively associated with fruits and vegetables consumption, and SBP with muscular fitness.
Table 3. Parameter estimates and variance components for each MS marker and Z-MS

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>Z-MS</th>
<th>WC</th>
<th>HDL-C</th>
<th>TRI (log transformed)</th>
<th>SBP</th>
<th>GLU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intercept (BB)</strong></td>
<td>3.88±0.50***</td>
<td>64.38±1.73</td>
<td>51.37±3.75</td>
<td>4.16±0.13***</td>
<td>109.98±3.21</td>
<td>82.60±2.24</td>
</tr>
<tr>
<td>SS</td>
<td>-0.64±0.26*</td>
<td>-8.26±0.92</td>
<td>2.76±1.98</td>
<td>0.14±0.07*</td>
<td>-1.97±1.78</td>
<td>-0.47±1.12</td>
</tr>
<tr>
<td>BS</td>
<td>0.14±0.22</td>
<td>-3.82±0.89</td>
<td>-0.04±1.79</td>
<td>0.01±0.06</td>
<td>0.61±1.66</td>
<td>1.78±0.92</td>
</tr>
<tr>
<td>Twins</td>
<td>-0.06±0.23</td>
<td>-0.43±0.77</td>
<td>-0.79±1.78</td>
<td>-0.14±0.06*</td>
<td>0.21±1.45</td>
<td>1.48±0.99</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.52±0.12***-4.47±0.37***</td>
<td>2.42±0.86***</td>
<td>-0.05±0.03*</td>
<td>-1.32±0.73*</td>
<td>-1.51±0.55**</td>
<td></td>
</tr>
<tr>
<td>Age²</td>
<td>-0.01±0.02</td>
<td>0.10±0.05</td>
<td>-0.15±0.11</td>
<td>0.00±0.00</td>
<td>-0.32±0.10**</td>
<td>-0.08±0.07</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>0.36±0.03***</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td>Maturity Offset (y)</td>
<td>0.53±0.15**</td>
<td>7.55±0.47***</td>
<td>-2.58±1.15</td>
<td>0.04±0.04</td>
<td>2.88±0.98**</td>
<td>-0.13±0.70</td>
</tr>
<tr>
<td>Fruits and vegetables</td>
<td>0.37±0.20</td>
<td>1.29±0.72</td>
<td>1.41±1.49</td>
<td>-0.04±0.05</td>
<td>3.45±1.23**</td>
<td>1.44±0.96</td>
</tr>
<tr>
<td>Sugary drinks</td>
<td>-0.20±0.14</td>
<td>0.88±0.53</td>
<td>-0.37±1.09</td>
<td>-0.03±0.04</td>
<td>-0.70±0.94</td>
<td>-0.72±0.69</td>
</tr>
<tr>
<td>TPA (arbitrary units)</td>
<td>-0.07±0.06</td>
<td>0.08±0.21</td>
<td>1.00±0.43*</td>
<td>-0.01±0.01</td>
<td>0.27±0.36</td>
<td>-0.21±0.26</td>
</tr>
<tr>
<td>Muscular fitness (z-score)</td>
<td>0.24±0.07**</td>
<td>-0.71±0.26**</td>
<td>-0.72±0.52</td>
<td>-0.01±0.02</td>
<td>1.37±0.43**</td>
<td>0.85±0.32**</td>
</tr>
<tr>
<td>Cardiorespiratory fitness (min)</td>
<td>0.04±0.04</td>
<td>0.47±0.15**</td>
<td>-0.03±0.32</td>
<td>0.02±0.01</td>
<td>0.14±0.26</td>
<td>0.29±0.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variance components (σ²)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Between siblings'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>1.16±0.32</td>
<td>22.20±5.78</td>
<td>68.49±22.05</td>
<td>0.05±0.02</td>
<td>63.27±23.73</td>
<td>3.18±4.47</td>
</tr>
<tr>
<td>SS</td>
<td>1.46±0.41</td>
<td>5.13±3.84</td>
<td>78.47±18.12</td>
<td>0.09±0.03</td>
<td>33.98±12.57</td>
<td>24.87±9.73</td>
</tr>
<tr>
<td>BS</td>
<td>0.39±0.37</td>
<td>0</td>
<td>42.62±20.15</td>
<td>0.05±0.02</td>
<td>29.22±11.16</td>
<td>14.59±8.31</td>
</tr>
<tr>
<td>Within siblings'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>0.96±0.19</td>
<td>16.22±3.32</td>
<td>80.83±16.32</td>
<td>0.09±0.02</td>
<td>104.12±20.53</td>
<td>30.47±5.73</td>
</tr>
<tr>
<td>SS</td>
<td>1.48±0.28</td>
<td>18.21±3.92</td>
<td>56.34±10.32</td>
<td>0.15±0.03</td>
<td>48.05±9.82</td>
<td>46.66±8.63</td>
</tr>
<tr>
<td>BS</td>
<td>2.54±0.41</td>
<td>48.21±10.02</td>
<td>133.22±20.70</td>
<td>0.12±0.02</td>
<td>72.81±11.10</td>
<td>50.00±9.31</td>
</tr>
<tr>
<td>Deviance</td>
<td>1720.77</td>
<td>2929.64</td>
<td>3570.30</td>
<td>492.61</td>
<td>3419.05</td>
<td>3134.95</td>
</tr>
</tbody>
</table>

p<0.05; *p<0.01; ***p<0.001; BB – brother-brother; SS – sister-sister; BS – brother-sister; TPA – total physical activity; BMI – body mass index
DISCUSSION

Based on a multilevel statistical model, this study investigated sibling resemblance in MS markers as well as the influence of biological and behavioural characteristics in the expression of these markers. Siblings resemble each other in a wide variety of traits, yet the effect sizes of their similarities vary across studies (Miranda-Lora et al., 2017; Park, Park, & Cho, 2006; Poulsen, Vaag, Kyvik, & Beck-Nielsen, 2001; Santos et al., 2013; Sung et al., 2009; Zarkesh et al., 2014) which can also be partially explained by differences in sample sizes and composition, age range, reference population, analytical strategies and covariate adjustments. Although results for familial resemblance in MS are available in extended or nuclear families and twins, few studies have addressed this issue with pediatric populations (Miranda-Lora et al., 2017; Park et al., 2006; Poulsen et al., 2001; Santos et al., 2013; Sung et al., 2009; Zarkesh et al., 2014). We were able to find only one study that investigated differences in sibling resemblance in MS according to their sex, i.e., brother-brother, sister-sister and brother-sister, but used an adult sample (van Dongen et al., 2013). Thus, we are limited in our ability to compare our results to those of previous studies.

Brother-brother pairs were more alike than SS and BS pairs for Z-MS (overall measure of metabolic risk), suggesting that they were more prone to exhibit either genetic and/or shared environmental similarities. We could only retrieve one study that investigated familial resemblance, including siblings, in the overall measure of MS cluster (Miranda-Lora et al., 2017); however, the authors did not differentiate by type of sibling pair. The reported correlation was 0.28, somewhat lower than in the present study. The genetic component of sibling resemblance can be inferred from genome-wide association data that identified several variants responsible for increasing MS markers, namely lipid metabolism, glucose sensing, insulin signaling, and appetite control (Chang, Cheng, Yu, & Chuang, 2013). We also speculate that the sib-type differences we observed could be related to the effects of sex chromosomes in the expression of anatomical and hormonal differences (Lusis, Attie, & Reue, 2008). However, genetic factors are not solely responsible for the expression of MS, as shared environmental factors are also important (Eckel et al., 2005). Further,
there is also evidence that a number of gene–environment interactions modulate the risk for developing MS (Ordovas & Shen, 2008), such that individuals who have a genotype that predicts an increased risk of MS but exposed to a healthy environment remain healthy because this exposure tends to compensate for their genetic predisposition.

Results for individual MS markers show different results across sib-types. For example, for WC, BB pairs’ correlation was $\rho=0.58$, SS pairs (0.22) and BS pairs was almost zero. Published results show different trends, as van Dongen et al. (2013) reported slightly higher values for SS pairs ($\rho=0.33$) than in BB ($\rho=0.28$) or BS pairs ($\rho=0.23$), and Katzmarzyk et al. (2000) reported that all sib-pair types had the same correlation ($\rho=0.25$). These differences may be associated with variation in sample characteristics (age range and size) and analysis strategy. It is also possible that genetic factors play a role, since Randall et al. (2013) showed sexual differences in genetic loci for anthropometric traits, and seven of these were significantly associated with WC.

In HDL-C, TRI and SBP when adjustments for biological and behavioural covariates were made, same-sex siblings had higher correlation values than opposite-sex siblings. Different results were reported by van Dongen et al. (2013) where lower values were observed (in these MS markers and in all sib-types). However, the Dongen et al. study is based on an adult sample and the analysis was only adjusted for age and sex. Our results suggest that when we removed the effect of biological and behavioural characteristics the same-sex siblings were more similar in their genetic and shared environment predispositions than opposite-sex siblings. Although, the genetic mechanisms are complex on these markers as well as their interaction with other factors, the genetic effects are relatively well-documented by means of heritability estimates and candidate genes, genome-wide linkage, and most recently genome-wide associations studies in HDL (Weissglas-Volkov & Pajukanta, 2010), TRI (Johansen, Kathiresan, & Hegele, 2011) and SBP (Arora & Newton-Cheh, 2010). Moreover, there is evidence that different levels of circulating sex hormones in boys and in girls are paramount factors to modulate levels of HDL-C, TRI and SBP (Agirbasli et al., 2009; Aydin & Winters, 2016) which can also explain the differences found in the sib-type in our sample.
For GLU, after adjustment for biological and behavioural covariates, the resemblance between all sib-types decreased. These results indicated that biological and behavioural characteristics may have a greater influence on sibling resemblance. Moreover, SS pairs were systematically more alike than BB and BS pairs, independent of the adjustment. On the other hand, van Dongen et al. (2013) showed higher resemblance in same-sex siblings than opposite-sex siblings. However, this study based on the adult sample and the analysis was only adjusted for age and sex. Previous studies with family data showed a higher variation in siblings’ correlations, ranging from 0.10 to 0.49. Available genetic studies showed approximately ~83 loci for one or more glycemic traits (Mohlke & Boehnke, 2015). Nonetheless, we speculate that changes in siblings’ resemblance after adjusting for biological and behaviours characteristics may be indicative that there exists a complementary role of the behavioural and environmental factors modulating the genetic susceptibility.

Siblings’ biological characteristics were differentially associated with MS markers. Chronological age is negatively associated with all MS markers (except TRI) as well as with MS score. For example, Sekokotla, Goswami, Sewani-Rusike, Iputo, and Nkeh-Chungag, 2017 investigated adolescents aged 13-18 years and showed that for each increasing year of age the risk for MS decreases by 2.29 times. BMI was found to be positively associated with all individual MS markers (except TRI) and Z-MS which is similar to previous reports (Camhi & Katzmarzyk, 2011; Sardinha et al., 2016). Although maturity offset data are scarce, we found one study that investigated this association and showed that early maturing adolescents are more at risk than late maturing ones (Werneck et al., 2016), possibly because of physiological and psychosocial factors (Alberga, Sigal, Goldfield, Prud'homme, & Kenny, 2012).

Some health behaviours are favorably associated with some MS markers. Specifically, low fruit and vegetable consumption was positively related to SBP. Clinical trials have shown a protective effect of fruit and vegetable consumption on SBP (Couch et al., 2008). Although the mechanisms by which fruits and vegetables influence blood pressure remain undefined (Shi, Krupp, & Remer, 2013), they are important sources of nutrients, namely fiber, potassium, magnesium, and antioxidants which have been suggested to potentially reduce...
blood pressure (Shi et al., 2013). In our study, physical activity was positively associated with HDL-C. Previous studies are in line with our results (LeBlanc & Janssen, 2010; Moschonis et al., 2013) and is a consistent finding showing that PA is positively associated with health (Warburton, Nicol, & Bredin, 2006). For example, Moschonis et al. (2013), showed that more than approximately 45 min of MVPA per day was significantly associated with reduced likelihood of dyslipidemias in children (aged 9-13 years).

Surprisingly, our data indicated that more muscular fit siblings had higher values for Z-MS, SBP and GLU. Notwithstanding, those who presented better performances (in both muscular and cardiorespiratory fitness) had lower WC values. Previous studies showed that physical fitness (a product of both cardiorespiratory and muscular fitness) play important protective roles in the development of metabolic syndrome (Ford & Li, 2006). Yet, most of the available reports are based on adult samples; nevertheless, studies with children and youth showed inconsistent results. For example, Artero et al. (2011) reported that muscular and cardiorespiratory fitness were independently associated with metabolic risk in adolescents. On the other hand, Demmer et al., 2016 showed a positive association between hand grip strength and SBP in boys and girls, after adjusting for BMI. On the other hand, Rioux et al., 2017 after adjusting for age, BMI z-score and cardiorespiratory fitness reported no association between muscular strength and cardiometabolic risk factors in boys, only in girls. Additionally, Zaqout et al., 2016 using longitudinal data showed no associations between hand grip strength or the sum of physical fitness and a cluster of cardiometabolic risk factors. These apparent differences may be linked to population diversity (Portuguese, Americans and other European countries), sample sizes, age range as well as PF tests used.

This study is not without limitations. First, the sample does not include adolescents from all Portuguese regions making it difficult to generalize to the overall population. Yet, this is a common situation with family and twin studies. Second, the use of self-reports to obtain information concerning physical activity and dietary habits are prone to errors even when collected under controlled conditions as is the case of our study. Third, if twin zygosity was known, variance components as well as intraclass correlation estimates might change. In any
case, we provided results with twins (with unknown zygosity) and without, and they suggest an overall similar trend. Third, in our analysis no adjustments were made for putative indicators of shared sibling environments and this limits more adequate interpretations of the influences of these factors.

**CONCLUSIONS**

In conclusion, same-sex siblings are more similar in their MS markers than opposite-sex siblings. Younger sibs, those with greater BMI and biological maturation have higher levels of MS markers. Further, siblings who do not consume fruits and vegetables every day have a higher SBP. Overall, more active siblings have higher good cholesterol (HDL-C), and more muscular fit siblings have higher Z-MS, SBP and GLU. Our results suggest that sibling resemblance in MS markers is evident, it is possible to minimize this adverse condition through lifestyle modifications, namely through education-based programs at the school and family levels aiming for healthy diets and improvements in their physical activity levels, namely to moderate-to-vigorous ones.

Although previous reports focused their attention on familial resemblance in MS markers, very few used youth samples and no study investigated the issue in youth siblings splitting by differences in pair-sex composition (i.e., brother-brother, brother-sister and sister-sister). This is relevant information when developing and implementing interventions in youth, school-based as well as family-based. Given the complexity of factors regulating the expression of MS markers, it is also important to consider individual characteristics when designing individual programs to anticipate success. Furthermore, familial healthy lifestyles linked to favorable contexts play important roles in MS prevention. We also anticipate that future research could adopt a study design simultaneously integrating multilevel model analyses coupling top-down and bottom-up approaches to clear identify the set of key-variables that best predict MS development at the family level.
REFERENCES


Sibling similarity in metabolic syndrome


Sibling similarity in metabolic syndrome


Sibling similarity in metabolic syndrome


Profile Resemblance in Health-Related Markers: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

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ABSTRACT

The co-occurrence of health-related markers and their associations with individual, family, and environmental characteristics have not yet been widely explored in siblings. We aimed to identify multivariate profiles of health-related markers, analyze their associations with biological, sociodemographic, and built environment characteristics, and estimate sibling resemblance in these profiles. The sample includes 736 biological siblings aged 9–20 years. Body fat was measured with a portable bioelectrical impedance scale; biological maturation was assessed with the maturity offset; handgrip strength, standing long jump, one-mile run, and shuttle run were used to mark physical fitness. Health behaviours, sociodemographic, and built environmental characteristics were recorded by questionnaire. Latent profile analysis and multilevel logistic regression models were used; sibling resemblance was estimated with the intraclass correlation ($\rho$). Two multivariate profiles emerged: “P1 = fit, lower fat and poorer diet” (86.7%) and “P2 = higher fat and lower fit, but better diet” (13.3%). Siblings whose fathers were less qualified in their occupation were more likely to belong to P2 (OR = 1.24, $p = 0.04$); those whose fathers with Grade 12 and university level education were more likely to fit in P2 compared to peers living with fathers having an educational level below Grade 12 (OR = 3.18, $p = 0.03$, and OR = 6.40, $p = 0.02$, Grade 12 and university level, respectively). A moderate sibling profile resemblance was found ($0.46 \leq \rho \leq 0.55$). In conclusion, youth health-related markers present substantial differences linked with their body composition, physical fitness and unhealthy diet. Furthermore, only father socio-demographic characteristics were associated with profile membership. Sibling’s profile resemblance mirrors the effects of genetics and shared characteristics.
INTRODUCTION

Behaviours like physical inactivity, sedentariness, and unhealthy dietary intake are risk factors associated with increases in the worldwide incidence of non-communicable diseases (World Health Organization, 2011). On the contrary, the adoption of health-related behaviours like regular involvement in moderate-to-vigorous physical activities, consuming healthy diets, combined with adequate physical fitness (Smith et al., 2014), and healthy body fat levels (May, Kuklina, & Yoon, 2012) are likely to reduce the risk of premature death by non-communicable diseases. These markers, both healthy and unhealthy, are acquired and developed during childhood within the family orbit, become more pronounced during adolescence, and tend to persist into adulthood (Wiium, Breivik, & Wold, 2015).

Area of Residence

There is growing evidence suggesting that geographic area of residence is an important correlate of healthy youth development, since it may provide opportunities, as well as conditions that impact health (Villanueva et al., 2013). Further, a domain of current interest is the built environment and how access to public transportation, shops/markets, traffic, safety, and available areas for recreation or physical activity practice can contribute to individual health (Mitas, Sas-Nowosielski, Groffik, & Fromel, 2018; Patnode et al., 2010; Sallis et al., 2018). For example, Mitas et al. (2018) showed that adolescents who perceived their neighborhood environment as the safest were significantly more likely to meet the recommendations for leisure-time walking. Furthermore, safe neighborhoods encourage and facilitate youth involvement in unsupervised sports’ participation (Mitas et al., 2018). Sallis et al. (2018) reported that walkability was positively and significantly related to objectively-measured physical activity and negatively related to sedentary time and TV time in adolescents aged 12–16 years.

Familial Environment

The family environment also plays a role in the acquisition and development of lifestyle behaviours in youth, with significant impacts on their health (Jones, 2018; Manios et al., 2015). Information gathered on nuclear
families, twins, or siblings is a useful way to better understand the magnitude of environmental and genetic factors on lifestyle behaviours and other health-related markers. For example, Nelson, Gordon-Larsen, North, and Adair (2006) used sibling data to show that household environments accounted for 8%–10% of the total variation in adolescent fast food intake and sedentary behaviours and 50% of the total variation in adolescents being overweight. Additionally, a systematic review by Fisher, Smith, van Jaarsveld, Sawyer, and Wardle (2015) of twin studies reported that the shared environment had a strong effect size on physical activity levels (60%), with a smaller contribution from genetic factors (21%).

**Clustering**

Much of the available research has not considered predictors from both the neighborhood and the family environment, or even in association with correlates from other domains, of the expression of behaviours and traits in youth. Latent profile/class analysis is a very useful way to tackle this issue, given that it allows for the classification of individuals into distinct profiles/classes based on the co-occurrence of traits and behaviours (Williams & Kibowski, 2016). For example, based on physical activity and sedentary variables, Patnode et al. (2011) identified three distinct classes for boys and girls with significant differences between classes for a number of demographic indicators. Furthermore, by adding dietary intake information, Iannotti and Wang (2013) identified three distinct classes whose membership was related to age, sex, race/ethnicity, and socioeconomic status. Similarly, Pereira et al. (2015) added data on sleep time and were only able to identify two distinct classes significantly related to individual characteristics (sex and maturity offset), but not to sociodemographic factors (maternal education and household income).

**Profile and Sibling Approach**

We were not able to identify studies that simultaneously focused on lifestyle behaviours and other traits related to health (e.g., physical fitness and body composition) and that also explored the roles of biological, sociodemographic characteristics, and built environment in the prediction of profiles/class membership. Further, given population heterogeneity regarding health outcomes, which are governed by a plethora of factors (biological,
In health-related markers, we believe that using a person-centered approach will provide strong clues to identify putative profiles for different people, which, in turn, may provide novel insights and a holistic view regarding health-related markers and ways to develop and promote them. Siblings share a substantial part of their life, but may also exhibit different developmental trajectories and healthy/unhealthy lifestyle profiles. Thus, using siblings as a template to explore the co-occurrence of lifestyle behaviours and other traits related to health may provide important clues about the effects of common and unique characteristics that are associated with youth health. Consequently, relevant information may be provided to families, educators, and health professionals in terms of prevention and planning interventions. Hence, the present study aims: (1) to identify multivariable profiles of health-related markers, namely lifestyle behaviours (physical activity, healthy, and unhealthy food habits), physical fitness, and body composition; (2) to investigate the associations among biological, sociodemographic, and built environment characteristics and the multivariable profiles; (3) to estimate sibling similarity in these multivariable profiles. Based on these, the following questions were addressed: (1) Can we identify multivariate profiles of health-related markers in youth? If so, how many will emerge? (2) How are biological, sociodemographic and built environment characteristics associated with profile membership, i.e., how strong are their effect sizes? (3) How sizeable is sibling resemblance in their multivariate profiles?

**METHODS**

**Study Participants**

The current study sample was from the Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health, which investigates physical growth, body composition, physical fitness, physical activity, metabolic syndrome, and health behaviours in a cohort of siblings (Pereira et al., 2017). The subjects in this study were part of a larger study, the Portuguese Healthy Family Study (Pereira et al., 2017), in which participants were randomly sampled from schools in mainland (north and central) Portugal and from the Azores islands. Those who had their siblings studying in the same school were invited to take part in the study (~4100), and the response rate was ~80%. Written informed consent was obtained from
legal guardians, and the project was approved by the Ethics Committee of the University of Porto and school authorities (CEFADE 09.2015). The final sample consisted of 3285 siblings 9–20 years of age. However, for the present study, the sample comprised 736 biological siblings (350 females and 386 males) from 370 nuclear families with complete data on lifestyle behaviours, physical fitness, and body composition. No statistically-significant mean differences were observed between included and excluded siblings in height, weight, and % body fat.

**Health-Related Markers**

**Body Composition**

Percentage body fat (%Fat) was estimated with youth in light clothing and using a reliable and valid instrument (Kabiri, Hernandez, & Mitchell, 2015), a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body Composition Analyzer Tanita Corporation, Tokyo, Japan).

**Physical Fitness**

Physical fitness was assessed with four tests: (1) handgrip strength, using a hand dynamometer (Takei Digital Grip Strength Dynamometer, Model T.K.K.5401, Tokyo, Japan) with participants gripping the dynamometer with maximum force for 5–10 s; (2) standing long jump, where participants jumped as far as possible from a standing position; (3) 1-mile run/walk test, where participants ran/walked the distance in the shortest time possible; (4) shuttle-run (SHR), with participants running as fast as possible to another line (9 m away) where two small blocks were placed, picking up a block, returning to place it behind the starting line, and then repeating the route for the second block. A standardized z-score for each physical fitness test result was computed, which were summed to obtain a physical fitness z-score for each subject. The signs in the 1-mile run/walk and shuttle-run were reverted. These tests have been shown to be reliable and valid (Safrit, 1990).

**Dietary Intake**

Diet intake data were obtained from a food frequency questionnaire (FFQ) adapted and modified from the Health Behaviour in School-aged Children Survey (HBSC) (Currie et al., 2008) using typical Portuguese food items. This questionnaire was previously used in multi-country studies (Katzmarzyk et al.,
Youth were asked about various types of food consumed in a typical week. For each item, the reported answers were converted into weekly portions as follows: “never” = 0; “less than once per week” = 0.5; “once per week” = 1; “2–4 days per week” = 3; “5–6 days per week” = 5.5; “once a day, every day” = 7; and “more than once a day” = 10, as previously advocated (Mikkilä et al., 2015). Food items related to healthy diet were as follows: fruits, vegetables, dark-green vegetables, orange vegetables, fruit juice, skim milk, low-fat milk, whole milk, cheese, other milk products, bread or whole grains, beans, lentils, bean curd, eggs, fish. Food items related to unhealthy diet were as follows: sweets, sugar-sweetened sodas, cakes, pastries, donuts, diet sodas, ice cream, potato chips, French fries, fast foods, sports drinks, energy drinks, fried food. Portions of each food item were summed, and two scores were derived for healthy and unhealthy diets.

**Physical Activity**

Total physical activity (TPA) was obtained with the Baecke questionnaire (Baecke, Burema, & Frijters, 1982), a reliable and valid instrument (Philippaerts, Westerterp, & Lefevre, 1999). This questionnaire includes three specific domains based on a total of 16 questions: work/school PA, leisure-time PA, and sports participation. TPA was estimated based on the sum of these three specific domains. For each domain, each score ranged from 1 (minimal) to 5 (maximal), such that the TPA score varied between 3 and 15. Participants answered the questionnaire during regular physical education classes under the supervision of their school-teacher, as well as by a trained research team member.

**Screen Time**

Information about screen time was obtained using the U.S. Youth Risk Behaviour Surveillance Survey (U.S. Centers for Disease Control and Prevention) questionnaire by self-administered questions: “How long do you watch TV per day?” and “How long do you use your computer or play non-active video games per day?” Answers ranged from <30 min, 30 min–1 h, 1 h–1 h 30, 1 h 30–2 h to >2 h, subsequently categorized from 0–4 (−/+). Individual scores were summed and obtained a total score for screen time, as reported in different studies (Arango et al., 2014; Rey-Lopez et al., 2012; Rey-Lopez et al., 2011).
Biological Maturation

Biological maturation was assessed with the maturity offset (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002), which estimates the temporal distance (in decimal years) from age-at-peak height velocity (PHV). A positive (+) maturity offset indicates the number of years the participant is beyond PHV, whereas a negative (−) maturity offset represents the number of years the participant is before PHV. This method has been widely used in children and youth (Costa, Fragoso, & Teles, 2017; Morrissey, Janz, Letuchy, Francis, & Levy, 2015; Pereira et al., 2015).

Sociodemographic Characteristics

Sociodemographic characteristics were based on the occupation and education of both parents (mothers and fathers). Parents’ occupation was categorized into ten groups (from 0–9) according to the Portuguese National Classification of Occupations (2010), where Group 0 is the highest socioeconomic status (SES) and Group 9 is the lowest. Categories are as follows: (0) armed forces; (1) central administration/politicians and executive directors; (2) specialists of intellectual and scientific activities; (3) technicians and intermediate-level jobs; (4) back-office jobs; (5) security, seller, and individual services; (6) farmer and qualified workers of farm, fish, and forest; (7) qualified industry and building jobs: (8) machine and equipment operators; and (9) nonqualified jobs. Parents’ education was obtained according to the following categories: (1) <Grade 12; (2) Grade 12/diploma for technical qualification (equivalent to high school); (3) university level.

Built Environment

Built environment information was obtained via questionnaire. We applied the Portuguese version of the Environmental module (environmental perception of the residential area) of the International Physical Activity Study, a reliable and valid instrument (Alexander, Bergman, Hagströmer, & Sjöström, 2006; Craig et al., 2003), previously used in the Portuguese population (Delgado, 2005). This questionnaire includes questions about the traffic system, accessibility to public transportation and shops/markets, housing density, perceived safety of the neighborhood, the presence of sidewalks and bike paths, and recreational facilities. The response item options were as follows: completely disagree,
partially disagree, partially agree, or completely agree. For the purposes of this study, options were dichotomized into two categories: 0 = disagree (completely disagree, partially disagree); 1 = agree (partially agree or completely agree).

**Statistical Analysis**

Descriptive statistics were computed as means (±standard deviations) and frequencies. Then, as advocated (Cabanas-Sanchez et al., 2018; Hart et al., 2016), we removed the effects of age and sex from the six health-related markers (percent body fat, global physical fitness, physical activity, healthy and unhealthy food habits) using a multiple regression analysis. Residuals from these regressions were standardized using a z-score transformation. These analyses were done in SPSS 23. Then, these standardized residuals were exported to Mplus software 7.4 in order to identify the best fitting number of latent profiles using iterative maximum likelihood estimation techniques (Muthén & Muthén, 1998-2015). A series of models was fitted to the data with a sequence of putative profiles, and as advocated, we relied on the Akaike information criteria (AIC), the Bayesian information criteria (BIC), as well as the Vuong–Lo–Mendell–Rubin likelihood ratio test to compare models and determine the number of latent profiles (Wang & Wang, 2012). Further, we also relied on recommendations from Geiser (2013) to set the algorithm iterations to avoid local maxima.

Using the best solution on profiles and given the nested structure of the data (subjects nested within sibships), we used a multilevel logistic model to predict profile membership (P1 is the reference) and sequentially-tested three models of increasing complexity. Model 1 (M1) only included biological maturation; in Model 2 (M2), we added father and mother education, as well as their occupation; finally, Model 3 (M3) included built environment covariates. Parameter estimates were obtained via maximum likelihood as implemented in Stata 13 software. Model comparison was done with the deviance statistic as is customary (Snijders & Bosker, 2012). Differences in deviances follow a Chi-squared distribution with degrees of freedom equal to differences in the number of parameter estimates. Finally, intraclass correlations (ρ) and the corresponding 95% confidence intervals (95% CI) as suitable measures of sibling similarity were computed from the variance components (Snijders & Bosker, 2012).
RESULTS

Descriptive statistics for biological characteristics, body composition, physical fitness, lifestyle behaviours, sociodemographic characteristics, and built environment are presented in Table 1. On average, sibling pairs had similar chronological ages and biological maturation. However, sister-sister (SS) pairs had more body fat than brother-brother (BB) and brother-sister (BS) pairs. Further, BB pairs were more fit and active, but had higher screen time and higher unhealthy diet scores than SS and BS pairs. Healthy diet scores were similar across all types of siblings. A slight difference was observed for mothers’ and fathers’ occupation, favoring BS pairs. For education level, the highest percentages of responses for BB and BS pairs were observed in “Grade 12/diploma/technical qualification” for both the mother and father, while for SS pairs, the highest percentages category was “<Grade 12”, also in mother and father. Regarding the built environment, the perception about all items was similar in all sib-types.
Table 1. Sample descriptive characteristics (means, standard deviations (SD), and frequencies (%)) for sibling pairs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Brother-Brother (n = 200) Mean ± SD</th>
<th>Sister-Sister (n = 167) Mean ± SD</th>
<th>Brother-Sister (n = 369) Mean ± SD</th>
<th>All Sibs Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronological age (years)</td>
<td>13.1 ± 1.8</td>
<td>12.6 ± 1.6</td>
<td>12.9 ± 1.7</td>
<td>12.9 ± 1.7</td>
</tr>
<tr>
<td>Biological Maturation (years)</td>
<td>−0.14 ± 1.9</td>
<td>−0.33 ± 1.3</td>
<td>−0.24 ± 1.6</td>
<td>−0.23 ± 1.6</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20.0 ± 6.4</td>
<td>25.8 ± 5.6</td>
<td>23.2 ± 6.6</td>
<td>22.9 ± 6.7</td>
</tr>
<tr>
<td><strong>Physical fitness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handgrip strength (kgf)</td>
<td>27.0 ± 9.2</td>
<td>22.0 ± 5.5</td>
<td>23.9 ± 7.3</td>
<td>24.3 ± 7.7</td>
</tr>
<tr>
<td>Standing long jump (cm)</td>
<td>162.4 ± 31.6</td>
<td>133.7 ± 28.3</td>
<td>151.5 ± 31.1</td>
<td>150.4 ± 32.3</td>
</tr>
<tr>
<td>1-mile run/walk (min)</td>
<td>8.6 ± 2.0</td>
<td>10.2 ± 2.2</td>
<td>9.2 ± 2.2</td>
<td>9.3 ± 2.2</td>
</tr>
<tr>
<td>Shuttle-run (s)</td>
<td>11.2 ± 1.8</td>
<td>12.2 ± 2.1</td>
<td>11.4 ± 1.9</td>
<td>11.5 ± 1.9</td>
</tr>
<tr>
<td><strong>Lifestyle behaviours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total physical activity</td>
<td>8.4 ± 1.5</td>
<td>7.7 ± 1.3</td>
<td>8.1 ± 1.5</td>
<td>8.1 ± 1.5</td>
</tr>
<tr>
<td>Screen time</td>
<td>3.6 ± 2.5</td>
<td>2.9 ± 2.1</td>
<td>3.4 ± 2.2</td>
<td>3.3 ± 2.3</td>
</tr>
<tr>
<td>Unhealthy diet</td>
<td>31.5 ± 16.0</td>
<td>23.9 ± 11.5</td>
<td>28.6 ± 14.6</td>
<td>28.3 ± 14.6</td>
</tr>
<tr>
<td>Healthy diet</td>
<td>57.6 ± 16.3</td>
<td>57.0 ± 15.0</td>
<td>57.8 ± 14.4</td>
<td>57.6 ± 15.1</td>
</tr>
<tr>
<td><strong>Sociodemographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>5.9 ± 2.4</td>
<td>6.1 ± 2.7</td>
<td>5.2 ± 2.7</td>
<td>5.6 ± 2.5</td>
</tr>
<tr>
<td>Father</td>
<td>5.9 ± 2.4</td>
<td>6.1 ± 2.4</td>
<td>5.4 ± 2.4</td>
<td>5.7 ± 2.6</td>
</tr>
<tr>
<td>Education</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Grade 12</td>
<td>28.8</td>
<td>48.0</td>
<td>31.7</td>
<td>34.7</td>
</tr>
<tr>
<td>Grade 12/diploma/technical</td>
<td>42.9</td>
<td>24.0</td>
<td>36.0</td>
<td>39.1</td>
</tr>
<tr>
<td>qualification</td>
<td>28.2</td>
<td>28.0</td>
<td>32.3</td>
<td>23.7</td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Grade 12</td>
<td>32.0</td>
<td>48.6</td>
<td>34.8</td>
<td>37.1</td>
</tr>
<tr>
<td>Grade 12/diploma/technical</td>
<td>36.0</td>
<td>40.3</td>
<td>40.3</td>
<td>39.1</td>
</tr>
<tr>
<td>qualification</td>
<td>32.0</td>
<td>11.1</td>
<td>24.9</td>
<td>23.7</td>
</tr>
<tr>
<td><strong>Built Environment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to shops/markets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>21.5</td>
<td>19.7</td>
<td>23.5</td>
<td>22.2</td>
</tr>
<tr>
<td>Agree</td>
<td>78.5</td>
<td>80.3</td>
<td>76.5</td>
<td>77.8</td>
</tr>
<tr>
<td>Access to public transportation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>14.1</td>
<td>17.1</td>
<td>20.2</td>
<td>18.0</td>
</tr>
<tr>
<td>Agree</td>
<td>85.9</td>
<td>82.9</td>
<td>79.8</td>
<td>82.0</td>
</tr>
<tr>
<td>Traffic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>54.8</td>
<td>70.4</td>
<td>62.8</td>
<td>62.4</td>
</tr>
<tr>
<td>Agree</td>
<td>45.2</td>
<td>29.6</td>
<td>37.2</td>
<td>37.6</td>
</tr>
<tr>
<td>Safety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>59.3</td>
<td>65.8</td>
<td>58.3</td>
<td>60.4</td>
</tr>
<tr>
<td>Agree</td>
<td>40.7</td>
<td>34.2</td>
<td>41.7</td>
<td>39.6</td>
</tr>
<tr>
<td>Presence of sidewalk and bike</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>49.7</td>
<td>45.4</td>
<td>50.0</td>
<td>48.8</td>
</tr>
<tr>
<td>Agree</td>
<td>50.3</td>
<td>54.6</td>
<td>50.0</td>
<td>51.2</td>
</tr>
<tr>
<td>Recreational facilities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>54.2</td>
<td>63.2</td>
<td>52.7</td>
<td>55.4</td>
</tr>
<tr>
<td>Agree</td>
<td>45.8</td>
<td>36.8</td>
<td>47.3</td>
<td>44.6</td>
</tr>
</tbody>
</table>

kgf: kilogram-force

Figure 1 illustrates the best profile solution merging lifestyle behaviours (physical activity, screen time, and healthy/unhealthy food) and biological markers (physical fitness and body fat). Based on the statistical tests previously
Profile resemblance in health-related markers

mentioned (AIC, BIC, and the Vuong–Lo–Mendel–Rubin likelihood ratio test) (Table 2), a two-latent profile model best fitted the data, and the counts and percentages of individuals in each profile were: Profile 1, \( n = 638 \) (86.7%), and Profile 2, \( n = 98 \) (13.3%). We labeled Profile 1 (our reference profile) as “fit, lower fat, and poorer diet” and Profile 2 as “higher fat and lower fit, but better diet”.

![Figure 4. Profiles’ display for biological health markers, as well as lifestyle behaviours](image)

Table 2. Criteria used to identify the best number of latent profiles

<table>
<thead>
<tr>
<th>Fit Measures</th>
<th>Number of Profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No. of parameters</td>
<td>12</td>
</tr>
<tr>
<td>AIC</td>
<td>11,907.918</td>
</tr>
<tr>
<td>BIC</td>
<td>119,630.198</td>
</tr>
<tr>
<td>VLMR LRT</td>
<td>-</td>
</tr>
<tr>
<td>( p )-value</td>
<td>-</td>
</tr>
<tr>
<td>Adjusted LRT test</td>
<td>-</td>
</tr>
<tr>
<td>( p )-value</td>
<td>-</td>
</tr>
</tbody>
</table>

AIC: Akaike information criteria; BIC: Bayesian information criteria; VLMR: Vuong–Lo–Mendell–Rubin likelihood ratio test; LRT: Likelihood-ratio test

Table 3 displays results from the multilevel logistic regression models. In \( M_1 \), results showed that more mature siblings were less likely to belong to Profile 1 (OR=1.18±0.10, \( p<0.05 \)). In \( M_2 \), with family-level covariates, a better fit was obtained relative to \( M_1 \) (\( \chi^2=84.26 \) with 6 df, \( p<0.001 \)). In this model, biological maturation ceased to be a significant predictor of profile membership (\( p > 0.05 \)).
Siblings whose fathers were less qualified in their occupation were more likely to belong to Profile 2 (OR=1.24±0.12, p< 0.05). Additionally, those whose fathers had Grade 12 and university-level education were also more likely to fit in Profile 2 compared with peers living with fathers having below Grade 12 education (OR=3.18±1.72 and OR=6.40±5.09, p<0.05, Grade 12 and university level, respectively). Built environmental covariates were added in Model 3, but this model did not improve the model fit over M2 (χ²=4.28 with 6 df, p=0.135). This indicates that built environmental covariates did not reach a statistically-significant level to explain siblings’ profile membership.

Sibling resemblance in profile membership was estimated with intraclass correlations, and values were moderate (0.46≤ρ≤ 0.55). Further, with increases in model complexity, i.e., from M1 to M3, resemblance tended to increase somewhat.
Table 3. Multilevel logistic regression in siblings profile membership

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model 1 (M₁)</th>
<th></th>
<th>Model 2 (M₂)</th>
<th></th>
<th>Model 3 (M₃)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio (SE)</td>
<td>95% CI</td>
<td>Odds Ratio (SE)</td>
<td>95% CI</td>
<td>Odds Ratio (SE)</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Fixed effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (P2) §</td>
<td>0.07 (0.02) ***</td>
<td>0.04–0.13</td>
<td>0.01(0.01) ***</td>
<td>0.00-0.09</td>
<td>0.01 (0.01) ***</td>
<td>0.00-0.09</td>
</tr>
<tr>
<td>Maturity offset</td>
<td>1.18 (0.10) *</td>
<td>1.00–1.40</td>
<td>1.21 (0.13) ns</td>
<td>0.99-1.48</td>
<td>1.22 (0.13) ns</td>
<td>0.98–1.50</td>
</tr>
<tr>
<td>Father occupation</td>
<td>1.24 (0.12) *</td>
<td>1.02-1.51</td>
<td>1.24 (0.13) *</td>
<td>1.01–1.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother occupation</td>
<td>0.93 (0.08)  ns</td>
<td>0.79-1.11</td>
<td>0.93 (0.09)  ns</td>
<td>0.78–1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father education (Grade 12)</td>
<td>3.18 (1.72) *</td>
<td>1.10–9.19</td>
<td>3.38 (1.94) *</td>
<td>1.10–10.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father education (university level)</td>
<td>6.40 (5.09) *</td>
<td>1.35-30.38</td>
<td>7.25 (6.13) *</td>
<td>1.38–38.02</td>
<td></td>
<td></td>
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<tr>
<td>Mother education (Grade 12)</td>
<td>0.91 (0.48) ns</td>
<td>0.33–2.55</td>
<td>0.92 (0.51) ns</td>
<td>0.31–2.72</td>
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<tr>
<td>Mother education (university level)</td>
<td>0.79 (0.58) ns</td>
<td>0.18–3.34</td>
<td>0.70 (0.55) ns</td>
<td>0.15–3.29</td>
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<tr>
<td>Shops/markers (agree) ¥</td>
<td></td>
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<tr>
<td>Public transportation (agree) ¥</td>
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<td>Traffic (agree) ¥</td>
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<tr>
<td>Sidewalks/bike paths (agree) ¥</td>
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<td>Safety (agree) ¥</td>
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<tr>
<td>Recreational facilities (agree) ¥</td>
<td></td>
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<tr>
<td><strong>Random effects (variance components)</strong></td>
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<td></td>
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<tr>
<td>Between siblings’</td>
<td>2.84 (1.17)</td>
<td></td>
<td>3.58 (1.51)</td>
<td></td>
<td>4.08 (1.76)</td>
<td></td>
</tr>
<tr>
<td>Intraclass correlation</td>
<td>0.46 (0.28–0.66)</td>
<td></td>
<td>0.52 (0.32–0.71)</td>
<td></td>
<td>0.55 (0.35–0.74)</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant; § P1 is the reference; ∞ <grade 12 is the reference; ¥ not agree is the reference
DISCUSSION

To the best of our knowledge, this is the first study that aimed to identify children and adolescents’ multivariate profiles by focusing simultaneously on a set of health-related markers including lifestyle behaviours, physical fitness, and body composition using latent profile analysis. Furthermore, we have also investigated the putative links between these profiles with biological, sociodemographic, and built environmental characteristics along with the estimation of sibling resemblance in such profiles. Two distinct multivariate profiles emerged from our analysis. The differences between these two profiles were related to body composition, physical fitness, and unhealthy diet, which, in turn, can be interrelated with the development of non-communicable diseases during children’s and adolescents’ period of physical growth and development (World Health Organization, 2011). Additionally, these health-related markers apparently did not manifest in the same way and intensity in all subjects. For example, although 8 subjects (data not shown) from our sample shared the same physical activity score (0.26), they also differed substantially in other health-related markers, namely: unhealthy diet (-1.20 to 1.00), healthy diet (-1.00 to 2.02), physical fitness (-0.73 to 0.80), and body composition (-1.14 to 2.23).

Previous research within the person-centered paradigm reported that different sets of health-related markers tended to cluster differently in subgroups of subjects who shared identical characteristics within the same group (Cabanas-Sanchez et al., 2018; Fernandez-Alvira et al., 2013; Tabacchi, Faigenbaum, Jemni, & Thomas, 2018). However, these reports were based on cluster or latent class analyses and were most often divergent. For example, Hartz et al. (2018) using physical activity, sedentary time, and diet identified three distinct clusters for boys and girls aged 12–19 years, whereas Cabanas-Sanchez et al. (2018) found five clusters based on moderate-to-vigorous physical activity, screen time, non-screen sedentary time, diet, and sleep. Further, Patnode et al. (2011) studied three classes emerging from 12 behaviours related to physical activity, sports participation, and sedentary activities. Notwithstanding the relevance of previous studies, they apparently did not consider viewing youth from a broader perspective because their study relied mostly on the lifestyle behaviours,
neglecting thus the significance of other important factors like physical fitness and body composition.

Our data showed that profiles were mostly conditioned on the relationship between body fat, physical fitness, and unhealthy diet. Unexpectedly, however, youth with lower body fat and higher physical fitness also tended to show higher levels of unhealthy diet habits. This probably signposts that physical fitness exerts a protective effect on body fat increases while attenuating the effects of unhealthy diet. This was partially corroborated by the data of Garcia-Pastor, Salinero, Sanz-Frias, Pertusa, and Del Coso (2016) showing that adolescents with higher body fat content also had lower levels of cardiorespiratory and muscular endurance fitness than their leaner counterparts, whereas the links between physical activity or diet with body fat was less evident. Furthermore, Artero et al. (2014) reported that adolescents from nine European countries with better muscular fitness also had lower chronic inflammation most probably due to lower levels of fatness. However, overweight and obese adolescents displayed less adverse profiles if they maintained appropriate levels of muscular fitness. In sum, both physical fitness and body composition seem to be more important key markers distinguishing healthy from unhealthy profiles than physical activity, healthy diet, and lower levels of sedentariness.

We also showed that, from sociodemographic characteristics, only father’s occupation and education were predictors of profile membership. Further, none of the potential built environment correlates, as well as biological maturation significantly predicted profile membership. Although there was evidence indicating that different facets of the built environment influenced physical activity, diet, body composition, and physical fitness (Collins, Al-Nakeeb, Nevill, & Lyons, 2012; Masoumi, 2017; Sallis & Glanz, 2006; Vanhelst et al., 2013), we were not able to identify a study that investigated these associations using multivariate health profiles. Furthermore, previous studies rarely explored, in the same article (and data analysis strategy), how likely different facets of the built environment are to influence health-related markers. As such, comparisons with our study are problematic. However, we were able to identify a study (McDonald et al., 2012) that used latent class analysis to identify classes of neighborhoods based on built environment characteristics and then tested how adolescent physical activity, sedentary behaviour, and screen time differed by neighborhood type/class.
(McDonald et al., 2012). The results were similar to those of our study, i.e., no differences in adolescent physical activity, sedentary behaviour, and screen time by neighborhood class were observed. This calls for more research on multivariate health profile analysis to better understand the impact of the built environment on youngsters’ health (Ding & Gebel, 2012).

Sociodemographic characteristics were previously associated with the co-occurrence of health behaviours (Fernandez-Alvira et al., 2013; Liu, Kim, Colabianchi, Ortaglia, & Pate, 2010; Ottevaere et al., 2011). For example, Fernandez-Alvira et al. (2013) using children’s data from seven European countries found associations between parental education and cluster membership, i.e., children of lower educated parents were more likely to be allocated in the low activity/sedentary cluster than in the active cluster. Ottevaere et al. (2011) also used cluster analysis and reported that adolescents from low educated parents had diets of lower quality and spent more time in sedentary activities. On the other hand, Liu et al. (2010) examined adolescents’ co-varying patterns of physical activity and sedentary behaviour and highlighted that adolescents pertaining to a class with moderate physical activity and high sedentary behaviour were more likely to belong to low income families than their peers from other classes. In our study, we used a set of sociodemographic indicators, and our results are not without some controversy. Indeed, father’s occupation was positively associated with a better profile, but father’s education was negatively linked. This may suggest that treating both educational and occupational categories as indicators of the same dimension or fundamental cause might ignore their sometimes sizeable independent and distinct contributions to health. Furthermore, different socioeconomic indicators have different effects across life and obviously can influence health in different ways. For example, education level is typically addressed until early adulthood (approximately ages 18–25) with no further changes. Yet, final occupation level tends to be acquired later and is more prone to change than education level (Galobardes, Shaw, Lawlor, Lynch, & Davey Smith, 2006).

We identified a moderate sibling resemblance in their multivariate profile. In addition, with the inclusion of covariates, the intraclass correlations increased, suggesting a potential effect of the genetic and shared characteristics on these health-related markers. Regardless, we were only able to retrieve one study
based on the person-centered approach that examined the co-occurrence of health-related markers in families (Niermann, Spengler, & Gubbels, 2018). Indeed, Niermann et al. (2018) used cluster analysis to identify patterns of health behaviours within families based on triads (father, mother, and child) and reported lower intraclass correlation values than in our sample. Furthermore, previous studies investigating sibling resemblance for each individual health-related marker, namely physical fitness (Malina & Mueller, 1981; Pawlak, 1984; Pereira et al., 2017), physical activity (Jacobi et al., 2011; Pereira et al., 2018; Seabra, Mendonca, Goring, Thomis, & Maia, 2008), body composition (Feng, Zang, Xu, & Xu, 2008; Katzmarzyk et al., 2000), and diet (Bogl et al., 2017), showed low-to-moderate sibling similarities. However, the effect sizes of such sibships’ likeness varied across studies, which can also be partially explained by differences in sample sizes and composition, age range, reference population, analytical strategies, and covariate adjustments. In spite of this, several genome-wide association (GWA) studies using health-related markers (de Vilhena e Santos, Katzmarzyk, Seabra, & Maia, 2012; Lightfoot, 2011; Lu & Day, 2016; Willems & Wright, 2017) reported their relationships with candidate genes. For example, Lightfoot (2011) and de Vilhena e Santos et al. (2012) performed systematic reviews and highlighted different genetic determinants involved in the regulation of physical activity, namely: dopamine receptor 1 (Drd1) and helix loop helix 2 (Nhlh2), as well as Ace, Gln223ARrg, MC4R, and DRD2, respectively. Further, a GWA approach also allowed Lu and Day (2016) to identify 12 loci related to % body fat, whilst Willems and Wright (2017) identified 16 loci associated with grip strength. Moreover, Schnurr et al. (2016) found a common genetic etiology between whole % body fat and cardiorespiratory fitness. However, we could not find any genetic study related to the co-occurrence of all six health markers that could eventually lead us into a deeper understanding of sibling resemblance in their profile membership.

Notwithstanding the relevance of these results, our study has limitations: (1) participants were not recruited from all Portuguese regions, which limits the generalization of the results to the whole Portuguese population; yet, this situation is common in family and twin studies; (2) the use of questionnaires to obtain information about physical activity, screen time, healthy, and unhealthy diet is prone to errors, even though the questionnaires have been applied in controlled
conditions. Further, these questionnaires are frequently used and have been shown to be reliable in previous studies (Ambrosini, de Klerk, O'Sullivan, Beilin, & Oddy, 2009; Katzmarzyk et al., 2013; Santos et al., 2014; Schmitz et al., 2004).

CONCLUSIONS

In conclusion, two distinct multivariate profiles emerged from our analysis, and the differences between such profiles are related to body composition, physical fitness, and unhealthy diet. Moreover, none of the potential built environment correlates and biological maturation were associated with profile membership, but father’s occupation and education were. Finally, a moderate resemblance in sibling profiles was found and increased when the effects of sociodemographic characteristics and built environment were controlled for, which may mirror the effects of genetic make-up and shared characteristics. Taken together, these results point to the importance of targeting specific groups when developing adequate intervention programs to improve their health. Since the last century, many intervention programs were proposed and implemented to improve children’s and adolescents’ health. Yet, substantive frameworks underlying their elaboration and effectiveness are still unclear. Therefore, we claim that such intervention programs should be at least empirical evidence based, targeting different classes/profiles of adolescents. In other words, a risk-prevention program that is effective in one culture, population, or specific group may not be, or even much less so, effective in others.
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CHAPTER IV

RESEARCH BASED ON LONGITUDINAL DATA
Change and stability in sibling resemblance in obesity markers. The Portuguese sibling study on growth, fitness, lifestyle and health

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ABSTRACT

Obesity markers evolve over time and these changes are shared within the family orbit and governed by individual and environmental characteristics. Available reports often lack an integrated approach, in contrast to a multilevel framework that considers their concurrent influence. Hence, this study aims to: (1) describe mean changes in obesity markers [body fat (%BF), body mass index (BMI) and waist circumference (WC)] across sib-ships; (2) analyze tracking of individuals within their sib-ship in these markers during 2 years of follow-up; (3) probe consistency in sibling resemblance in these markers, and (4) analyze the joint influence of individual and familial characteristics in these markers. The sample comprises 168 biological Portuguese siblings [brother-brother (BB), sister-sister (SS), brother-sister (BS)] aged 9-17 years. %BF, BMI and WC were measured using standardized protocols, and biological maturation was assessed. Physical activity, diet, screen time and familial characteristics were obtained by questionnaires. Multilevel models were used to analyze the clustered longitudinal data. Sibling resemblance was estimated with the intraclass correlation. On average all sib-types increased in BMI and WC over 2 years of follow-up, and SS pairs increased in %BF. Individuals within sib-ships track high in all obesity markers across time. Consistency in siblings’ resemblance was also noted, except for BB pairs in %BF which decreased at follow-up. More maturing sibs tend to have higher values in all markers. Greater screen time was associated with higher %BF, whereas those consuming more sugary drinks had lower %BF and BMI values. Siblings whose mothers had less qualified occupations tended to have lower BMI values. Longitudinal individual tracking and sibling resemblance for obesity markers was found. Yet, different trajectories were also identified depending on the marker and sib-type. Individual and familial characteristics exert different influences on each obesity marker.
INTRODUCTION

There is a strong call to investigate how changes in obesity markers such as percent body fat (%BF), body mass index (BMI) and waist circumference (WC) occur during childhood and adolescence to better comprehend their etiology and tendencies as well as to identify important time windows for fruitful interventions (World Health Organization, 2015). Further, it is well known that adolescence is a period of rapid and systematic changes in body size, shape and composition, where BF distribution and patterns vary considerably with age, sex, and biological maturation (Wells, 2007). It is also considered a third critical period for developing obesity (Gonzalez-Muniesa et al., 2017). The etiology of obesity is multifactorial and complex, stemming from the additive and interactive links between the individual (biological and behavioural) and its environmental (familial, social and geographic) characteristics (Hruby et al., 2016).

Family members share a multitude of traits which make them highly suitable candidates to tease out the influences of genes and the environment on obesity markers (Silventoinen, Rokholm, Kaprio, & Sorensen, 2010). For example, twin data showed that BMI heritability estimates range from 70 to 90% in both adolescent boys and girls across the globe (Elks et al., 2012). However, other studies using complex twin models showed inconsistent results regarding the additive components of genetic, unique and shared environmental factors for BMI and WC across different populations (Ji et al., 2014; Silventoinen et al., 2017; White, Slane, Klump, Burt, & Pivarnik, 2014). Furthermore, intraclass correlation coefficients (ICC) from twin studies consistently show higher monozygotic twins’ resemblance than in dizygotic twins of both sexes for BMI (Ji et al., 2014; Lajunen et al., 2009a; Wardle, Carnell, Haworth, & Plomin, 2008), WC (Ji et al., 2014; Wardle et al., 2008) and %BF (White et al., 2014). Additionally, data from nuclear families revealed that ICC values depend on the kinship structure (Jelenkovic, Poveda, & Rebato, 2010; P. T. Katzmarzyk et al., 2000; Treuth, Butte, Ellis, Martin, & Comuzzie, 2001; Wu et al., 2003), and may vary from 0.15 (spouses) to 0.44 (father-daughter) in BMI; 0.11 (Father-daughter) to 0.53 (siblings) in WC, and 0.05 (spouses) to 0.34 (both father-daughter and mother-daughter) in %BF.
Apparently, there is a paucity of studies related to changes in obesity markers grounded on twins and nuclear family data. Further, available results show discrepancy in the sizes of both genetic and environmental effects (Haberstick et al., 2010; Hunt et al., 2002; Lajunen et al., 2009). For example, Lajunen et al. (2009) using a Finish twin cohort reported that the amount of BMI variation explained by additive genetic factors changed from 0.69 to 0.83 in boys and 0.58 to 0.74 in girls. Moreover, shared environment decreased from 0.15 to 0.00 in boys and 0.21 to 0.03 in girls, whereas unique environment tended to have similar values across time, varying from 0.15 to 0.17 in boys and 0.21 to 0.23 in girls aged 11-17 years. However, Haberstick et al. (2010) using a combined sample of twins and siblings showed that the explained variance by the genetic effects slightly decreased in boys from 0.96 to 0.89, whereas in girls remained stable across time (0.97). Moreover, the effect of the unique environment decreased in both sexes (0.51 to 0.29 and 0.66 to 0.33, in boys and girls, respectively). Additionally, Hunt et al. (2002) using familial data from the Canada Fitness Survey reported a significant level of resemblance among families based on heritability estimates for body mass, BMI, skinfolds and WC. Although, the intraclass correlation coefficients between family members were in general low at baseline, it also remained lower with the 7-years change with slight variations depending on the kinship structure and obesity markers.

We contend that a fruitful approach to investigate changes and/or stability in obesity markers should be simultaneously grounded on longitudinal data, with related individuals, using individual-based and environmental-based characteristics as covariates. For this, the multilevel model approach to investigate siblings’ obesity markers in a 2-year follow-up period and their associations with biological and familial covariates is well suited to provide novel and relevant information that can be used for planning and developing more effective intervention programs within the family orbit. Therefore, we aim: (1) to describe mean changes in obesity markers (%BF, BMI and WC) across sibships; (2) to analyze tracking of individuals within their sibship in obesity markers during 2 years of follow-up; (3) to investigate consistency in sibling resemblance in their obesity markers; and (4) analyze the joint influence of individual and familial characteristics in these markers.
METHODS

Study design and participants

The study sample comprised young siblings (aged 9-17 years) from the Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health. In brief, this study investigates physical growth, body composition, physical fitness, physical activity, metabolic syndrome, and health behaviours in a cohort of siblings based on cross-sectional and longitudinal information (Pereira et al., 2017). All participants enrolled in this study were part of a larger project named The Portuguese Healthy Family Study (Santos et al., 2013). For the present article, we used available longitudinal data on 474 siblings followed for three consecutive years (2011-2013). However, the final sample was only composed by 168 siblings (74 females and 94 males) from 86 families (58 siblings and 26 twins) which had complete data at baseline (2011) and follow-up (2013) for biological, behavioural and familial characteristics. No statistically significant mean differences (p>0.05) were observed between included and excluded siblings in height, weight and percentage of body fat (%BF), body mass index (BMI) and waist circumference (WC).

The assessment of twins’ zygosity usually requires collecting biological samples (e.g., blood or cheek swabs) but it can be fairly assessed with reliable questionnaires. Yet, due to limited time as well as other operational constraints inherent to each school setting during data collection, we were not able to send a putative cross-culturally validated questionnaire to twins’ mothers, aiming to classify their zygosity.

All recruitment and data collection were approved by the Ethics Committee of the University of Porto and school authorities and the written informed consent was acquired from legal guardians for all participants.

Measures

Anthropometry and body composition

Height, weight and WC were measured using standardized protocols established by the International Society for the Advancement of Kinanthropometry (Ross & Ward, 1986). %BF was estimated using a reliable and
valid instrument (Kabiri, Hernandez, & Mitchell, 2015) – a portable bioelectrical impedance scale (TANITA BC-418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan). All youth were barefoot and in light clothing. BMI was computed using the standard formula: BMI= Weight (kg)/Height (m)^2

**Individual characteristics**

**Biological Maturation**

Maturity offset was used to assess biological maturation. This procedure is valid and reliable (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002) and has been commonly used in children and youth (Morrissey, Janz, Letuchy, Francis, & Levy, 2015). Briefly, the maturity offset estimates the temporal distance (in decimal years) from age-at-peak height velocity (PHV). A positive (+) maturity offset indicates the number of years the participant is beyond PHV, whereas a negative (-) maturity offset represents the number of years the participant is before PHV.

**Diet**

Diet consumption was obtained using a food frequency questionnaire (FFQ) adapted and modified from the Health Behaviour in School-aged Children Survey (HBSC) (Currie et al., 2008) using typical Portuguese food items. This questionnaire has been broadly applied in multi-country studies (Peter T. Katzmarzyk et al., 2013). Youth were asked about various types of food consumed in a typical week. Food items related to healthy diet are: fruits, vegetables, dark-green vegetables, orange vegetables, fruit juice, skimmed milk, low-fat milk, whole milk, cheese, other milk products, bread or whole grains, beans, lentils, bean curd, eggs, fish. Food items related to unhealthy diet comprehend sweets, sugary drinks, cakes, pastries, donuts, diet sodas, ice cream, potato chips, French fries, fast foods sports drinks, energy drinks, fried food. For each item, the reported answers were converted into weekly portions as follows: “never” = 0; “less than once per week” = 0.5; “once per week” = 1; “2–4 days per week” = 3; “5–6 days per week” = 5,5; “once a day, every day” = 7; and “more than once a day” = 10, as previously advocated (Mikkila et al., 2015).
Physical activity

Total physical activity (TPA) was assessed with the Baecke questionnaire (Baecke, Burema, & Frijters, 1982). This is a reliable and valid instrument (Philippaerts, Westerterp, & Lefevre, 1999) and includes three specific domains (work/school PA, leisure-time PA, and sports participation) which are based on a total of 16 Likert-type questions. TPA was estimated based on the sum of these three specific domains. For each domain, each score ranges from 1 (minimal) to 5 (maximal), such that the TPA score varies between 3 and 15. Participants answered the questionnaire during regular physical education classes under the supervision of their school-teacher as well as by a trained research team member.

Screen time

Screen time data were obtained via the U.S Youth Risk Behaviour Surveillance Survey (U.S. Centers for Disease Control and Prevention, 1999) questionnaire by self-administered questions: “How long do you watch TV per day?” and “How long do you use your computer or playing non-active video games per day? Answers ranged from <30m, 30m-1h, 1h-1h30,1h30-2h to >2h, being subsequently categorized from 0 to 4 (-/+). Individual scores were summed to obtain a total score for screen time, as reported in different studies (Arango et al., 2014; Rey-Lopez et al., 2012).

Familial characteristics

Parents’ occupation

Parents’ occupation was categorized into ten groups (from 0 to 9) according to the Portuguese National Classification of Occupations (2010), where group 0 is the highest SES and group 9 is the lowest. Categories are as follows: (0) armed forces (1) central administration/politicians and executive directors; (2) specialists of intellectual and scientifically activities; (3) technicians and intermediate level jobs; (4) back-office jobs; (5) security, seller and individual services; (6) farmer and qualified workers of farm, fish and forest; (7) industry and building qualified jobs: (8) machine and equipment operators; and (9) nonqualified jobs.
Parental support for physical activity

Participants answered a questionnaire concerning the perceived support for PA received from their parents based on the Sallis, Grossman, Pinski, Patterson, and Nader (1987) questionnaire. This validated questionnaire includes a list of items relating to parental encouragement for children’s PA practice (Sallis et al., 1987). The response options for all questions range from 0 (never) to 5 (very often), and the sum of the responses was computed to obtain a score for parental support.

Statistical analysis

The analysis was conducted in three steps. Firstly, IBM-SPSS software version 25 was used to compute basic descriptive statistics (mean±standard deviations. To address the first aim of the study, we ran a null model (without covariates) with three-levels for each characteristic. The aim was to test for mean changes between baseline and follow-up through analysis of z-test scores. To deal with aim two, a model with three-levels [repeated measures (baseline and follow-up) nested within individuals, which are themselves nested within sibling pairs)] was used to compute an intraclass correlation as a measure of individual tracking (ICCitrk) in all obesity markers within sib-ships. To answer aims three and four, we also relied on a multilevel model but based on two hierarchical levels, i.e., repeated measures (baseline and follow-up) nested within sibling pairs. The within- and between sib-ship variances were estimated separately, and we obtained different intraclass correlations (ρ), with their corresponding 95% confidence intervals (%95 CI), for the three sib-types for each time point (baseline and follow-up) according to an approach described by Hedeker, Mermelstein, and Demirtas (2012). Separate models were estimated for %BF, BMI and WC using the following set of covariates: Sib-types [Brother-Brother (BB), Sister-Sister (SS), Brother-Sister (BS) pairs] and Sib-type by timepoint (BB as a reference in both). Also, since there were a relatively small sample of twins (n=23 twin pairs of the total of 84 sibling pairs), models were further adjusted for this condition (code: 0=no twin sib-ship; 1=twin sib-ship) as well as an interaction between twin and timepoint. Finally, we also adjusted for a series of other covariates, namely maturity offset, screen time, fruit and vegetable consumption,
sugary drink consumption, physical activity, parent support for PA, father and mother occupation. In all models, BB pairs at baseline was the reference category. When needed, covariates were centered at their respective means as generally advocated (Hox, 2010). All parameters were estimated by maximum likelihood procedures (Goldstein, 2003), and the significance level was set at 5%.

RESULTS

Descriptive statistics for the biological, behavioural and familial characteristics are shown in Table 1. As expected, all sib-types are older and more mature at follow-up compared to baseline (p<0.05). Mean behavioural and familial characteristics are similar across the sib-types at both baseline and follow-up (p>0.05), except for BB in unhealthy that have lower values in follow-up compared to baseline (p<0.05) and for BS in parental support for PA that reported higher support in follow-up compared to baseline (p<0.05).

On average, all sib-types increased their BMI and WC at follow-up compared to baseline (p<0.05). SS pairs had more %BF at the follow-up compared to baseline (p<0.05), but BB and BS pairs did not change significantly (p>0.05). When the whole sample was considered, all subjects, on average, have higher %BF, BMI and WC at follow-up when matched to baseline (p<0.05).
## Table 1. Descriptive statistics [means and standard deviations (SD) and frequencies (%)] for all sib-types at baseline and follow-up

<table>
<thead>
<tr>
<th>Individual characteristics</th>
<th>Brother-brother (n=50) Baseline</th>
<th>Brother-sister (n=84) Baseline</th>
<th>Sister-sister (n=34) Baseline</th>
<th>All Sibs (n=168) Baseline</th>
<th>Brother-brother (n=50) Follow-up</th>
<th>Brother-sister (n=84) Follow-up</th>
<th>Sister-sister (n=34) Follow-up</th>
<th>All Sibs (n=168) Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td><strong>Chronological age (yr)</strong></td>
<td>12.3±1.4</td>
<td>14.6±1.6</td>
<td>19.98***</td>
<td>12.2±1.4</td>
<td>14.3±1.3</td>
<td>14.02***</td>
<td>11.8±1.3</td>
<td>14.2±1.3</td>
</tr>
<tr>
<td><strong>Biological maturation (yr)</strong></td>
<td>-0.9±1.5</td>
<td>0.8±1.7</td>
<td>16.72***</td>
<td>-0.9±1.2</td>
<td>0.6±1.2</td>
<td>13.64***</td>
<td>-0.9±1.1</td>
<td>0.8±0.9</td>
</tr>
<tr>
<td><strong>Body fat (%)</strong></td>
<td>19.4±5.9</td>
<td>19.4±6.0</td>
<td>-0.06ns</td>
<td>21.8±6.0</td>
<td>22.3±5.9</td>
<td>1.21ns</td>
<td>25.1±4.4</td>
<td>27.8±5.3</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>19.7±3.5</td>
<td>20.6±3.7</td>
<td>6.54***</td>
<td>18.9±2.6</td>
<td>19.7±2.6</td>
<td>3.94***</td>
<td>19.2±3.1</td>
<td>20.7±3.4</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>67.3±9.2</td>
<td>70.2±10.2</td>
<td>8.15***</td>
<td>64.4±5.7</td>
<td>66.1±5.5</td>
<td>4.50***</td>
<td>63.1±6.6</td>
<td>65.8±6.8</td>
</tr>
<tr>
<td><strong>Behavioural characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Screen time</strong></td>
<td>3.4±2.6</td>
<td>3.6±2.2</td>
<td>0.63ns</td>
<td>3.0±2.2</td>
<td>2.9±1.9</td>
<td>-0.26ns</td>
<td>2.8±2.2</td>
<td>2.4±2.1</td>
</tr>
<tr>
<td><strong>Total physical activity</strong></td>
<td>8.2±2.0</td>
<td>8.5±1.6</td>
<td>1.09ns</td>
<td>7.6±2.2</td>
<td>8.0±1.4</td>
<td>1.62ns</td>
<td>7.8±1.3</td>
<td>7.7±1.9</td>
</tr>
<tr>
<td><strong>Healthy diet</strong></td>
<td>44.0±18.3</td>
<td>42.5±18.7</td>
<td>-0.48ns</td>
<td>42.1±5.2</td>
<td>39.6±13.5</td>
<td>-1.47ns</td>
<td>41.0±13.9</td>
<td>38.4±20.4</td>
</tr>
<tr>
<td><strong>Unhealthy diet</strong></td>
<td>29.1±19.3</td>
<td>24.4±15.5</td>
<td>-2.08</td>
<td>22.6±14.1</td>
<td>21.2±14.2</td>
<td>-1.13ns</td>
<td>15.0±7.4</td>
<td>13.5±7.5</td>
</tr>
<tr>
<td><strong>Familial characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father occupation</td>
<td>6.0±2.7</td>
<td>6.0±2.7</td>
<td>-0.00ns</td>
<td>5.6±2.5</td>
<td>5.6±2.5</td>
<td>1.01ns</td>
<td>6.0±2.0</td>
<td>6.1±2.1</td>
</tr>
<tr>
<td>Mother occupation</td>
<td>6.7±2.2</td>
<td>7.1±2.1</td>
<td>0.86ns</td>
<td>5.6±2.7</td>
<td>5.6±2.7</td>
<td>-1.75ns</td>
<td>6.2±2.8</td>
<td>6.2±2.8</td>
</tr>
<tr>
<td>Parental support for PA</td>
<td>11.8±8.1</td>
<td>11.3±7.3</td>
<td>-0.42ns</td>
<td>10.5±6.5</td>
<td>11.9±6.0</td>
<td>2.00</td>
<td>11.2±6.1</td>
<td>12.7±5.9</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001; ns=non-significant*
Table 2 shows tracking coefficients for individual siblings within their respective sib-ships. Across all obesity markers tracking is high, ranging from 0.90 to 0.97 (BB), 0.75 to 0.95 (SS) and 0.73 to 0.97 in (BS).

<table>
<thead>
<tr>
<th>Tracking coefficients</th>
<th>%BF</th>
<th>WC</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICCitrk (95%CI)</td>
<td>ICCitrk (95%CI)</td>
<td>ICCitrk (95%CI)</td>
</tr>
<tr>
<td>Brother-brother</td>
<td>0.90 (0.83-0.95)</td>
<td>0.96 (0.92-0.98)</td>
<td>0.97 (0.94-0.98)</td>
</tr>
<tr>
<td>Sister-sister</td>
<td>0.91 (0.81-0.96)</td>
<td>0.97 (0.93-0.99)</td>
<td>0.75 (0.57-0.87)</td>
</tr>
<tr>
<td>Brother-sister</td>
<td>0.97 (0.94-0.98)</td>
<td>0.93 (0.87-0.97)</td>
<td>0.73 (0.55-0.85)</td>
</tr>
</tbody>
</table>

The intra-class correlation (ρ) values of sibling resemblance are in Table 3 and show that, regardless of the obesity marker, SS pairs resemble each other more than BB and BS pairs at both baseline and follow-up. Additionally, BS pairs were less similar in all markers at both timepoints. Moreover, there are slight differences in siblings’ ρ values from baseline to follow-up in all markers, suggesting stability in siblings’ resemblance across time.

<table>
<thead>
<tr>
<th>Tracking coefficients</th>
<th>%BF</th>
<th>WC</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
</tr>
<tr>
<td>Brother-brother</td>
<td>0.45 (0.19-0.75)</td>
<td>0.39 (0.13-0.73)</td>
<td>0.55 (0.21-0.84)</td>
</tr>
<tr>
<td>Sister-sister</td>
<td>0.37 (0.12-0.72)</td>
<td>0.36 (0.11-0.72)</td>
<td>0.53 (0.21-0.83)</td>
</tr>
<tr>
<td>Brother-sister</td>
<td>0.24 (0.04-0.71)</td>
<td>0.23 (0.03-0.72)</td>
<td>0.62 (0.31-0.86)</td>
</tr>
</tbody>
</table>

Finally, Table 4 contains results showing the relevance of covariates on sibs resemblance. The BB pairs tended to have lower values of %BF (β=22.63±1.91) as compared to SS pairs at both baseline (β=4.06±1.35) and follow-up (β=5.88±1.64). They also had higher WC values (β=71.57±2.21) when compared to SS and BS in both baseline (β=4.87±1.79 and β=2.84±1.47, for SS and BS respectively), and follow-up (β=4.58±2.03 and β=3.68±1.55, for SS and BS respectively). No differences were found for twins at baseline and follow-up in all obesity markers as well as in BMI in all sib-types compared to BB pairs at baseline. Further, more mature
subjects tended to exhibit higher values of %BF ($\beta=0.49\pm0.23$), BMI ($\beta=0.86\pm0.12$) and WC ($\beta=2.30\pm0.27$). Those who had greater screen time were also those that had, on average, more %BF ($\beta=0.27\pm0.14$), whereas those consuming more unhealthy food had lower %BF and BMI values ($\beta=-0.10\pm0.02$ and $\beta=-0.03\pm0.01$, for %BF and BMI, respectively). Siblings whose mothers had less qualified occupations tended to have lower BMI ($\beta=-0.15\pm0.08$). Additionally, healthy diet, physical activity, parental support for PA and father occupation did not significantly associate with siblings’ %BF, BMI and WC ($p>0.05$).

Table 4. Parameter estimates and variance components for each obesity marker

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>%BF</th>
<th>BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (BB baseline)</td>
<td>22.63±1.91***</td>
<td>22.09±0.95***</td>
<td>71.57±2.21***</td>
</tr>
<tr>
<td>BB follow-up</td>
<td>-1.42±1.55</td>
<td>-0.38±0.80</td>
<td>-0.02±2.09</td>
</tr>
<tr>
<td>SS baseline</td>
<td>4.06±1.35***</td>
<td>-1.04±1.80</td>
<td>-4.87±1.79*</td>
</tr>
<tr>
<td>SS follow-up</td>
<td>5.88±1.64***</td>
<td>-0.70±0.95</td>
<td>-4.58±2.03'</td>
</tr>
<tr>
<td>BS baseline</td>
<td>2.11±1.25</td>
<td>-1.00±0.59</td>
<td>-2.84±1.47'</td>
</tr>
<tr>
<td>BS follow-up</td>
<td>1.96±1.33</td>
<td>-1.22±0.65</td>
<td>-3.68±1.55'</td>
</tr>
<tr>
<td>Twins Baseline</td>
<td>1.80±1.05</td>
<td>0.39±0.55</td>
<td>1.11±1.21</td>
</tr>
<tr>
<td>Twins Follow-up</td>
<td>0.72±1.47</td>
<td>0.28±0.78</td>
<td>-0.15±1.65</td>
</tr>
<tr>
<td>Maturity offset</td>
<td>0.49±0.23</td>
<td>0.86±0.12***</td>
<td>2.30±0.27***</td>
</tr>
<tr>
<td>Screen time</td>
<td>0.27±0.14'</td>
<td>0.10±0.07</td>
<td>0.14±0.16</td>
</tr>
<tr>
<td>Healthy diet</td>
<td>0.01±0.02</td>
<td>0.00±0.01</td>
<td>-0.02±0.02</td>
</tr>
<tr>
<td>Unhealthy diet</td>
<td>-0.10±0.02***</td>
<td>-0.03±0.01</td>
<td>-0.01±0.02</td>
</tr>
<tr>
<td>Physical activity</td>
<td>-0.22±0.15</td>
<td>-0.04±0.10</td>
<td>-0.09±0.22</td>
</tr>
<tr>
<td>Parental support for PA</td>
<td>-0.05±0.05</td>
<td>-0.03±0.03</td>
<td>-0.07±0.06</td>
</tr>
<tr>
<td>Father occupation</td>
<td>-0.21±0.15</td>
<td>0.02±0.02</td>
<td>-0.03±0.17</td>
</tr>
<tr>
<td>Mother occupation</td>
<td>-0.01±0.14</td>
<td>-0.15±0.08'</td>
<td>-0.07±0.06</td>
</tr>
</tbody>
</table>

Variance components ($\sigma^2$)

<table>
<thead>
<tr>
<th>Between siblings'</th>
<th>%BF</th>
<th>BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB baseline</td>
<td>16.82±8.42</td>
<td>2.47±2.14</td>
<td>22.82±13.47</td>
</tr>
<tr>
<td>SS baseline</td>
<td>7.52±4.35</td>
<td>4.63±2.24</td>
<td>16.24±8.85</td>
</tr>
<tr>
<td>BS baseline</td>
<td>0.87±4.80</td>
<td>0.43±0.92</td>
<td>0.32±4.37</td>
</tr>
<tr>
<td>BB Follow-up</td>
<td>12.59±7.21</td>
<td>2.46±2.27</td>
<td>27.94±16.78</td>
</tr>
<tr>
<td>SS Follow-up</td>
<td>11.57±6.07</td>
<td>6.00±2.93</td>
<td>16.59±10.37</td>
</tr>
<tr>
<td>BS Follow-up</td>
<td>0</td>
<td>1.11±0.93</td>
<td>0</td>
</tr>
</tbody>
</table>

Within siblings'

| BB baseline        | 20.20±6.02 | 7.69±2.23  | 39.22±11.43 |
| SS baseline        | 6.23±2.33  | 2.80±0.99  | 14.26±5.04 |
| BS baseline        | 29.67±6.70 | 5.29±1.20  | 27.04±6.10 |
| BB follow-up       | 19.83±5.81 | 8.41±2.39  | 49.66±14.14 |
| SS follow-up       | 8.04±2.91  | 3.54±1.32  | 18.94±6.90 |
| BS follow-up       | 32.20±5.00 | 4.71±1.04  | 25.19±3.94 |

Log likelihood | -1026.99  | -803.19   | -1073.55   |

p<0.05 * p<0.01 ** p<0.001; BB – brother-brother; SS – sister-sister; BS – brother-sister;
DISCUSSION

A high individual tracking within sibships was observed in all obesity markers. Previous studies with unrelated subjects that also examined tracking in obesity markers during adolescence to adulthood showed moderate-to-high values across time (Aarestrup et al., 2016; Eisenmann, Welk, Wickel, & Blair, 2004). For example, Eisenmann et al. (2004) reported a moderate to relatively high tracking [expressed by auto-correlations (ρ)] in all obesity markers, namely: ρ=0.64 in BMI, ρ=0.44 %BF (derived from skinfolds) and r=0.79 in WC from adolescence to adulthood. Moreover, Ronque et al. (2018) also showed high tracking in a 3-yr follow-up study (BMI=0.94 and sum of skinfolds=0.86) in adolescents. Altogether, these overall findings indicate that the ways these obesity markers express themselves in sibs show stability in their relative position and hence, despite their inherent plasticity during growth, track across time.

We also showed that, even with a slight variation within sibpairs depending on the type of obesity marker, SS pairs tend to be more consistent than BB and BS pairs in their resemblance. These departures from similarity could be partially explained by sex-differences in physiological, biochemical and hormonal mechanisms during growth in overall body size and composition (Neigh & Mitzelfelt, 2016), and may clarify the absence of sameness in %BF and WC, and the low BMI similarity in opposite-sex siblings. Available reports with twins (Ortega-Alonso, Pietilainen, Silventoinen, Saarni, & Kaprio, 2012; Silventoinen, Kaprio, & Yokoyama, 2011) and nuclear families (Hunt et al., 2002) related to BMI described different changes depending on sex and period of life. For example, in pre-pubertal MZ twins (1-11 yrs) it was shown that BMI resemblance tended to increase in both boys and girls, and the same occurred to DZ boys; however, this resemblance tended to decrease in DZ girls and maintained its magnitude in opposite-sex twins (Silventoinen et al., 2011), whereas older twins aged 16.1 to 24.5 yrs tended to stay stable in their BMI changes (Ortega-Alonso et al., 2012). In the sole nuclear family longitudinal study we were able to retrieve Hunt et al. (2002) using family data with a 7 yr follow-up (boys and girls mean ages at baseline was 12.4 and 11.8 yrs, respectively) also analyzed a phenotype termed ρ (changes) in three obesity markers (BMI, skinfolds and WC) and reported lower ρ values than those shown in the present study. Furthermore, the authors reported within-pair ρ changes (BMI=0.07, skinfolds=0.00 and WC=0.34) for all sib-types. Moreover,
Haberstick et al. (2010) informed that, from adolescence to early adulthood (16-22 yrs), BB pairs tend to exhibit a similar pattern of BMI (from 0.36 (baseline) to 0.34 (follow-up)), whilst SS and BS pairs show a slight increase (from 0.49 to 0.55 and 0.38 to 0.43, respectively).

We claim that the discrepancy found in these results and the ones obtained in our study may be related to several factors: (i) the adjustments made for confounders, (ii) differences in study design, (iii) follow-up time, and (iv) statistical analyses. In any case, what these results tend to show is the importance of both genetic and environmental factors and probably their interactions on changes in obesity markers within the family orbit. We also contend that given the evident lack of data on genome-wide association (GWA) and gene-environment interaction studies in all markers (except for BMI) we are not yet able to present a clear picture of what is happening (Felix et al., 2016; Graff et al., 2012; Zhao et al., 2017). Indeed, using BMI data Felix et al. (2016) GWA study signaled that the direction of the effect size for all 15 single nucleotide polymorphisms (SNPs) was identical in children and in adults. However, Graff et al. (2012) showed that obesity susceptibility loci may have a comparatively stronger role during adolescence than adulthood, with variations across race/ethnic subpopulations. Additionally, using European and African American samples, Zhao et al. (2017) examined social/psychosocial factors that may modify the effect of sets of genetic variants on BMI and reported that socioeconomic status (parental education) was found to modify the genetic effect in the gene/region around SNP rs9540493 on BMI only in European Americans.

Siblings’ individual and familial characteristics were differently associated with obesity markers, except for biological maturation that was positively associated with all markers, suggesting that those ahead in their biological maturation have greater chances of developing obesity, and this in line with previous findings (Wang & Adair, 2001; Werneck et al., 2017). However, changes in means of obesity markers across time only differed at baseline in siblings from different types. Thus, BB pairs showed less %BF than SS pairs and higher WC than BS and SS pairs. These results indicate that despite sib-pairs (depending on sex) starting from different mean values for the obesity markers, these values tend to come closer in time. In the particular case of BMI and WC, our results confirm other studies (Chaves, Baxter-Jones, Souza, Santos, & Maia, 2015; Cole, Freeman, & Preece, 1995). Nonetheless, available data (Chaves et al., 2015; McCarthy, Cole, Fry, Jebb, & Prentice, 2006) related to %BF
showed different tendencies: boys proportionately decrease their body fat whereas girls increase theirs across age. Indeed, our study revealed the same tendency (see Table 3) although results were not statistically significant. A plausible reason underlying this lack of significance may be related to the fact that we only have 2 years of follow-up, and we need to expand the time window of follow-up to clarify this tendency.

Screen time only associated with %BF, and siblings who spent more time in front of screens tended to have higher %BF. However, previous studies have shown contradictory results. For example, Chinapaw, Proper, Brug, van Mechelen, and Singh (2011) in a systematic review found a lack of evidence for a positive longitudinal relationship between screen time and BMI, %BF and WC. Likewise, Fulton et al. (2009) reported that even after adjusting for age and sexual maturation, screen time was unrelated to obesity markers, while Delmas et al. (2007) showed positive associations in boys but not in girls. On the other hand, previous systematic reviews reported strong evidence that spending large proportions of awake time on screens was positively associated with obesity markers (Carson et al., 2016). There are some inconsistencies between such studies, perhaps as a consequence of employing different analytical strategies along with the use of different samples and/or study designs. Regardless, the direction and magnitude of the positive associations between screen time and obesity depends on the marker, and our results showed that screen time strongly influenced %BF, which may contribute to the development of obesity. Additionally, in future studies we recommend the use of different indicators of obesity in the same sample to better understand these associations. Unexpectedly, and contrary to most available literature (Bes-Rastrollo, Sayon-Orea, Ruiz-Canela, & Martinez-Gonzalez, 2016; Keller & Bucher Della Torre, 2015), healthy diet were not associated with all obesity markers and unhealthy diet were negatively associated with %BF and BMI in our sample. Still, our findings were corroborated by previous studies (Janssen et al., 2005; McNaughton, Ball, Mishra, & Crawford, 2008; Parnell et al., 2008). For example, McNaughton et al. (2008) reported no associations between BMI or waist circumference and any of the dietary patterns. On the other hand, Parnell et al. (2008) reported that obese children had a significant lower intake of sugar than overweight or normal weight children. One possible explanation for these unexpected results may be related to the perception of children and adolescent
with the excess of %BF and weight, which tend to underreport their real consumption. On the other hand, youth who are normal weight may not be restricting their unhealthy foods compared with youth having excess of fat, which can try to reduce their weight by diminishing the consumption of these type of foods.

Finally, from all familial characteristics, only mother's occupation was associated with BMI. Indeed, siblings whose mothers had less qualified occupations presented lower BMI. Socioeconomic status (SES) and its corresponding indicators (e.g. household income, parents' education and occupation), were strongly associated with obesity markers in previous studies. However, the direction and magnitude of these associations are heterogeneous and inconsistent, ranging from negative to positive associations depending on the country and obesity marker (Due et al., 2009). Nevertheless, our results are similar to those presented by Gurzkowska et al. (2014) in a study conducted on Polish adolescents. Although the authors had not performed any adjustment for other confounders, they found that higher SES was associated with higher weight, BMI and WC. On the other hand, a study conducted by Costa de Oliveira Forkert et al. (2017) in European (Germany, Sweden, Greece, Italy, Spain, Hungary, Belgium, France and Austria) and Brazilian adolescents found different results for WC. Here, both parental education and father's occupation levels were negatively associated with WC in European girls, while in boys only mother's occupation level was inversely associated with WC. However, among Brazilian adolescents no significant associations were found. Notwithstanding, this study only adjusted its analysis for age and focused on European vs Brazilian, which could explain the different directions of associations compared to our study. Altogether, these results suggest that SES is an important factor that influences not only individual development, at large, but also its obesity development. However, it is noteworthy to mention that the direction of SES influence is not consistently universal, and programs for preventing obesity should be defined and tailored according to SES specificities.

Notwithstanding the relevance of our results, this study is not without limitations. For example, (i) participants were not recruited from all Portuguese regions, which restricts the generalization of the results to the whole Portuguese population; however, this issue is common in family and twin studies; (ii) the use of questionnaires to obtain information about physical activity, screen time and diet is
prone to errors, although the questionnaires have been applied in controlled conditions. Further, these questionnaires are frequently used, and previous studies have confirmed their reliability; (iii) It may be possible that the variance components, as well as intraclass correlation estimates, could be different if we had precise information about twin zygosity. Yet, in results not shown, we tested different models with and without twin data and no substantive differences were found suggesting that such absence did not compromise our results.

**CONCLUSIONS**

In overall terms, our findings report increases in BMI and WC for all sib-types for 2 years of follow-up, as well as for %BF in SS pairs. Obesity markers tend to track across time within sib-ships, whereas sibling resemblance tends to be consistent over time. Additionally, our data reinforce the idea that both individual and familial characteristics may exert divergent influences on each obesity marker development. Hence, we suggest that prevention strategies need to consider the complex and intertwined relationships of both familial and biological characteristics that may provide new insights in obesity control. Therefore, when developing suitable interventions aiming to prevent obesity it is crucial to consider a target marker, fashioning different programs for distinct groups based on their individual and familial characteristics. Finally, we need more longitudinal studies based on larger family-based data relying on a multilevel approach combined with appropriate statistical models. We are convinced that this endeavor will help to build a more comprehensive understanding of this complex interaction between nature and nurture in obesity phenomena.
REFERENCES


Wang, Y., & Adair, L. (2001). How does maturity adjustment influence the estimates of overweight prevalence in adolescents from different countries using an


Change and stability in sibling physical fitness: The Portuguese sibling study

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ABSTRACT

This study aims to: (1) describe mean changes in muscular and motor fitness components in siblingships over 2 years; (2) analyze individual tracking of fitness within siblingships; (3) investigate sibling resemblance in fitness over time, and (4) examine the joint influence of biological, behavioural and familial characteristics on fitness.

The sample comprises 166 Portuguese biological sibling pairs (brother-brother, sister-sister, brother-sister) aged 9-17 years assessed at baseline and 2 years later. Physical fitness components were measured with standardized tests. Percentage of body fat and biological maturation were assessed, and physical activity, diet, screen time and familial characteristics were obtained by questionnaires. Multilevel models were used to analyze the clustered longitudinal data.

Crude results showed that, on average, all sibling types increased their muscular and motor fitness components from baseline to follow-up (except sister-sister pairs in standing long jump and shuttle-run). When adjusted for covariates, the mean changes were no longer significant. Individual tracking was moderate-to-high for the muscular component but low-to-moderate for the motor component. Consistency in sibling resemblance was higher in sister-sister pairs than brother-brother and brother-sister pairs. More mature sibs were fitter in both components whereas siblings with higher body fat percentage were less fit. Screen time, physical activity and parental occupation were not associated with fitness components. We conclude that biological characteristics were more strongly associated with fitness components than individual behaviours and familial characteristics. Further, the muscular component tracked better than the motor component. Sister-sister pairs had greater resemblance in fitness over time compared to brother-brother or sister-brother pairs.
INTRODUCTION

Physical fitness is currently regarded as an important asset in adolescents’ health (Ortega, Ruiz, Castillo, & Sjostrom, 2008), and can be defined as the ability to carry out daily task with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies (President’s Council on Physical & Sports, 1971). Bouchard and Shephard (1994) expanded this definition with a focus on health-related fitness, which comprises five components including morphological, muscular, motor, cardiorespiratory, and metabolic fitness (Chen, Hammond-Bennett, Hypnar, & Mason, 2018; Chu, Chen, Pontifex, Sun, & Chang, 2019; Minatto, Petroski, & Silva, 2016). Much research has focused on roles of cardiorespiratory and morphological (especially adiposity) fitness as markers for non-communicable diseases (Al-Mallah, Sakr, & Al-Qunaibet, 2018; Elagizi et al., 2018). Additionally, a growing body of research has emphasized the benefits of muscular and motor fitness on a variety of health-related outcomes (Moradi et al., 2019; Ortega et al., 2008; Perez-Sousa, Olivares, Garcia-Hermoso, & Gusi, 2019).

Although systematic decreases in physical fitness levels have been reported in some countries (Albon, Hamlin, & Ross, 2010; Craig, Shields, Leblanc, & Tremblay, 2012; Tomkinson & Olds, 2007), there is also evidence that positive secular trends are also emerging in different fitness components (Dos Santos et al., 2015; Moliner-Urdiales et al., 2010). Further, variation in physical fitness levels within and between populations has been attributed to differences in biological, behavioural and familial characteristics, including body fat (Szmodis et al., 2019), biological maturation, physical activity levels (Chen et al., 2018), screen time (Aguilar, Vergara, Velasquez, Marina, & Garcia-Hermoso, 2015) and sociodemographic factors (D. P. Guedes, Miranda Neto, Lopes, & Silva, 2012).

Hence, the study of physical fitness levels in adolescents is an important issue given the acknowledged implications on their present and future health-related outcomes (Fraser et al., 2018). Further, longitudinal reports provide meaningful information, and available data have demonstrated moderate-to-high tracking for different fitness components from childhood to adolescence (Falk et al., 2001; Roth, Schmidt, Seidel, Woll, & Bos, 2018), during adolescence (Da Silva, Beunen, Prista,
Change and stability in sibling physical fitness

& Maia, 2013; Souza et al., 2016), and from adolescence to adulthood (Fraser et al., 2017; Van Oort et al., 2013). Yet, studies seldom adjust for potential confounders such as biological maturation, behavioural characteristics (physical activity and sedentariness), or familial characteristics (sociodemographic factors).

Family members share a multitude of contextual factors and using family data can provide substantial insights regarding the influence of individual and contextual characteristics on the ways that fitness levels unfold over time. We were able to find only one study that examined familial resemblance in musculoskeletal fitness over 7 years, and correlations were 0.27 and 0.22 for trunk flexibility and sit-ups, respectively, for all family member types (Katzmarzyk, Gledhill, Perusse, & Bouchard, 2001). Additionally, values ranged from -0.11 (brother-sister) to 0.39 (brother-brother and father-daughter) for grip strength, and between -0.34(father-daughter) to 0.54 (brother-sister) for push-ups (Katzmarzyk et al., 2001).

Full siblings share a substantial portion of their genes identical-by-descent (50% on average) and also have a common familial environment, neighborhood histories and school contexts (Falconer & Mackay, 1996). Yet, they may differ in chronological age, sex and health behaviours, as well as in their physical growth and biological maturation (Malina, Bouchard, & Bar-Or, 2004). Altogether, these factors are also linked to similarity and/or dissimilarity in their current physical fitness levels and changes over time. Hence, we believe that using sibling pairs (sib-ships) may be helpful in disentangling the nature-nurture aspects of developmental trajectories in physical fitness. Further, using the multilevel statistical model as an analytical template will be of great help given that familial data are necessarily correlated, while considering the influence of both individual and familial characteristics. Thus, we hope to provide novel insights concerning changes and/or tracking in physical fitness components in sib-ships.

We anticipate that this body of knowledge would certainly provide novel insights for effective interventions regarding healthy fitness maintenance across the life span. Therefore, this study aims to: (1) describe mean changes in muscular and motor fitness components across different sib-ships over 2 years; (2) analyze tracking of individuals within their sib-ship in physical fitness components over 2 years; (3) investigate consistency in sibling resemblance in physical fitness components over
time; and (4) probe the joint influence of biological, behavioural and familial characteristics in these components. Further, we hypothesize that (1) on average there is an increase in muscular and motor components across sib-ships, (2) individual tracking during 2 years of follow-up is moderate-to-high, (3) sibling resemblance is more consistent in same-sex sibs, and (4) individual and familial characteristics are significantly associated with siblings’ physical fitness.

METHODS

Participants

The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health is a family-based study aimed to investigate physical growth, body composition, physical fitness, physical activity, metabolic syndrome, and health behaviours in a large cohort of siblings based on cross-sectional and longitudinal information (Pereira et al., 2017). The study sample comprises 474 siblings (aged 9-17) assessed during three consecutive years (2011-2013). However, for the purpose of the present study we sampled 166 subjects with complete information (76 females and 90 males) from 83 families (58 siblings and 25 twins) assessed over three consecutive years (2011-2013).

Subjects had complete data at baseline (2011) and follow-up (2013) for biological, behavioural and familial characteristics, and there were no statistically significant mean differences (p>0.05) in height, weight, percentage body fat (%BF), and physical fitness between included and excluded siblings. Moreover, we do not have information on the twins’ zygosity as this usually requires genetic analysis from biological samples (blood for example). As is well-known, zygosity can be fairly assessed with reliable questionnaires instead of using biological samples (e.g., blood or cheek swabs). Yet, given limited time as well as operational constraints within each school setting to collect the data, we were not able to send twin mothers a putative cross-culturally validated questionnaire to classify their zygosity. All recruitment and data collection methods were approved by the Ethics Committee of the lead institution and school authorities, and written informed consent was acquired from legal guardians for all participants.
Physical fitness

Physical fitness was assessed with the following tests: (1) handgrip strength (HG) using a hand dynamometer (Takei Digital Grip Strength Dynamometer, Model T.K.K.5401, Tokyo, Japan) – all participants pressed with maximum isometric effort which was maintained for 5 to 10 seconds, and results were recorded in kilogram-force (kgf); (2) standing long jump (SLJ) – all participants attempted to jump as far as possible, landing with both feet on the ground and without falling backwards; results were recorded in centimeters (cm); (3) 50 yard dash (50YD) – all participants ran the 50 yards in the shortest time possible, and results were recorded in seconds (s); (4) shuttle-run (SR) – all participants ran as fast as possible to the other line, picked up a block and returned to place it behind the starting line, then they started the routine again and repeat it 2 times. Here, the results were also recorded in seconds (s). In the present study, tests were grouped into two components – muscular and motor. To assess the muscular component, we used the HG and SLJ that evaluate strength and power abilities, respectively (Malina et al., 2004). To assess the motor component, the 50YD and the SR were used to measure speed and agility abilities, respectively (Malina et al., 2004).

Individual test results were firstly transformed into z-scores and then an unweighted sum of z-scores was computed to describe each component. Scores were appropriately converted to z-scores to account for scale differences between different fitness tests. Care was taken to reverse signs in 50YD and SR performance as shorter times indicate better performance on these tests.

Covariates

The maturity offset was used as a marker of biological maturation (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002). This procedure expresses the estimated distance (in decimal years) each subject is from age-at-peak height velocity.

Body fat percentage (%BF) was estimated using a reliable and valid instrument (Kabiri, Hernandez, & Mitchell, 2015) – a portable bioelectrical impedance scale (TANITA BC- 418 MA Segmental Body Composition Analyzer Tanita Corporation, Japan). All youth were barefoot and in light clothing.

Total physical activity (TPA) was assessed with the Baecke questionnaire (Baecke, Burema, & Frijters, 1982). This is a reliable and valid instrument (Dartagnan...
Change and stability in sibling physical fitness

P. Guedes, Lopes, Guedes, & Stanganelli, 2006; Philippaerts, Westerterp, & Lefevre, 1999), and has been systematically used in research in Portugal (Antunes et al., 2015; Freitas et al., 2017; Santos et al., 2014). The questionnaire includes three specific domains (work/school PA, leisure-time PA, and sports participation) and is based on a total of 16 Likert-type questions. TPA was estimated based on the sum of these domains.

Screen time data were obtained via the Youth Risk Behaviour Surveillance System (YRBSS) (U.S. Centers for Disease Control and Prevention, 2012) questionnaire by self-administered questions: “How many hours do you watch TV?” and “How many hours do you play video or computer games or use a computer for something that is not school work?” Answers ranged from <30m, 30m-1h, 1h-1h30,1h30-2h to >2h, being subsequently categorized from 0 to 4 (+/-). Individual scores were summed to obtain a total score for screen time (ST), as reported in different studies (Arango et al., 2014; Rey-Lopez et al., 2012).

Parental occupation was categorized into ten groups (from 0 to 9) according to the Portuguese National Classification of Occupations (2010), where group 0 comprises the highest SES and group 9 the lowest. Categories are as follows: (0) armed forces (1) central administration/politicians and executive directors; (2) specialists of intellectual and scientific activities; (3) technicians and intermediate level jobs; (4) back-office jobs; (5) security, seller and individual services; (6) farmer and qualified workers of farm, fish and forest; (7) industry and building qualified jobs; (8) machine and equipment operators; and (9) nonqualified jobs.

**Statistical analysis**

IBM-SPSS software v.25 was used to compute basic descriptive statistics (mean±standard deviations). A multilevel approach consisting of three levels in the analysis was used to account for the data structure, i.e., repeated measures (baseline and follow-up) nested within individuals, which are themselves nested within sibling pairs.

To address the first aim of the study, we ran a null model (without covariates) with three levels for each characteristic, in order to test for mean changes between baseline and follow-up, and a z-test was used. For the second aim we estimated the between individual and sib-ship variances as well as the between sib-ship variances.
Thus, different intraclass correlations describing tracking (ICCitrk), with their corresponding 95% confidence intervals (95% CI), were estimated for individuals within sib-ships. These ICCs indicate the association of the repeated observations within subjects. For the third aim, we estimated separately the within- and between sib-ship variance at each timepoint, as well as the between individual sib-ship variance. Therefore, different intraclass correlation ($\rho$) values with their corresponding 95% confidence intervals (95% CI) were obtained for the three sib-types at each time point (baseline and follow-up) as markers of the association within sibling pairs. These procedures are in accordance with those described by Hedeker, Mermelstein, and Demirtas (2012). Separate models were estimated for the muscular and motor fitness components using the following set of covariates: Sib-types [Brother-Brother (BB), Sister-Sister (SS), Brother-Sister (BS) pairs] and sib-type by timepoint (BB as a reference in both). Also, due to a relatively small sample of twins (n=25 twin pairs in a total of 83 sibling pairs), models were further adjusted for this condition (code: 0=no twin sib-ship; 1=twin sib-ship) as well as for an interaction between twin and timepoint. Finally, our models were also adjusted for a series of other covariates, namely: maturity offset, %BF, ST, TPA, and father’s and mother’s occupation. In all models, BB pairs at baseline were the reference category. When needed, covariates were centered at their respective means as generally advocated (Hox, 2010). All parameters were estimated by maximum likelihood procedures as implemented in STATA 15, and the significance level was set at 5%.

RESULTS

Descriptive statistics for sibling characteristics are presented in Table 1. As expected, participants were older and more mature at follow-up compared to baseline for all sib-types (p<0.05). Moreover, only SS pairs increased %BF at follow-up when compared to baseline (p<0.05). The same trend was verified when we consider all sib-types as a whole. Mean behavioural (ST and TPA) and familial characteristics (father and mother occupation) were similar across sib-types at both baseline and follow-up (p>0.05). All sib-types increased their muscular and motor fitness scores from baseline to follow-up (p<0.05). However, no significant changes were identified for SS pairs over time in the standing long jump and shuttle-run test (p>0.05).
Table 1. Descriptive statistics [means and standard deviations (SD)] for all sib-types at baseline and follow-up

<table>
<thead>
<tr>
<th>Individual characteristics</th>
<th>Brother-brother Mean±SD</th>
<th>Brother-brother Mean±SD</th>
<th>z</th>
<th>Brother-sister Mean±SD</th>
<th>Brother-sister Mean±SD</th>
<th>z</th>
<th>Sister-sister Mean±SD</th>
<th>Sister-sister Mean±SD</th>
<th>z</th>
<th>All Sibs Mean±SD</th>
<th>All Sibs Mean±SD</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological age (years)</td>
<td>12.9±1.9</td>
<td>14.1±1.9</td>
<td>21.70***</td>
<td>12.2±1.4</td>
<td>13.4±1.5</td>
<td>24.88***</td>
<td>12.1±1.5</td>
<td>13.5±1.5</td>
<td>16.73***</td>
<td>12.4±1.6</td>
<td>13.7±1.7</td>
<td>36.54***</td>
</tr>
<tr>
<td>Biological maturation (years)</td>
<td>0.1±1.6</td>
<td>1.6±1.6</td>
<td>17.41***</td>
<td>-0.6±1.3</td>
<td>0.5±1.2</td>
<td>22.71***</td>
<td>-0.6±1.2</td>
<td>0.5±1.0</td>
<td>13.93***</td>
<td>-0.4±1.4</td>
<td>0.7±1.4</td>
<td>31.62***</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20.1±6.3</td>
<td>20.1±5.9</td>
<td>0.00</td>
<td>22.8±7.2</td>
<td>23.2±7.3</td>
<td>1.13</td>
<td>25.0±4.9</td>
<td>26.6±5.8</td>
<td>2.97***</td>
<td>22.3±6.7</td>
<td>22.8±7.0</td>
<td>2.19**</td>
</tr>
<tr>
<td>Screen time (arbitrary units)</td>
<td>3.7±2.7</td>
<td>3.6±2.0</td>
<td>-0.45</td>
<td>3.4±2.2</td>
<td>3.1±2.0</td>
<td>-1.03</td>
<td>2.8±2.1</td>
<td>2.5±2.1</td>
<td>-0.73</td>
<td>3.4±2.4</td>
<td>3.2±2.1</td>
<td>-1.28</td>
</tr>
<tr>
<td>Physical activity (arbitrary units)</td>
<td>8.5±1.6</td>
<td>8.6±1.4</td>
<td>0.53</td>
<td>8.0±1.4</td>
<td>8.0±1.4</td>
<td>0.09</td>
<td>7.5±1.2</td>
<td>7.5±1.6</td>
<td>0.10</td>
<td>8.0±1.5</td>
<td>8.1±1.5</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Muscular fitness</strong></td>
<td></td>
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<tr>
<td>Hand grip strength (Kgf)</td>
<td>25.3±7.9</td>
<td>30.8±9.4</td>
<td>8.25***</td>
<td>21.8±6.1</td>
<td>26.0±6.8</td>
<td>9.23***</td>
<td>19.8±5.2</td>
<td>23.2±4.9</td>
<td>4.96***</td>
<td>22.6±6.9</td>
<td>27.1±8.0</td>
<td>13.09***</td>
</tr>
<tr>
<td>Standing long jump (cm)</td>
<td>162.8±29.5</td>
<td>175.0±30.4</td>
<td>4.80***</td>
<td>146.0±25.5</td>
<td>161.2±32.3</td>
<td>7.71***</td>
<td>137.1±20.5</td>
<td>138.9±19.4</td>
<td>0.75</td>
<td>149.9±27.7</td>
<td>161.6±32.0</td>
<td>8.28***</td>
</tr>
<tr>
<td>Muscular z-score</td>
<td>0.3±1.8</td>
<td>1.4±2.0</td>
<td>8.75***</td>
<td>-0.7±1.4</td>
<td>0.3±1.7</td>
<td>10.24***</td>
<td>-1.3±1.1</td>
<td>-0.8±1.1</td>
<td>3.41***</td>
<td>-0.5±1.6</td>
<td>0.5±1.9</td>
<td>13.28***</td>
</tr>
<tr>
<td><strong>Motor fitness</strong></td>
<td></td>
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<tr>
<td>50 yard dash (s)</td>
<td>8.0±1.1</td>
<td>7.8±1.1</td>
<td>-2.34</td>
<td>8.4±1.1</td>
<td>8.0±1.2</td>
<td>-4.01</td>
<td>8.7±0.6</td>
<td>8.4±0.7</td>
<td>-2.06</td>
<td>8.3±1.1</td>
<td>8.0±1.1</td>
<td>-4.98***</td>
</tr>
<tr>
<td>Shuttle-run (s)</td>
<td>11.5±1.8</td>
<td>10.9±1.2</td>
<td>-2.78</td>
<td>11.6±1.9</td>
<td>11.0±1.3</td>
<td>-3.65</td>
<td>12.5±1.9</td>
<td>11.9±1.1</td>
<td>-1.82</td>
<td>11.7±1.9</td>
<td>11.1±1.3</td>
<td>-4.69***</td>
</tr>
<tr>
<td>Motor z-score</td>
<td>0.1±1.7</td>
<td>0.7±1.6</td>
<td>3.22***</td>
<td>-0.3±2.0</td>
<td>0.4±1.8</td>
<td>5.06</td>
<td>-1.1±1.6</td>
<td>-0.5±1.1</td>
<td>2.05</td>
<td>-0.3±1.9</td>
<td>0.3±1.6</td>
<td>6.07***</td>
</tr>
<tr>
<td><strong>Familial characteristics</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Father occupation (arbitrary units)</td>
<td>5.5±2.8</td>
<td>5.5±2.8</td>
<td>0.00</td>
<td>5.5±2.5</td>
<td>5.5±2.5</td>
<td>1.01</td>
<td>5.7±2.1</td>
<td>5.8±2.2</td>
<td>1.79</td>
<td>5.5±2.5</td>
<td>5.5±2.5</td>
<td>1.98</td>
</tr>
<tr>
<td>Mother occupation (arbitrary units)</td>
<td>6.0±2.3</td>
<td>6.4±2.3</td>
<td>1.41</td>
<td>5.8±2.7</td>
<td>5.9±2.6</td>
<td>0.69</td>
<td>7.2±2.1</td>
<td>7.3±2.1</td>
<td>0.45</td>
<td>6.1±2.5</td>
<td>6.3±2.5</td>
<td>1.54</td>
</tr>
</tbody>
</table>

p<0.05; p<0.01; **p<0.001; *z-test*
Tracking coefficients for individual siblings within their respective sibships are shown in Table 2. Individual sisters within the SS pairs displayed higher tracking values (ICCitrk=0.76; 95%CI: 0.56-0.88) than brothers within the BB pairs (ICCitrk=0.61; 95%CI: 0.43-0.76) or subjects within the BS pairs (0.60; 95%CI: 0.45-0.74) in the muscular component. However, in the motor component a different pattern occurred, i.e., sisters within the SS pairs had the lowest (ICCitrk=0.27 95%CI: 0.08-0.59) followed by those within the BB (ICCitrk=0.40 95%CI: 0.19-0.65) and BS (ICCitrk=0.49 95%CI: 0.32-0.66) pairs.

Table 2. Individual siblings tracking (ICCitrk) coefficients and 95% CI within sibships for the muscular (z-Muscular) and motor (z-Motor) fitness components

<table>
<thead>
<tr>
<th>Tracking coefficients</th>
<th>z-Muscular ICCitrk (95%CI)</th>
<th>z-Motor ICCitrk (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother-brother</td>
<td>0.61 (0.43-0.76)</td>
<td>0.40 (0.19-0.65)</td>
</tr>
<tr>
<td>Sister-sister</td>
<td>0.76 (0.56-0.88)</td>
<td>0.27 (0.08-0.59)</td>
</tr>
<tr>
<td>Brother-sister</td>
<td>0.60 (0.45-0.74)</td>
<td>0.49 (0.32-0.66)</td>
</tr>
</tbody>
</table>

Intra-class correlations (ρ) for siblings’ resemblance are presented in Table 3. Here, SS pairs resembled each other more than BB and BS pairs at baseline and follow-up in both muscular and motor fitness components. Furthermore, SS pairs presented a consistent resemblance in 2-year follow-up in both components, and the same was evident for BS pairs in the motor component. On the other hand, BB pairs resemblance was inconsistent between the baseline and follow-up in both physical fitness components, and the same trend was observed for BS pairs in the muscular component.

Table 3. Intraclass correlation coefficients (ρ), as a measure of resemblance, with 95% CI for each sib-type

<table>
<thead>
<tr>
<th>Intraclass correlation</th>
<th>z-Muscular ρ (95% CI)</th>
<th>z-Motor ρ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.27 (0.00-0.99)</td>
<td>0.48 (0.17-0.80)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>0.64 (0.29-0.89)</td>
<td>0</td>
</tr>
<tr>
<td>SS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.94 (0.76-0.99)</td>
<td>0.83 (0.54-0.96)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>0.92 (0.77-0.98)</td>
<td>0.89 (0.46-0.99)</td>
</tr>
<tr>
<td>BS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.30 (0.00-1.00)</td>
<td>0.03 (0.00-1.00)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>0</td>
<td>0.09 (0.00-0.99)</td>
</tr>
</tbody>
</table>
Finally, the associations between biological, behavioural, family covariates, and the physical fitness components are shown in Table 4. All sibs exhibited similar scores in both components, except for z-Muscular in SS pairs in the follow-up, showing lower values than BB pairs at baseline ($\beta=-0.83\pm0.31$) and for z-Motor in BS pairs in the follow-up displaying higher values than BB pairs at baseline ($\beta=0.58\pm0.29$). Further, more mature subjects tend to have higher scores for both muscular ($\beta=0.87\pm0.04$) and motor ($\beta=0.48\pm0.06$) components. However, siblings with higher %BF tend to have lower scores for both muscular ($\beta=-0.07\pm0.01$) and motor ($\beta=-0.13\pm0.01$) components. Additionally, behaviour (screen time and physical activity) and familial (father and mother occupations) covariates were not associated with either fitness component ($p>0.05$).

Table 4. Parameter estimates and variance components for each physical fitness component across sib-types in time

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>z-Muscular Estimate±SE</th>
<th>z-Motor Estimate±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (BB baseline)</td>
<td>0.13±0.21</td>
<td>0.15±0.34</td>
</tr>
<tr>
<td>BB follow-up</td>
<td>0.21±0.16</td>
<td>0.09±0.27</td>
</tr>
<tr>
<td>SS baseline</td>
<td>-0.52±0.27</td>
<td>-0.18±0.41</td>
</tr>
<tr>
<td>SS follow-up</td>
<td>-0.83±0.31”</td>
<td>0.09±0.39</td>
</tr>
<tr>
<td>BS baseline</td>
<td>-0.20±0.15</td>
<td>0.31±0.29</td>
</tr>
<tr>
<td>BS follow-up</td>
<td>-0.01±0.17</td>
<td>0.58±0.29”</td>
</tr>
<tr>
<td>Twins Baseline</td>
<td>0.01±0.16</td>
<td>-0.18±0.27</td>
</tr>
<tr>
<td>Twins Follow-up</td>
<td>-0.03±0.17</td>
<td>0.16±0.27</td>
</tr>
<tr>
<td>Maturity offset</td>
<td>0.87±0.04”***</td>
<td>0.48±0.06”***</td>
</tr>
<tr>
<td>Body fat</td>
<td>-0.07±0.01”***</td>
<td>-0.13±0.01”***</td>
</tr>
<tr>
<td>Screen time</td>
<td>0.03±0.02</td>
<td>0.02±0.03</td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.05±0.03</td>
<td>0.01±0.05</td>
</tr>
<tr>
<td>Father SES</td>
<td>-0.02±0.03</td>
<td>-0.07±0.04</td>
</tr>
<tr>
<td>Mother SES</td>
<td>-0.02±0.02</td>
<td>-0.02±0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variance components</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Between siblings'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB baseline</td>
<td>0.04±0.09</td>
<td>0.87±0.46</td>
</tr>
<tr>
<td>SS baseline</td>
<td>0.84±0.33</td>
<td>1.30±0.58</td>
</tr>
<tr>
<td>BS baseline</td>
<td>0.03±0.06</td>
<td>0.03±0.23</td>
</tr>
</tbody>
</table>
### DISCUSSION

Altogether, our results partially corroborated our hypotheses. The null model shown in Table 1 substantiated our first hypothesis. On average, there were mean increases in muscular and motor components across sib-ships which corroborate previous findings (Falk et al., 2001; Roth et al., 2018) but using crude analyses (i.e., without covariates). However, these significant differences disappeared between baseline and follow-up, when adjusted for biological, behavioural and familial covariates (see Table 4). Unfortunately, we were not able to identify a study that generated, within the same analysis, unadjusted and adjusted mean changes in physical fitness components, to best understand the putative confounding effects of biological, behavioural and familial effects.Essentially, our results indicate that these confounders explain a substantial fraction of the variability in the outcome (i.e., mean changes in fitness components), or predict a considerable portion of the outcome. Moreover, we strongly believe that if adjustments for confounders produce different results when compared to crude analysis, an adjusted analysis should be used. Such valuable information should be kept in mind in future research to better understand true changes in physical fitness components over time.
Our second hypothesis, i.e., individual tracking during 2 years of follow-up, was moderately high and partially supported by our data. Indeed, tracking for the muscular component was moderately high (0.60≤ICCitrk≤0.76), but tracking for the motor component was low-to-moderate (0.27≤ICCitrk≤0.49). This finding is supported by previous research with unrelated subjects, since a moderately high tracking in adolescents’ muscular component (Da Silva et al., 2013; Fraser et al., 2017; Souza et al., 2016) as well as low-to-moderate in motor component (Da Silva et al., 2013; Souza et al., 2016) have been reported. However, Souza et al. (2016) using a multilevel approach to examine tracking in Portuguese adolescents in two age cohorts (10–12 and 12–14 years) found slightly lower values for the muscular component tests. The authors showed variation in tracking: from 0.48 to 0.57 (handgrip strength) and 0.49 to 0.54 (standing long jump) in girls, while boys stabilized their tracking values in handgrip strength (0.48) for both time points and increased from 0.48 to 0.51 in standing long jump.

In sum, these findings suggest that individual tracking is contingent on the physical fitness component, but that the muscular component tends to track better. Yet, these slight differences in tracking across studies are now known to be associated with methodological and statistical issues (Kowalski & Schneiderman, 1992). Further, they also express, in both components, a substantial degree of differences in movement complexity and motor control as well as physiological demands. In any case these results are valued for at least two reasons: (i) given the associations between physical fitness and health outcomes, a moderately high degree of tracking would suggest early identification and intervention as a strategy to ensure healthy levels of physical fitness across life; (ii) individuals who present development delays in their physical fitness may require a timely intervention to catch-up with satisfactory physical fitness levels.

Our third hypothesis (sibling resemblance is more consistent in same-sex sibs) was partially supported because SS pairs were more consistent in their resemblance in the 2-year follow-up, i.e., they were systematically more similar than BB and BS pairs. These departures from similarity could be partially explained by the well-known sex-differences in physical fitness levels (Malina et al., 2004). In fact, previous studies with unrelated subjects showed that
adolescence is a critical developmental period characterized by dramatic changes between the sexes, in which boys tend to systematically outperform girls in their physical fitness trajectories (Tomkinson et al., 2018). Yet, we still have with us such questions as how consistent genetic and environmental (i.e., shared and non-shared) factors are in explaining variance in muscular and motor fitness components? Do the primary factors that explain sex differences remain constant during adolescence? Unfortunately, there is a dearth of longitudinal studies trying to provide answers for these questions (Isen, McGue, & Iacono, 2014; Peeters et al., 2005). In any case, Isen, McGue, & Iacono, 2014 using twins’ longitudinal data tried to identify whether genetic and/or environmental sources of explained variance in the muscular fitness component were similar in males and females. Males tended to increase their variance, apparently explained by genetic and non-shared factors over time. Further, the variance explained by shared factors tended to disappear over time. In females, variances explained by genetic, shared and non-shared factors tend to stabilize over time. This may probably induce a greater consistency in SS pairs’ resemblance when compared to BB and BS pairs. Yet, further longitudinal research is needed to clarify these issues.

These findings may provide a meaningful body of knowledge to best understand these differences between different types of siblings. Further, results should be considered to target specific groups, when planning and developing intervention programs to improve physical fitness levels.

Finally, our last hypothesis (individual and familial characteristics are significantly associated with siblings’ physical fitness) was not supported by our data, because only biological characteristics were significantly associated with siblings’ physical fitness components, which is an apparently consistent finding (Malina et al., 2004). Biological maturation was positively linked with both components, i.e., more mature siblings tend to be fitter in muscular and motor fitness (Pereira et al., 2017). Indeed, more mature subjects tend to be more proficient in their movement skills and motor control, which are closely associated with the task structure and demands of each physical fitness test (Afonso et al., 2009). We also found a negative effect of %BF with siblings’ physical fitness components which is commonly reported across studies (Moliner-Urdiales et al.,
2011; Riendeau et al., 1958) and suggests that high %BF can negatively affect physical fitness. In sum, these results indicate that future intervention programs to improve physical fitness levels may be more effective if they include strategies to simultaneously improve body fat and physical fitness levels. Alternatively, targeting different groups based on their body fat levels may also be effective.

Behavioural factors (screen time and physical activity) were not significantly associated with physical fitness components in this study. Available reports linking physical fitness with screen time have shown inconsistent results (Smith et al., 2019). For example, we found studies reporting an inverse association between screen time and physical fitness (Edelson, Mathias, Fulgoni, & Karagounis, 2016), while others found no significant associations (Coledam, Ferraiol, & Oliveira, 2018). Moreover, despite an apparent lack of recent research relating physical activity with physical fitness, previous data revealed positive associations between vigorous physical activity and muscular and cardiorespiratory fitness (Martinez-Gomez et al., 2011), although this trend was not verified for moderate-to-vigorous physical activity (Loprinzi, Loenneke, & Hamilton, 2017). However, it is worth mentioning that previous studies did not consider the joint influence of other putative covariates, namely biological and familial, which may attenuate the effects of behavioural characteristics on physical fitness levels. Thus, we believe that such contentious results call for more research to better understand the complex relationships between lifestyle behaviours and physical fitness.

Finally, no significant associations were observed for familial characteristics. Although socioeconomic status (SES) has been identified as an important correlate of health outcomes, its relationship with physical fitness levels are scarcely explored (Freitas et al., 2007; Nevill et al., 2018; Sandercock et al., 2017). Moreover, available data from previous studies are divergent, and depend on how precisely SES was assessed, as well as if adjustments were made for the joint effects of other covariates. For example, Sandercock et al. (2017) in a study with Colombian adolescents (aged 14-16) showed that muscular component tests were positively related with SES. Contrarily, Nevill et al. (2018) using an allometric model, reported that after adjusting for body size, higher SES was
associated with lower handgrip strength and better jumping performance. This calls for more research aiming to scrutinize the influence of different SES markers in physical fitness development changes.

This study is not without limitations. First, the use of indirect methods (questionnaires) to obtain information about behaviour and familial characteristics is prone to errors. Nevertheless, these techniques are commonly used, and previous studies have confirmed their validity and reliability. Also, these questionnaires were applied in controlled conditions. Second, the sample does not include participants from all Portuguese regions, which limits the generalization of the results towards the whole Portuguese population; however, this issue is common in family and twin studies. Third, if twin zygosity was known, variance components as well as intraclass correlation estimates might change. In any case, we tested models with and without twins and they suggest an overall similar trend. Fourth, the use of two out of five fitness components proposed by the Bouchard and Shephard (1994) model does not provide a fully comprehensive picture of sibling resemblance in the broad spectrum of health-related physical fitness. However, we tried to fill a major gap identified in available research, namely in using the muscular and motor components.

In conclusion, despite an apparent improvement in both physical fitness components over two years, this increase vanished when the effects of biological, behavioural and familial characteristics were considered in the analysis. Furthermore, subjects tend to be more stable, i.e., track more, in muscular fitness compared to motor fitness. Sister-sister pairs are more consistent in their resemblance than brother-brother and brother-sister pairs for both physical fitness components. Finally, biological factors were more influential in both physical fitness components than behavioural and familial factors. These results suggest that despite the known influence of genetic factors in regulating individual physical fitness levels, biological factors like body fat and biological maturation exert important influences. We contend that our findings stress the importance of considering individual characteristics when developing physical fitness interventions. In other words, the same intervention for all individuals may not cause similar changes in physical fitness components. Thus, this empirical
evidence should be considered when developing family and school-oriented physical fitness programs. Additionally, there is a need for more longitudinal studies based on larger family-data using a multilevel approach combined with appropriate statistical models.
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CHAPTER V

GENERAL DISCUSSION, LIMITATIONS AND FUTURE RESEARCH AVENUES
GENERAL DISCUSSION

The challenging question - why are individuals from the same family more different than alike? – was the motto of the present thesis and helped shape its approach, methodology, results, and implications. The main aim was to describe and interpret the intricate intertwined relationships between individual, familial and environmental characteristics in a varied set of phenotypes using sib-ships.

The thesis was conceived and supported by two major pillars: Urie Bronfenbrenner’s (1994) bioecological model and the multilevel statistical model (Courgeau, 2003) to accommodate the network of variables underlying human development in a unified body of knowledge. It is assumed that the use of such models and their specific tools will provide an integrated understanding of this multifaceted phenomenon. Moreover, the present thesis relies on a sib-ship design using both cross-sectional and longitudinal information to have a clearer understanding of sibling resemblance. Using the multilevel model, with its statistical elegance, was paramount to investigate the “effects” of individual, familial and environmental characteristics on a set of phenotypes, in addition to controlling for the non-independence of the sibling data.

Given the complexity of the topic and the array of phenotypes investigated - body shape and composition, physical fitness, metabolic syndrome, and health behaviours – a set of specific aims were fabricated for cross-sectional and longitudinal data. The present chapter yields responses for each aim as well as a general reading of the key findings. Further, the limitations of the thesis are also presented as well as putative suggestions for future research.

Research based on siblings cross-sectional data

Aim 1: to investigate sibling resemblance in body shape, physical fitness, physical activity and metabolic syndrome adjusted for individual, familial and environmental characteristics.

A systematical wrestling with this aim prompted us to write four papers. A summary of the main findings is below (Table 1).
Table 1. Summary of the main findings addressing aim 1

**Paper I – Multilevel modelling of somatotype components: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health.**

- Somatotype familiality, as a gestalt, is of moderate effect size;
- Same-sex siblings had stronger resemblance than opposite-sex siblings for all somatotype components;
- Age was associated with all components, although with different directions depending on the component;
- Physical activity was positively associated with mesomorphy.

**Paper II – Resemblance in physical activity levels: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health.**

- Sister-sister pairs had higher resemblance in physical activity than brother-brother and brother-sister pairs;
- Age and physical fitness were associated with total physical activity although with different directions;
- Parental support for physical activity was positively associated with total physical activity;
- Behavioural and environmental characteristics were not associated with total physical activity levels.

**Paper III – A multilevel analysis of health-related physical fitness. The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health.**

- Same-sex sibling pairs had higher resemblance than opposite-sex sibling pairs for all physical fitness tests (except brother-brother in the 1-mile run/walk);
- Age and biological maturation were positively associated with all fitness tests (except for age in the shuttle-run and biological maturation in the 1-mile run/walk);
- Physical activity and sedentary behaviour do not play strong roles in predicting physical fitness;
- Higher socioeconomic status was negatively associated with the standing long jump and the shuttle-run.

**Paper IV – Sibling similarity in metabolic syndrome: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health**
General Discussion, limitations and future research avenues

- Same-sex siblings had higher resemblance than opposite-sex siblings in their metabolic syndrome markers;
- Age, biological maturation and BMI were positively associated with metabolic syndrome markers;
- Fruits and vegetables were negatively associated with higher systolic blood pressure;
- Physical activity positively associated with HDL-cholesterol;
- Muscular fitness was negatively linked with the metabolic syndrome score, systolic blood pressure and glucose;
- Both muscular and cardiorespiratory fitness were positively associated with waist circumference.

Sibling resemblance and the links of shared factors have long fascinated social (Hauser & Wong, 1989; Mazefsky et al., 2008; Plomin et al., 2001; Yeh et al., 2016) and biological research (Berge et al., 2016; Carlier et al., 1994; Draisma et al., 2008). Altogether, the findings of the studies that composed this thesis allowed the inference that despite the complex structure of the family, their members presented, in general, a moderate significant degree of similarity in their somatotype, physical fitness, physical activity and metabolic syndrome. This body of research suggests that these traits run in families.

However, it is also important to scrutinize whether the expression of such similarity is due to nature (genetics) or nurture (environment), or if it results from the combined effects of both nature and nurture. Previous GWAS studies provided significant evidence that variation across populations in physical and biological characteristics is associated with polymorphisms in several genes (Mills & Rahal, 2019; Visscher et al., 2017). For example, in stature, there are thousands of genetic variants distributed across the genome, which contribute in different magnitudes to an individual's height (Guo et al., 2018). Furthermore, fat mass and obesity associated gene (FTO) has been repeatedly replicated and is affecting obesity by regulating appetite (Church et al., 2010). Additionally, a recent review (Kalantari et al., 2016) indicated an interaction between FTO and lifestyle behaviours, or, in other words, an interaction between nature (FTO
gene) and nurture (lifestyle behaviours). Research on this topic is vast and has provided clear evidence that allows for a better understand of why siblings are similar. Definitely, the main challenge here is to understand the differences that shape siblings. Siblings share, on average, 50% of their genes identical-by-descent, which means that these also makes them 50% different. However, they also share a common family environment, which can contribute for their similarity. For example, parental support for physical activity is a common environmental characteristic that may influence siblings’ similarity (Liu et al., 2017; Salas et al., 2018). In this sense, associations between parental support and sibling physical activity may reflect the key role of parents as social models in shaping youth behaviour. Moreover, the familial characteristics that siblings share such as socioeconomic status, education level, among others, may also impact the degree of their similarity (Arias et al., 2018; Dickson et al., 2016; Escarce, 2003). Nonetheless, the presence of non-shared factors such as age and sex, as well as their lifestyle behavioural choices comprise strong candidate variables that can also influence their dissimilarity. Throughout the present thesis, when sequential models of increasing complexity were tested, i.e., adjusted for individual, familial and environmental covariates, changes in sibling resemblance were observed, in addition to different variations depending on the sib-type. Some characteristics tended to maintain their resemblance; however, others increased or decreased their intraclass correlation value, revealing a fluctuation in the impact of genetic, individual, familial and environmental characteristics on the expression of these phenotypes.

It is important to note that at the same time that individuals grow, mature and develop, changes are also occurring in their communities, as well as their social networks (e.g., families and personal relationships (Cairns & Cairns, 2005). The main challenge here is to capture the integrated levels that shape human development as well as to understand their underlying mechanisms, i.e., the interdependence of characteristics within and without the individual. Put differently, how reciprocal are interactions between individuals and their environments that condition their differences.
Although this line of thought goes back to the 1960s (Anastasi, 1958), researchers at that time did not have at their disposal the analytical tools that are available today. The multilevel model used in this thesis provided a degree of flexibility in the analysis of family data that has not been previously implemented. More specifically, with this elegant line of thinking and multilevel tool, the study of the phenomena was done considering the non-independent or clustered data, that is, children nested in families as well as the addition of a set of predictors from individual, familial and environmental levels. The data derived from these four studies also allowed an in-depth examination of the individual, familial and environmental correlates of body shape, physical activity, physical fitness and metabolic syndrome. Despite previous evidence concerning the influence of behavioural and environmental determinants on the expression of these phenotypes (Artero et al., 2011; Baxter et al., 2006; Bohr et al., 2013; Ekelund et al., 2001; Longkumer, 2014; Stabelini Neto et al., 2014), the results obtained in the present thesis shed light on a key take-home message: it is the biological characteristics, namely age, biological maturation and physical fitness components that are crosscut to all phenotypes used in this thesis. Importantly, these factors make children and youth, growing up in the same family, different from one another. Behavioural, familial and environmental characteristics exerted dissimilar influences on them. These findings suggest that these factors are unsystematic, idiosyncratic (i.e., peculiar or individual) and random but also important in the expression of some phenotypes (Plomin, 2011). That is, while some factors may exert effects on one characteristic, they can also be unrelated to others. However, when the focus is directed to human development as a whole, there is a need to consider all these factors together, even though they do not affect all phenotypes to promote healthy development trajectories.

Furthermore, having the theoretical models proposed by Urie Bronfenbrenner as a general template these results can therefore be interpreted in light of the Process-Person-Context-Time (PPCT) model. Thus, the variation in somatotype, physical activity levels, physical fitness components and indicators of metabolic syndrome (Process) are influenced by individual characteristics (Person), namely: chronological age, biological maturation,
physical fitness and health behaviours. Simultaneously, such characteristics interact with the features of immediate environments (Context – Micro-level) i.e., parental support for physical activity and socioeconomic status. Indeed, an examination of siblings’ differences based on this model allowed for an assessment of the influence of individual, familial and environmental characteristics through their influence on proximal processes (variability in somatotype, physical activity levels, physical fitness components and indicators of metabolic syndrome). Such a robust approach is needed to best understand these phenomena. Put in these terms, such findings are easier to comprehend since they provide valuable information not only for siblings and for their families, but also to better understand what makes all youth different.

Undoubtedly, human development consists of a dynamic process, characterized by a network of multiple factors. Consequently, it requires adequate explicative models and congruent tools that allow looking at the process as a whole. This calls for examining the combined influence of individual, familial and environmental characteristics, instead of inspecting the effect of each characteristic separately.

Results from the present thesis claim the attention of agents to consider the complexity and variability inherent to human development, particularly the determinants that exert significant changes in children and youth health trajectories.

Aim 2: To examine the joint effects of familial and environmental characteristics in multivariate profiles of health-related markers.

To answer this aim a paper entitled “Profile resemblance in Health-Related Markers: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health” was written. The main findings are shown in Table 2.
Table 2. Summary of the main findings addressing aim 2

Paper V – Profile resemblance in health-related markers: The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health

- A moderate sibling resemblance in profiles was found;
- Two distinct multivariate profiles emerged and the differences between them are related to body fat, physical fitness and unhealthy diet;
- The majority of the sample (86.7%) was located in profile 1: “fit, lower fat and poorer diet”;
- Only father’s education and occupation were significant predictors of profile membership.

In this paper we intended to shift the paradigm i.e., not to look at each phenotype in isolation, but viewing youth from a broader perspective based on their health-related markers and using a person-centered approach, namely latent profile analysis. This allowed for the identification of profiles (i.e., groups) of subjects in a sample that show similar patterns of variables. At the same time, and according to the hierarchical structure of the data, the logistic multilevel model was used to investigate the putative links between these profiles with biological, sociodemographic, and environmental characteristics along with the estimation of sibling resemblance in these profiles.

The main findings of this study showed that the moderate sibling resemblance increased when the model was adjusted for biological, behavioural and environmental characteristics, indicating a putative effect of genetic and shared factors. Moreover, two profiles emerged and the differences between them were related to body fat, physical fitness and unhealthy diet. Interestingly, physical activity, screen time and healthy diet did not seem to distinguish subjects. Altogether, these findings point to a novel view of human individuality and development with a wide range of implications for individuals and society. The key question here was: knowing that subjects are genetically different, if we control for familial and environmental differences does youth development become the same? The answer is “no” because even if subjects are provided with similar environments, their genetic differences would remain the same and
consequently youth will be different in their development. The results of the present thesis shed light onto the importance of creating specific groups based on genetic characteristics so that individuals are provided with equal opportunities. By equal opportunities is meant that, despite their differences, there is a need to create suitable conditions to foster and develop healthy trajectories.

Notwithstanding the importance of these results to create specific groups of youth when intervention programs are to be planned for improving health, the main question was: which were the biological, familial and environmental characteristics that best predict profile membership? According to the results, it was sociodemographic characteristics, namely father’s education and occupation that seemed to predict belonging to the profile. However, it is important to note that the two indicators presented inverse signs: while father’s occupation influenced positively the better profile, the father’s education displayed a negative effect. Nevertheless, if genetics provide most of the systematic variance and if environmental effects are unsystematic and unstable this implies that parents probably influence more on youth outcomes at conception than throughout their life. One possible explanation for these outcomes may be related to the nature of nurture rather than nurture given that they are 50 percent similar genetically. Furthermore, youth make their own environments, regardless of their parents, particularly within schools, friends, etc., and all of these factors may interact with their genetic propensities. So, most of what happens to youth is beyond their genetic heritage and may involve a set of random experiences over which parents have no control (Plomin, 2018).

Unfortunately, the present thesis did not simultaneously use both top-down and bottom-up approaches to better understand these mechanisms in a longitudinal perspective.

These results can also be interpreted through the lens of the bioecological model as they emphasize the importance of both individual [biological-body fat and physical fitness), behavioural characteristics (unhealthy diet)] and contextual systems [sociodemographic characteristics (father’s occupation and education)] and the interdependent relations between these two systems in order
to promote healthy development. In other words, the results of the cross-sectional studies suggest how the interplay of individual, family and environmental characteristics affect children and adolescent’s health, therefore, they need to be considered in funding decisions and promotion of policies to improve youth health trajectories.

**Research conducted based on longitudinal data:**

Aim 3: To analyze the tracking of individuals within their sib-ship in obesity markers and physical fitness components during 2 years of follow-up;

Aim 4: To probe the consistency in sibling resemblance in these markers and components;

Aim 5: To analyze the combined influence of individual and familial characteristics on trajectories of these phenotypes.

To help address these aims two studies were conducted. The main findings are presented in Table 3.

Table 3. Summary of the main findings of the studies addressing aims 3-5

**Paper VI – Change and stability in sibling resemblance in obesity markers. The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health**

- Obesity markers tend to track across time within sib-ships, whereas sibling resemblance tends to be consistent across time;
- Biological maturation was positively associated with all obesity markers;
- Screen time negatively impacted body fat percentage;
- Unhealthy diet was associated with body fat percentage and BMI;
- Mother’s occupation only influenced BMI.

**Paper VII - Change and stability in sibling resemblance in physical fitness. The Portuguese Sibling Study on Growth, Fitness, Lifestyle and Health**

- Individual tracking was more pronounced for muscular fitness than motor fitness;
- Sister-sister pairs were more consistent than brother-brother and brother-sister pairs in both physical fitness components;
- Biological maturation and body fat percentage positively influenced both physical fitness components;
- Behavioural and familial characteristics do not seem to influence muscular and motor fitness trajectories.

Longitudinal data comprises a collection of repeated observations of the same subjects, taken from a larger population, over “prolonged” periods of time – more often years or decades (Smith, 2012). Such a research design allows for an understanding of the degree and direction of change over time in a specific characteristic. The Portuguese Sibling Study that followed siblings over three consecutive years, aimed to infer a set of conclusions about their stability and change across time in obesity markers and physical fitness components.

One of the first important findings was that obesity markers were more predictable than physical fitness components especially in the motor component, with tracking coefficients ranging from low to moderate. Once again, a possible explanation may be related to the higher genetic effect in obesity markers and how they track across time. This evidence was mirrored in a recent meta-analysis of GWAS studies (Bradfield et al., 2019) that demonstrated a large overlap of nominally significant SNPs (Single Nucleotide Polymorphisms) found in both meta-analysis studies of pediatric obesity and adult BMI points. Furthermore, another important issue to consider relates to the gene-environment interaction to modulate the expression of obesity markers across time. However, previous studies using longitudinal data and gene-environment interaction analysis did not yield robust conclusions (Wang et al., 2019). It seems that, at this moment, most evidence of genetic effects are clearer than environmental effects. Such valuable information can be used by agents to control obesity markers in children and adolescents, as well as to improve physical fitness levels. Here, a special focus should be given to the different trajectories of these phenotypes across time in order to define accurate windows of opportunity for successful intervention for the change or maintenance of these health-related indicators. Moreover, the same trend was observed in sibling resemblance, which might be key to promote healthy family’s trajectories. These
results suggest that shared factors in obesity markers between siblings maintain across time, however, the same trend was not verified for the physical fitness components. Possibly, due to the complexity of the mechanisms underlying the expression of these components, there can be other key factors that interact with shared factors, hence inducing such variations in siblings’ resemblance over time.

Finally, following the same line of thought as Urie Bronfenbrenner, in this phase of the thesis the aim was to consider the patterning of environmental transitions and/or changes across time (chronosystem) acting on the person but also on the environment, to investigate their impact in obesity markers and physical fitness trajectories. Once again, the results allowed to best illuminate the influence of biological maturation, screen time and diet (Person), as well as mother’s occupation (Context) on the trajectories of obesity markers (Process). On the other hand, the results also suggest that physical fitness components trajectories (Process) are influenced by the biological maturation and body fat (Person).

This body of results provide important guidance on social, economic and health policy decisions to make a healthy society and to maintain these good choices across the life course. More specifically, family and school-oriented programs for controlling these markers need to consider this empirical evidence to develop more assertive programs. There is no doubt that the cornerstone for lifelong good health is laid down in childhood. In this period of life, children spend most of their time at schools. In fact, school comprises a key social context where children and adolescents spend approximately one-third of their waking time, in which several key behaviours that can systematically developed (Cunningham & Zavodny, 2011). Therefore, schools have a rich opportunity to improve health by adopting strategies (e.g., engagement in a wide variety of activities) considering individual differences within students and also provide suitable places where different children can grow and develop.
LIMITATIONS

The empirical results reported in this thesis should be considered in the light of potential limitations. These are as follows: (1) the participants were not recruited from all Portuguese regions which limits the generalization of findings to the whole Portuguese population; (2) the use of questionnaires to collect information concerning behavioural and familial characteristics may be susceptible to errors; (3) SES was acquired based on the Portuguese National Classification of Occupations (2010), where only the nine major categories were considered; (4) the indirect estimation of maturity has its limitations and can be prone to errors; (5) the 1-mile run/walk test only provided an indirect estimation of cardiorespiratory fitness; (6) the inclusion of a 3-level model, i.e., sib-ships nested within their schools, was not considered; (7) no adjustments were made for putative indicators of shared sibling environments, which may have limited further understanding concerning their importance; (8) only two years of follow-up were included; possibly the inclusion of more than three points in time may provide valuable and rich information about development trajectories.
FUTURE RESEARCH AVENUES

The bottom line of science is replication, which means that studies need to be replicated in order to afford reliable knowledge. This thesis provided meaningful empirical evidence regarding the intricate intertwined relationship between individual, familial and environmental characteristics in a varied set of phenotypes using sib-ships. Despite the relevance of its findings, some issues still deserve attention for future research:

1. Do genetic or environmental factors have a greater influence on human development?
2. Do inherited traits or life experiences play a higher role in shaping individuals?

Seeking answers for these questions requires a combination of top-down and bottom-up approaches. Moreover, it might worth trying to expand the analysis to family pedigrees to better capture the familial resemblance, as well as different degrees of relationship in phenotypes involved in human development, but also the examination of shared and non-shared factors. Thus, the following questions could be asked:

3. Which factors most influence health-related markers in all levels of information (i.e., microsystem, mesosystem, exosystem and macrosystem)?
4. Do these levels of information interact among themselves? If so, what is their effect size?
5. Are there mediating factors that impact in the magnitude of individual, familial and environmental influences in human development?

The use of 4-levels of information: individuals nested within families nested within schools, nested within countries to make cross-cultural comparisons may be helpful to answer the following issues:

6. Do factors associated with variability in human development change across time?
7. Do the characteristics associated with health-related markers stabilize across time?

It will be imperative to utilize longitudinal studies of large, representative samples of siblings, ideally covering multiple developmental periods (i.e., childhood to adulthood), in order to adequately address the issues of stability and change in health-related markers.
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