AUGUSTO CÉSAR FERREIRA DE MORAES

High blood pressure and clustering of risk factors in adolescents: a multicenter study

Tese apresentada para dupla titulação à:

Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Medicina Preventiva

Orientador: Prof. Dr. Heráclito Barbosa de Carvalho

Universidad de Zaragoza

Departamento de Fisiatría y Enfermería

Orientador: Prof. Dr. Luis Alberto Moreno Aznar

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DEDICATION

Dedico esta minha tese a minha Leoa **Mariana Ianello Giassetti "de Moraes"** mostrando-me o valor humano, moral da vida. Sobre tudo enxugando minhas lágrimas nos momentos difíceis e aplaudindo minhas pequenas grandes conquistas nessa trajetória, que foi de suma importância para eu me tornar DOUTOR.

"...It's times like these you learn to live again
It's times like these you give and give again
It's times like these you learn to love again
It's times like these time and time again."

ACKNOWLEDGEMENTS

Para que fosse possível eu realizar mais esta conquista pessoal, algumas pessoas foram e são primordiais em minha vida e principalmente nessa trajetória.

Primeiramente, além de dedicar quero fazer um agradecimento a minha rainha, um exemplo de vida, profissional e dedicação a sua família e amigos, a professora **Romilda Marcondes de Moraes, MINHA MÃE,** obrigado por sempre estar do meu lado me ajudando, dando conselhos em todos os quesitos. Que mesmo com todas as dificuldades que a vida lhe impôs, e ainda impões, sempre está sorrindo e pronta para ajudar ao próximo, você é meu maior exemplo **MINHA MÃE!!!**

Aos meus irmãos: **Nelson, Vinicius e Fernanda** que indiretamente me incentivaram a terminar minha pós-graduação, obrigado por vocês existirem.

Ao Professor Dr. **Heráclito Barbosa de Carvalho**, que abriu as portas para mim da pós-graduação na Medicina Preventiva da FMUSP, após muitos não acreditarem no meu trabalho e competência e me orientou neste trabalho, e mais que acreditou no meu trabalho, confiou em mim em todas as empreitadas que propôs, e soube como um verdadeiro maestro me orientar neste caminho., **Tatico Valeu!!!**

Agradeço ao Professor Dr. **Luis Alberto Moreno Aznar** (sei que ele não prefere que seja destacado como professor), que desde o primeiro email (em fev/2011) foi muito solicito e abriu as possibilidades de trabalhar com o estudo HELENA, abriu as portas do GENUD e da UNIZAR, para eu realizar um dos meus sonhos desde "niño" quer era morar fora do Brasil, por um tempo. Que foi um perfeito guia turístico e histórico de Zaragoza, que por força da teoria do caos é *La Ciudad del Imperador Augustus Caesar*, que inclusive me acompanhou nas compras de roupas para o forte inverno europeu quando cheguei em Zaragoza, **Luis muito Obrigado!**

Aos meus grandes amigos de pós-graduação: **Alex Cassenote, Gabriel Andreuccetti, Francisco Leonardo e Arnaldo Souza**, pelo companheirismo, pelas conversas, pelas viagens em congressos e turismo e pelas festas que fizeram parte da minha pós-graduação e que com certeza vou lembrar para o resto da vida. **Caipira**, nossa trip pela Califórnia jamais esqueceremos muleque, e valeu pelos empréstimos de $$$ quando tive os problemas com a bolsa! **Carterinha**, nós em Londres lá no parque olímpico foi F...., até o Usain saiu pra ver nós, hehehehehehhe! **Barack,** tamo junto moleque, aquela viajem pro delta com os Capitães e a trip pra San Antonio são inesquecíveis "Valeu Parcero!!!; Oh my God, Barack Obama!!!" hehehehe. **Piranha, meu artilheiro,** sensacional a visita em Bruxelas mano, o delirium não vai mais ser o mesmo depois que passamos por lá!

Agradeço a **Andréia Tenório** por me auxiliar com os papéis do certificado médico para o visto da Espanha, a **Talita** funcionária da CRInt da FMUSP, que auxiliou muito, escutou minhas queixas durante o processo de assinatura do convênio dupla titualação. Um agradecimento especial ao Prof. **José Otávio Auler** que durante seu mandato como Diretor da FMUSP, apoiou as atividades de internacionalização que desenvolvemos no meu doutorado, obrigado professor.

Aos meus amigos do grupo GENUD, **Pilar La Jefa**, GRACIAS por ter me ajudado desde sempre com "mios papeleos" e principalmente por saber um bom cardápio para os jantares do grupo. A **Las Viejas Glórias** do **GENUD (Silvia, Iris, Esther, Pilarita e Alba)**, me desculpe pelos sustos (hehehehehehh), foi espetacular cada momento que vivi com vocês (discussões científicas, auxilio no entendimento do idioma espanhol, visitas aos pueblos, viagens a Madrid e a Granada, etc) sempre terei uma lembrança muito carinhoso de vocês. **Doctores, Juan e Luis Gracia,** vocês também são inesquecíveis; **Tigre** valeu por ter me "salvo" em Madrid quando tive

problemas com meu cartão; y **Dr. Penguin** obrigado pelos ensinamentos de estatísticas e pelas gravações dos vídeos que são espetaculares! Obrigado **Angel, Alex y Alba** pelos grandes momentos em Biel e nos jantares do grupo; e **Alex e Angel** pelas discussões sobre futebol. Professores **German, Casajús e Gerardo**, as discussões com vocês sobre qualquer assunto sempre foram proveitosas e por ter aceito o convite em participar de minha banca, obrigado. A **Las Novatas** (**Tatiana/Mary/Sara**) que disfrutem desta jornada na Pós-Graduação, e obedeçam **Las Viejas Glórias.**

Aos novos membros do grupo YCARE (**Tara e Marcus**) bom ter convivido com vocês nessa fase final do meu doutorado, e sabem que continuaremos juntos nos novos projetos.

Leoa, o que tinha pra te dizer está descrito na dedicatória dessa tese, ah esqueci **TE AMO!!!!**

EPÍGRAPHS

Filho: – Então, está nervoso com a luta?

Pai: – Morrendo de medo.

Filho: – Não parece.

Pai: – Bem, não deveria mesmo.

Filho: – Então, não precisa fazer isso.

Pai: – Acho que preciso.

Filho: –Sabe, conviver com você não tem sido fácil. As pessoas me veem, mas pensam em você. E agora com tudo isso acontecendo, ficará pior ainda.

Pai: – Mas, não precisa ficar.

Filho: – Claro que precisa!

Pai: – Por quê? Você conseguiu muitas coisas, filho.

Filho: – O que? Meu sobrenome?!

Foi por isso que consegui um trabalho decente, é por isso que as pessoas me dão atenção. E agora que comecei a progredir...comecei a conseguir as coisas por mim mesmo, e isso acontece.

Agora, peço um favor, que não continue com isso. Vai acabar mal para você e vai acabar mal para mim.

Pai: – Acha que estou te prejudicando?

Filho: – Sim! De certo modo, sim!

Pai: – Essa é a última coisa que eu queria fazer.

Filho: – Eu sei disso, mas é o que está acontecendo!

– Não se importa com o que as pessoas pensam?

–Não te importa as pessoas falando de você como uma piada, e eu serei incluso nisso?

– Acha isso certo? Acha?!

Pai: – Você não vai acreditar nisso, mas você costumava caber aqui (apontando para palma da mão). Eu te levantava e dizia para sua mãe:

'Esse menino será o melhor de todo mundo. Esse menino será o melhor que qualquer pessoa já conheceu!'

– E você cresceu bem e admirável. Foi bom só observar, todos os dias eram como um privilégio.

– Então quando chegou a sua hora de ser um homem e tomar seu lugar no mundo...e foi o que você fez. Mas com o passar dos tempos você mudou.

– Deixou de ser você. Deixou as pessoas apontarem o dedo para sua cara e dizerem que você não é bom. E quando as coisas ficam difíceis, você começa a procurar alguém para culpar, como uma grande sombra.

– *Deixe-me te dizer uma coisa, que você já deve saber.*

– *O mundo não é um mar de rosas; é um lugar sujo, um lugar cruel, que não quer saber o quanto você é durão. Vai te colocar de joelhos e você vai ficar de joelhos para sempre se você deixar.* ***Você, eu, ninguém vai bater tão forte como a vida, mas não se trata de bater forte. Se trata de quanto você aguenta apanhar e seguir em frente, o quanto você é capaz de aguentar e continuar tentando. É assim que se consegue vencer.***

Agora se você sabe do teu valor, então vá atrás do que você merece, mas tem que estar preparado para apanhar. E nada de apontar dedos, dizer que você não consegue por causa dele ou dela, ou de quem quer que seja. Só covardes fazem isso e você não é covarde, você é melhor que isso.

– Sempre te amarei, não importa o que, não importa o que aconteça.

– Você é meu filho, é meu sangue. É a melhor coisa em minha vida.

– Mas até que comece a acreditar em si mesmo não terá vida!

– Não esqueça de visitar sua mãe! (a mãe morreu de câncer.)

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ABSTRACT

de Moraes ACF. **High blood pressure and clustering of risk factors in adolescents: a multicenter study.** [Thesis]. "São Paulo: Faculdade de Medicina, Universidade de São Paulo"; 2014.

Objective: To evaluate the relationship between blood pressure and socioeconomic, behavioral and blood biomarkers in adolescents from two cross-sectional studies.

Methods: The participants were chosen from two cross-sectional studies, one developed in Europe (n = 3308, HELENA Study) and another in Brazil (n = 991, BRACAH Study); the participants were selected via complex sampling. Systolic blood pressure (SBP) and diastolic (DBP) blood pressure (outcomes) were evaluated. Family socioeconomic variables (socioeconomic status of the family, education levels of the parents), lifestyle (physical activity, sedentary behavior, hours of sleep, feeding behavior), and blood biomarkers (iron, vitamins and genes, only the HELENA study) were evaluated. Additionally, the incidence of high blood pressure (HBP) in children and adolescents from the European IDEFICS cohort (n = 5061) was determined. Relationships were evaluated using multilevel linear regression.

Results: The prevalence and incidence of HBP in adolescents is high. The performance of ≥ 60 min of physical activity per day attenuates the effects of sedentary behavior on the blood pressures of adolescents from both studies and also minimizes the deleterious effects of genes on SBP in European adolescents. Protein intake (plant and animal) is negatively associated with DBP in European boys. Blood levels of folate and vitamin B6 are positively associated with blood pressure. Socioeconomic factors are inversely associated with blood pressure in European girls.

Conclusions: The prevalence and incidence of HBP is high in this age group. Control of blood pressure levels in adolescents is needed and may be achieved through the performance of ≥ 60 min of physical activity per day and through adequate protein intake.

Descriptors: Blood pressure; Lifestyle; Biomarkers; Risk factors; Adolescent; Multicenter study

RESUMO

de Moraes ACF. **Pressão arterial elevada e agregação de fatores de risco em adolescentes: um estudo multicêntrico.** [Tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2014.

Objetivo: Avaliar a associação dos níveis de pressão arterial sistólica e diastólica com os fatores socioeconômicos, comportamentais e biomarcadores sanguíneos em adolescentes de dois estudos transversais.

Métodos: Os participantes são provenientes de dois estudos transversais, um desenvolvido na Europa (n= 3.308, HELENA Study), e outro no Brasil (n= 991, BRACAH Study), selecionados por amostragem complexa. Foram avaliados a pressão arterial sistólica (SBP) e diastólica (DBP) (desfechos); variáveis socioeconômicas da família (nível socioeconômico da família, nível de escolaridade dos pais); variáveis do estilo de vida (atividade física, comportamento sedentário, horas de sono, comportamento alimentar); e biomarcadores sanguíneos (ferro, vitaminas e genes; apenas no estudo HELENA). Adicionalmente foi avaliada a incidência de pressão arterial alta (PAE) em crianças/adolescentes na coorte europeia IDEFICS (n= 5.061). As associações foram verificados por regressão linear multinivel.

Resultados: A prevalência e incidência de PAE é alta em adolescentes é alta; realizar ≥ 60 min/d de atividade física atenua o efeito do comportamento sedentário na pressão arterial em adolescentes de ambos os estudos, e também atenua o efeito deletério dos genes na SBP em adolescentes europeus. O consumo de proteínas (vegetais e animais) está associado negativamente com a DBP nos meninos europeus. Níveis sanguíneos de folato e vitamina B6 estão positivamente associados com a pressão arterial. E os fatores socioeconômicos estão inversamente associado apenas nas meninas europeias.

Conclusões: A prevalência e a incidência de PAE é alta nesta faixa etária. E o controle dos níveis pressóricos se faz necessário em adolescentes e pode ser realido por meio da prática de realizar $\geq$ 60 min/d de atividade física e o consumo adequado de proteínas.

Descritores: Pressão arterial; Estilo de vida; Biomarcadores; Fatores de risco; Adolescente; Estudo multicêntrico

RESUMÉN

de Moraes ACF. **Presión arterial elevada y agrupación de factores de riesgo en adolescentes: un estudio multicéntrico.** [Tesis]. "São Paulo: Facultad de Medicina, Universidad de São Paulo"; 2014.

Objetivo: Evaluar la asociación entre la presión arterial con los factores: socioeconómicos, estilo de vida y biomarcadores de la sangre en adolescentes de dos estudios transversales.

Métodos: Los participantes provienen de dos estudios transversales, uno desarrollado en Europa (n = 3308, Estudio HELENA), y otro en Brasil (n = 991, Estudio BRACAH), y fueron seleccionados por muestreo complejo. Se evaluó la presión arterial sistólica (PAS) y diastólica (PAD) (variable dependiente); variables socioeconómicas de la familia (nivel socioeconómico de la familia, nivel educativo de los padres); variables de estilo de vida (actividad física, comportamiento sedentario, horas de sueñohábitos dietéticos); y los biomarcadores de sangre (hierro, vitaminas y genes, sólo en el estudio HELENA). Además, analisamos la incidencia de la presión arterial elevada (PAE) en niños/adolescentes de la cohorte europea IDEFICS (n= 5061). Las asociaciones se analisaron mediante regresión lineal multinível.

Resultados: La prevalencia e incidencia de la PAE en los adolescentes es alta; realizar ≥ 60 min/día de actividad física atenúa el efecto del comportamiento sedentaria en la presión arterial en adolescentes de ambos estudios, viéndose atenuado el efecto de los genes sobre la PAS en los adolescentes europeos. La ingesta de proteínas de origen vegetal y animal se asocia negativamente con la PAD en los niños europeos. Los niveles sanguíneos de folato y vitamina B6 se asocian positivamente con la presión arterial.

Finalmente, los factores socioeconómicos se asocian inversamente sólo con la presión arterial en niñas europeas.

Conclusiones: La prevalencia y la incidencia de las PAE son elevadas en este grupo de edad. Parece necesario controlar de los niveles de presión arterial en adolescentes mediante la práctica de actividad física $\geq$ 60 min/día y la ingesta adecuada de proteínas.

Descriptores: Presión arterial; Estilos de vida; Biomarcadores; Factores de riesgo; Adolescente; Estudio multicéntricos.

PREFACE: REGULATIONS OF THE POSTGRADUATE COMMITTEE

Included below are the rules for the submission of dissertations and theses in the form of a compilation of articles, regulated by the Commission of Post-Graduate (CPG) at the School of Medicine, University of São Paulo (FMUSP).

1) At the discretion of the Program Coordinating Committee, dissertations and theses may be accepted based on Article/Paper compilations;

2) The master dissertation should contain at least one paper accepted for publication;

3) The doctoral thesis shall contain at least two articles accepted for publication;

4) The date of article submission must occur after the student's registration in the program;

5) The paper that is accepted or published should contain the stated purpose of the research project approved by the Ethics Committee on Research Involving Human Participants of the FMUSP and sent to CPG;

6) The student must be first author of the article;

7) The supervisor must be a co-author of the article;

8) The indexing of the journal that accepted or published the article should follow specific program rules concerning procedures for filing dissertations and theses.

Presentation:

Theses and dissertations should include the following mandatory items:

1) Delivery of a bound volume containing the research project approved by the Ethics Committee on Research Involving Human Participants of the FMUSP, with the presentation and critical analysis written in Portuguese or English;

2) Insert the manuscript that is accepted or published;

3) The contributions of the paper are analyzed, discussed and summarized.

1. RESEARCH PROJECT

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of premature death among adults [1] worldwide according to the Pan American Health Organization [2] and is considered a public health problem. Arterial hypertension (AH) alone is an important risk factor for the development of cardiovascular disease and increases its mortality and morbidity. This is particularly worrisome given the increasing number of hypertensive subjects [3,4]. An important risk factor for the development of CVD is high blood pressure [5].

High blood pressure (HBP) originates from the presence and aggregation of risk factors inherent to the individual (behavioral and biological) or the community in which the individual lives (socioeconomic, environmental, cultural and urbanization) [6-9]. Adolescents and children with high blood pressure have higher likelihoods of becoming hypertensive adults and may develop cardiovascular diseases such as atherosclerosis [10] and metabolic syndrome at earlier ages [11,12].

1.1 REASONS

In a review that highlights behaviors that entail significant health risk and are associated with CVD, Brenner et al. [13] cited physical inactivity, poor dietary habits, tobacco and alcoholic beverages. Epidemiological studies have shown that environmental factors profoundly influence the behavior patterns of adolescents [6].

Therefore, strategies designed to prevent the development of hypertension must be promoted in schools to reach as many children and adolescents as possible because these diseases appear to originate during this period [14 15]. Therefore, the

quantification and analysis of the contributions of socio-demographic variables to each risk factor associated with PAE is essential for the development of an intervention strategy, as well as a means of evaluating the effectiveness of said intervention. However, there are few studies in the literature that estimate the prevalence of high blood pressure in adolescents from different countries and levels of development.

Therefore, it is necessary to estimate the prevalence of HBP and identify possible risk factors for the disease, which may improve clinicians' knowledge of how the disease works and enable health care professionals and government officials to devise improved public health policies.

Therefore, this study aims to estimate the prevalence of high blood pressure in adolescents between 13 and 18 years of age from ten countries and to understand the clustering of risk factors associated with this outcome.

1.2 OBJECTIVES

1.2.1 General Objective

To estimate the prevalence of high blood pressure and its determinants in adolescents from 10 countries, including two developed countries and eight developing countries.

1.2.2 Specific Objectives

a) To analyze the relationship between PAE and socioeconomic, demographic and behavioral factors.

b) To estimate the prevalence of the following behaviors: physical activity, eating habits, smoking and alcohol consumption;

c) To develop a hierarchical conceptual model.

2. METHODS

2.1 Characterization Research

This was a cross-sectional school-based study of adolescents and high school students between the ages of 13 and 18 from 11 cities in the following ten countries: Brazil (Maringá), Austria (Vienna), Italy (Rome), Germany (Dortmound) France (Lille), Belgium (Ghent), Greece (Athens and Heraklion), Hungary (Pécs), Sweden (Stockholm) and Spain (Zaragoza). According to the World Bank, the following countries are classified as developed: Austria, Italy, Germany, France, Belgium, Sweden, Spain, Brazil, Greece and Hungary. Data from European countries were collected by the HELENA study (Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study), which is coordinated by **Professor Dr. Luis A. Moreno Aznar**; Faculty of Health Sciences, University of Zaragoza (Spain).

This study was submitted for approval by the Ethics Committee of the School of Medicine, University of São Paulo (CEP). For data collection, the study was approved by the Ethics Committee [16] and followed the guidelines of the Declaration of Helsinki [17].

2.2 Sample

The study population consisted of students of both sexes between 13 and 18 years of age enrolled in public and private school systems in each city. In Brazil, data were verified using additional data from the State Department of Education of Paraná [18]. Data from European countries were obtained from the corresponding National Institute of Statistics in each country [19].

To calculate the sample size, we used the following parameters: a 95% confidence level, a power of 80%, an estimated prevalence of 50% (± 3 percentage points), and a design effect of 2 because this was a complex sampling. Based on these parameters, it was determined that collecting data on 300 adolescents in each city was necessary. Based on previous adolescent health research studies, we added 10%, which accounted for losses and refusals, and 15% for multivariate analyses, resulting in a sample size of 360 adolescents from each city. However, the data collection planned for Brazil necessitated an overestimation of the sample because it was the only Latin American country included in the study. Therefore, it was determined that collecting data from 918 adolescents would be necessary.

The sample size was approved using proportional linking of the size of the strata (sex and age). The diversity of the sample was determined using a proportionate random distribution of all schools, taking into account their characteristics and their location within their city. A total of at least 360 X 13 = 4,680 adolescents were included.

Sampling was performed in the following two stages: type of school (primary sampling unit) and classrooms. We first recorded the number of schools with students within the desired age range and then randomly selected schools of the appropriate size and type (public or private). We then selected classes in each school via random sampling so that equal proportions of students from each age group were selected. Sampling and recruitment are summarized in Figure 1.

All students were eligible to participate in the study if they provided verbal consent, or the written consent of a responsible party was provided. Exclusion criteria included adolescents with orthopedic problems preventing anthropometric assessments, absence of parental consent and pregnancy.

2.3 Data Collection

Data were collected by a team of interviewers. Forty hours of training were undertaken in each country to standardize the questionnaires and anthropometric assessments [20]. Following training, two pilot studies were conducted one week apart in schools that were not part of the final sample and consisted of the following: a) final testing of the questionnaire, b) field work organization and c) evaluating the performance of each interviewer before data collection took place.

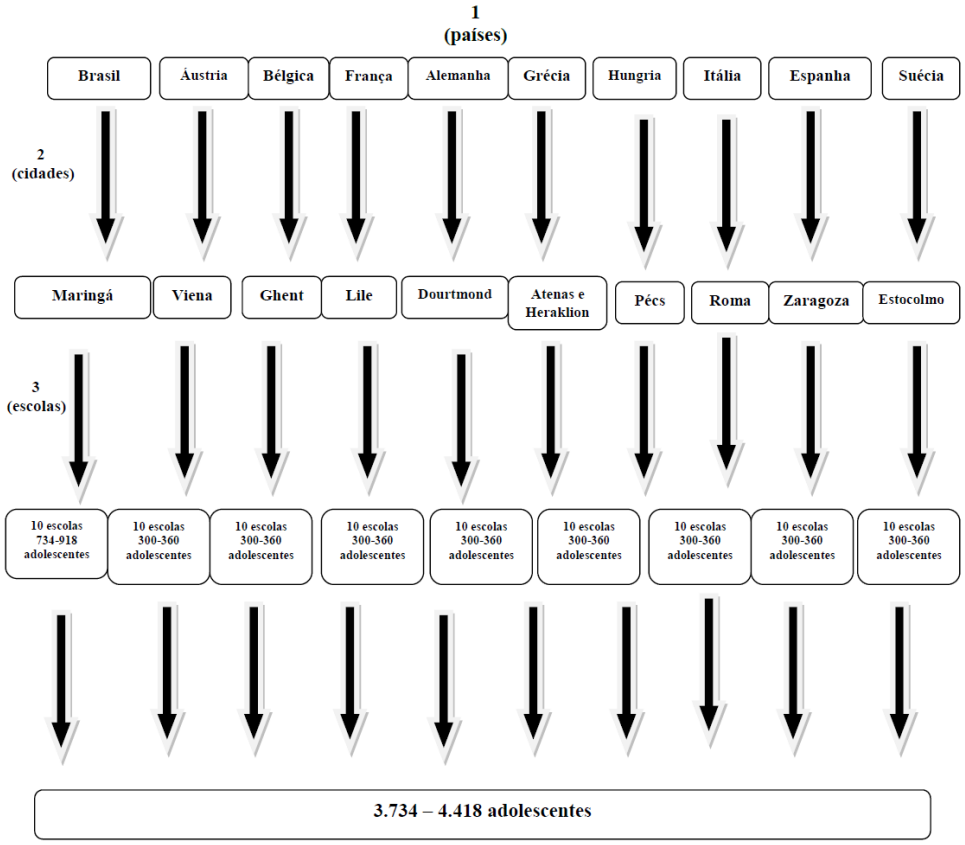

Figure 1. Flowchart sampling procedure. (1) The relationship between location and the presence of experienced researchers. (2) Stratified by sex, age and school in each city. (3) Stratified by age, sex and school in each city.

2.3 Variables

Outcome: The outcome studied was high blood pressure (HBP). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were each measured twice at minimum intervals of two minutes and were taken in the right arm of each individual following

five minutes of rest using an oscillometric electronic device equipped with automatic inflation and deflation; each teenager was seated. For data collection in Brazil, the model was validated for a 705-CP sample of adolescents by Furusawa et al. [21], and data collection in Europe was validated with a HEM-742 model was used by Christofaro et al. [22]. Following the completion of each measurement, blood pressures were determined based on the average of the two measurements: if the variation was greater than 5%, new measurements were taken. PAE was diagnosed in individuals with systolic blood pressures or diastolic blood pressures ≥ 95th percentile [23] for age and sex, adjusted for height [24].

Independent variables: The independent variables for this study included socio-demographic, behavioral and nutritional status indicators. Each is described below.

Socio-demographic indicators

- *Country;*
- *Sex:* self-reported by the adolescent;
- *Age:* determined based on the difference between the date of birth (reported by adolescents) and the date of data collection;

Socioeconomic indicators

- *School Characteristics:* public or private;
- *Parental education level:* university level (high); medium; or elementary (low);
- *Parental employment status:* both parents work or at least one at home;

Behavioral indicators

- *Physical Inactivity:* Information regarding physical activity was obtained using the International Physical Activity Questionnaire (International Physical Activity Questionnaire - IPAQ), which was modified for adolescents and referred to their behavior during the previous week [25-27]. Physical inactivity

was defined as <300 minutes per week of moderate vigorous physical activity consistent with current physical activity guidelines for adolescents [28].

- *Sedentary Behavior:* Information regarding time (in minutes) spent watching TV, using the computer and playing video games. Excessive was defined as TV time + computer + games ≥ 4 h / day.
- *Eating Behavior:* Verified using a questionnaire regarding weekly food frequency that was recommended by the WHO for epidemiological research in adolescents and high school students [29]. The questionnaire was translated and modified to fit the dietary habits of each country [30,31] after being subjected to a reliability study using Brazilian adolescents [32] and European [33] students. Consider the following question: "How many times in the last week have you eaten fruit?" This question assessed the consumption of fruits during a typical week. The questionnaire was self-administered in the classroom under the supervision of a researcher and pertained to the following five food groups: 1) vegetables, 2) fruit, 3) soda, 4) fried foods and 5) sweets (foods with high energy density such as cakes, biscuits and chocolates). Inadequate food intake was defined as ≥ 4 days / week in which fried foods, cakes, biscuits and sweets were eaten and ≤ 4 days / week in which fruit and vegetables were eaten. The amounts of each food consumed were not collected. The questionnaire also measured the number of meals (breakfast, morning snack, lunch, afternoon coffee, afternoon snack, dinner and supper) that were prepared and served at home. It also verified the impacts of specific diets (not on diets to lose weight and get fat), with the aim of identifying cases of reverse causality between outcomes and eating habits.

- *Regular consumption of tobacco and alcoholic beverages:* These parameters were measured using the questionnaire recommended by the World Health Organization (WHO) for epidemiological research in adolescents [34]. The questionnaire was modified and validated for Brazilian subjects by the Center for Epidemiological Research of the Federal University of Pelotas [35,36]. The adolescents were asked about their frequency of tobacco consumption (including the consumption of tobacco cigarettes, cigarillos and cigars) during the last thirty days. Adolescents who smoked at least one cigarette, cigarillo or cigar per day during the last thirty days were considered smokers, and adolescents who reported consuming two or more drinks per week for at least a month were considered regular consumers of alcohol.

Nutritional Status Indicators

- *General Obesity:* diagnosed by body mass index (BMI), which was calculated using the ratio of body mass in kilograms to the square of height in meters (kg / m2). Body mass was measured using a mechanical scale (maximum capacity: 150 kg; precision: 0.1 kg) brand. Height was measured using a stadiometer attached to an aluminum balance (precision: 0.01 m) [37]. Based on the cutoff points proposed by Cole et al. [38], teens were classified as strophic, overweight and obese.
- *Abdominal Obesity:* diagnosed based on waist circumferences, which were measured using a tape measure placed at half the distance between the iliac crest and the last rib in duplicate by calculating the average and assuming a maximum variation of 0.5 cm between the two measurements: the procedure was repeated if this variation was exceeded (precision 0.1 cm) [37].

Adolescents were classified as nonobese and obese based on cutoff points adjusted for sex and age [39].

3. PROPOSAL FOR DATA ANALYSIS

Continuous variables are presented as means with confidence intervals of 95% (95% CI), and qualitative variables are presented as percentages (%). The Shapiro-Wilk test was used to verify the normality of the data. The t test was used for unpaired samples, and the Mann-Whitney test was used to analyze differences between the sexes in the variables studied. To analyze possible differences between categorical variables, the chi-square test was used, as was the Yates correction for dichotomous independent variables, and the chi-square test was used for linear trends of ordinal or nominal or categorical variables to compare outcomes and independent variables.

Prevalence ratios (PRs) with confidence intervals of 95% (95% CI) as determined by the Poisson regression were used to quantify crude and adjusted relationships between outcomes and independent variables, a test recommended for outcomes with high prevalences [40].

Adjusted analyses were performed based on a previously developed hierarchical model that included the following four levels: 1) socio-demographic indicators, 2) socioeconomic indicators, 3) behavioral indicators, and 4) indicators of nutritional status. In this type of analysis (model), controlled variables are found at the same level or at higher levels [41]. To maintain the variables in this model, the level of significance was $p < 0.20$. Wald tests for heterogeneity for dichotomous or nominal variables and linear trends for ordinal variables and categorical variables were used.

Stata, version 11.0 (Stata Corporation, College Station, TX, USA), was used for all statistical analyses. Statistical significance was set at 5%.

4. FUNDING

The authors were not funded by any agency sponsoring this research. Permanent materials, including stadiometers, balances, and anthropometric tape devices used to determine blood pressures, were provided by the University Center of Maringá. Permanent materials, including sulfite sheets on which the questionnaires were printed, as well as transportation costs (gasoline and public transportation), were provided and paid for by one of the authors, Augusto César Ferreira de Moraes. Costs were contemplated at the time of data collection.

For data collection in Europe, the HELENA study received financial support from the European Community (Contract FOOD-CT-2005-007034). Researchers at the University of Zaragoza, Spain, received support from the MAPFRE Foundation [19].

5. SCHEDULE

The schedule below describes the stages of the project in months, beginning with the date of enrollment in the PhD program.

⇒ 1st to 7th month: completion of courses + qualification systematic review article;

⇒ 8th to 14th month: stage in Europe and hierarchical conceptual model + compilation of database research + presentation of internship report at the University of Zaragoza;

⇒ 15th to 22nd month: qualification + completion and submission of a systematic review;

⇒ 23rd to 33rd month: teaching stage (PAE) + data analysis and preparation of original papers + thesis + defense.

6. ARTICLES

This project was designed based on the principles proposed by Volpato [42]. These principles state that by imagining a research project, we have determined the magazines to which we intend to submit our articles; therefore, we must develop a

project of the same quality as the articles published in these journals. Therefore, we have listed six possible journals to which we intend to submit manuscripts under development in Table 6, sorted by journal, impact factor and QUALIS of the CAPES.

Table 6: Possible journals for submission of manuscripts, sorted by journal, impact factor and QUALIS/CAPES.

Journal	Impact Factor	QUALIS
Int J Epidemiol	5,262	A1
J Pediatr (US)	4,092	A1
Eur J Epidemiol	3,718	A2
Prev Med	3,172	A2
J Hum Hypertens	2,289	B1
BMC Public Health	2,223	B1

Each article under development that will be submitted and included in the thesis is described below, as follows:

7.1 Review Article: Title - "*Prevalence of high blood pressure in adolescents: a systematic review*." This article will aim to describe the prevalence of high blood pressure in adolescents and to identify the primary risk factors for hypertension based on a systematic review of the literature.

7.2 Original Article 1: Title - "*Risk behaviors and socioeconomic factors in adolescents: a multicenter study*." This article will aim to estimate the prevalence of specific behaviors and their relationship with socioeconomic factors in adolescents aged 13-18 years from ten countries.

7**.3 Original Article 2: Title -** "*High blood pressure and the clustering of risk factors in adolescents: a multicenter study*." This article will aim to estimate the prevalence of

high blood pressure and to analyze the effects of various risk factors in adolescents between 13-18 years of age from ten countries.

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1.1 RESEARCH PROJECT: DEVELOPMENT AND UPDATING OBJECTIVES AND METHODS

During the development of the project, I had daily meetings with my supervisors and also with the coordinators from each participating center of the HELENA study. After these meetings, we have decided to make some changes in the project, which are described below:

1) *Stratification analyzes by study and sex*: According to HELENA study rules, we cannot add any data from any other study to HELENA database; however, we could compare these data with other studies;

2) *Do not make the classification the values of SBP and DBP to HBP*: The classification considers high blood pressure percentile of height for age and sex. If we have performed this classification, we would have a classification bias, because the growth curves are different between Brazil and Europe;

3) *Cut-off point for classification of physical activity (PA)*: During the development of the papers from the thesis, we have verified that current PA recommendations for adolescents is ≥60 min/d, which is different from ≥300 min/wk that was defined at the beginning of the project. Therefore, in our papers we have used the most recent classification.

4) *Do not use tobacco and alcohol variables:* In our project, we had planned to use the consumption of alcoholic beverages and tobacco as risk factors for SBP and DBP increase. But, we decided to not use theses variables, because the literature shows that these data are underestimated when collected using questionnaires.

5) *Modifications in Proposed Papers:* After the changes described above, and the meetings with my supervisors and coordinators of each HELENA center, we have made modifications to the articles that would be developed during the PhD. Also, I had the

opportunity to work with unplanned exposures (serum concentrations of iron/vitamin and genetic polymorphisms);

6) *Modifications in proposed statistical analysis:* Statistical analyzes suffered small modifications for two reasons: i) Due to the changes in the characteristic of the outcome (from categorical to continual), the regression model was also modified: from Poisson to linear; ii) I learned to do statistical analysis using multilevel models, which perfectly fits the design of the project.

2. REVIEW ARTICLE

2.1 PREVALENCE OF HIGH BLOOD PRESSURE IN 122,053 ADOLESCENTS: A SYSTEMATIC REVIEW AND META-REGRESSION – PLUBLISHED IN MEDICINE

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ABSTRACT

Several studies have reported high prevalence of risk factors for cardiovascular disease in adolescents. To perform: i) systematically review the literature on the prevalence of high blood pressure (HBP) in adolescents; ii) analyse the possible methodological factors associated with HBP; and iii) compare the prevalence between developed and developing countries. We revised 10 electronic databases up to August 11st, 2013. Only original articles using international diagnosis of HBP were considered. The pooled prevalence's of HBP were estimated by random effects. Meta-regression analysis was used to identify the sources of heterogeneity across studies. Fifty-five studies met the inclusion criteria and total of 122,053 adolescents included. The pooled-prevalence of HBP was 11.2%; 13% for boys and 9.6% for girls ($p<0.01$). Method of measurement of BP and year in which the survey was conducted were associated with heterogeneity in the estimates of HBP among boys. The data indicate that HBP is higher among boys than girls, and that the method of measurement plays an important role in the overall heterogeneity of HBP value distributions, particularly in boys.

Key Words: Cross-sectional study; Adolescents; Blood pressure; Meta-regression

INTRODUCTION

Cardiovascular diseases (CVD) are the main sources of disease burden worldwide, and constitute a major public health problem in many countries [1]. High blood pressure (HBP) is an established major risk factor for stroke and coronary heart disease [2]. Studies have shown that blood pressure (BP) in childhood and adolescence are crucial factors in developing hypertension in adulthood [3].

Several studies have reported high prevalence of factors such as abdominal obesity [4], inflammation markers [5], metabolic syndrome,[6] and clustered metabolic risk [7], among the risk factors for CVD. Between the cardiovascular risk factors, some article highlights increased blood pressure values among adolescents as being particularly noteworthy [8, 9]. Because the prevalence of obesity has been increasing [4], we would expect to observe an increase in the prevalence of HBP, since there is a strong association between obesity and hypertension. Freedman et al. [10] et al also found that the prevalence of obesity increased but no increase in BP was observed.

Because of these major discrepancies in the literature and there has not been any systematic review verifying either the prevalence of HBP among adolescents or the for identifying the factors associated with this important aspect of adolescent health, we systematically reviewed the literature to collate the prevalence data of HBP among adolescents. Thus, we hypothesized the: i) the prevalence of HBP is high in adolescents and has increased over the past years; ii) the characteristics of the study are associated with HBP variation; and iii) the prevalence of HBP is different between developed and developing countries.

METHODS

Identification of studies

This study followed the systematic review methodology proposed in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [11]. The study is registered in the PROSPERO database (CRD42011001422). Searches involved 10 electronic databases: BioMed Central, Cinahl, Embase, ERIC, Medline/PubMed, PsyINFO, Scielo, Scopus, SportDiscus, and Web of Science. Articles listed in the databases through August 11st, 2013 were evaluated for inclusion in the analysis. This extended number of databases was used in order to minimise selection bias. The articles identified in the search were reviewed and contact made with the corresponding authors to solicit other relevant details, and studies that may have been missed in our search.

An ethics statement was not required for this work and no funding was received for this work, no funding bodies played any role in the design, writing or decision to publish this manuscript.

Three command groups (according to key words) were used for the database search. Within each group, we used the Boolean operator "OR" and between groups we used the Boolean operator "AND". In the first group we included terms related to blood pressure: *high blood pressure*, *blood pressure*, and *hypertension*. In the second group we included terms related to age: *adolescent, adolescence, young, youth, teenager,* and *teenage*. Given that the aim of the present review was to verify the prevalence of HBP, in the third group we added a set of commands to restrict study design to cross-sectional studies, because this type of epidemiological study is the most appropriate for studies that attempt to estimate the prevalence. These terms were: *prevalence studies, cross-sectional studies,* and *survey*.

Inclusion of studies

We included studies that published original data, in cases of duplicated data, the studies presenting outcomes related to our systematic review were retained and the articles that did not meet the inclusion criteria were excluded. The duplicates were removed using EndNote Web® reference management software, Thomson Reuters, Carlsbad, CA, USA.

Potentially relevant articles were selected by: i) screening the titles; ii) screening the abstracts; and iii) if abstracts were not available or did not provide sufficient data, the entire article was retrieved and screened to determine whether it met the inclusion criteria. To be included the study the article needed to: 1) have a representative population-based sample that included adolescents (aged between 10 - 19 years old; eg: if some studies had prevalence data of 10-15yo or 15-19yo, they were included); 2) be a cross-sectional design (because we are interested in verifying the prevalence of HBP, cross-sectional studies are the kind of epidemiological study more appropriate to check the prevalence, however we know the limitations and were considered); 3) have employed a probabilistic method to sample the population; 4) present the HBP prevalence; 5) be an original study presenting the prevalence of HBP for both genders; and 6) have diagnosed HBP according to international guidelines: SBP and/or DBP $\geq$ 95^{th} percentile for gender, age and height (currently just there are two guidelines; one of the American Academy of Pediatrics [12] and other European Society of Hypertension [13]). We also included those articles that did not present the prevalence *per se*, but contained an estimation of prevalence by gender. Also included were those articles that contained the confidence interval (95%CI) according to gender. The STROBE checklist for cross-sectional studies was applied by 2 members of the research team in assessing the percentage of items correctly related to the individual articles [14, 15] and, in case

of disagreement between the assessors, the article was evaluated by a 3rd member of the team (see Fig. 1). We not used the STROBE for to available the quality of the studies, just check the important methodological aspects this type of study.

Assessment, data extraction, and analysis

The evaluation and data extraction were performed independently by 2 members of the research team (ACdeM and MBL). Disagreements were resolved by consultation within the team until consensus was reached.

The data extracted from each study were: author, country, publication year, year of survey, journal in which the article was published, total study sample size, sample size of adolescents, age of subjects in years, proportion of girls, prevalence of HBP, and risk factors associated with HBP. The 95%CI was obtained from the articles [16-28] whenever possible, or was calculated using Stata 12.0 'cii' command (95%CI exact for binomial distribution) [29-62].

The pooled prevalence's of HBP (total sample and for each gender) were estimated by random effects. Test of heterogeneity (Q test) was used to evaluate whether the differences in prevalence estimates across studies were higher than expected by chance. Meta-regression analysis was used to identify the sources of heterogeneity across studies by I^2, initially to assess the contribution of each variable (year of survey; geographic location; characteristic of countries; study population; method of BP measurement) to the overall heterogeneity [63]. Those variables that were significantly associated with the heterogeneity ($p < 0.20$) were included in a multivariate hierarchical model [64]. At the first level, year of survey (1988 - 1998, 1999 - 2004 and 2005 - 2009) was introduced, at the second level were geographic location (North America, Europe, Asia, Latin America, Oceania, Middle East, and Africa), characteristic of countries according International Monetary Fund classification

(developed or developing), and study population (community or school); at the third level were the devices used to measure BP (sphygmomanometer or automatic digital monitor). A p value of <0.05 was considered statistically significant in all the analyses.

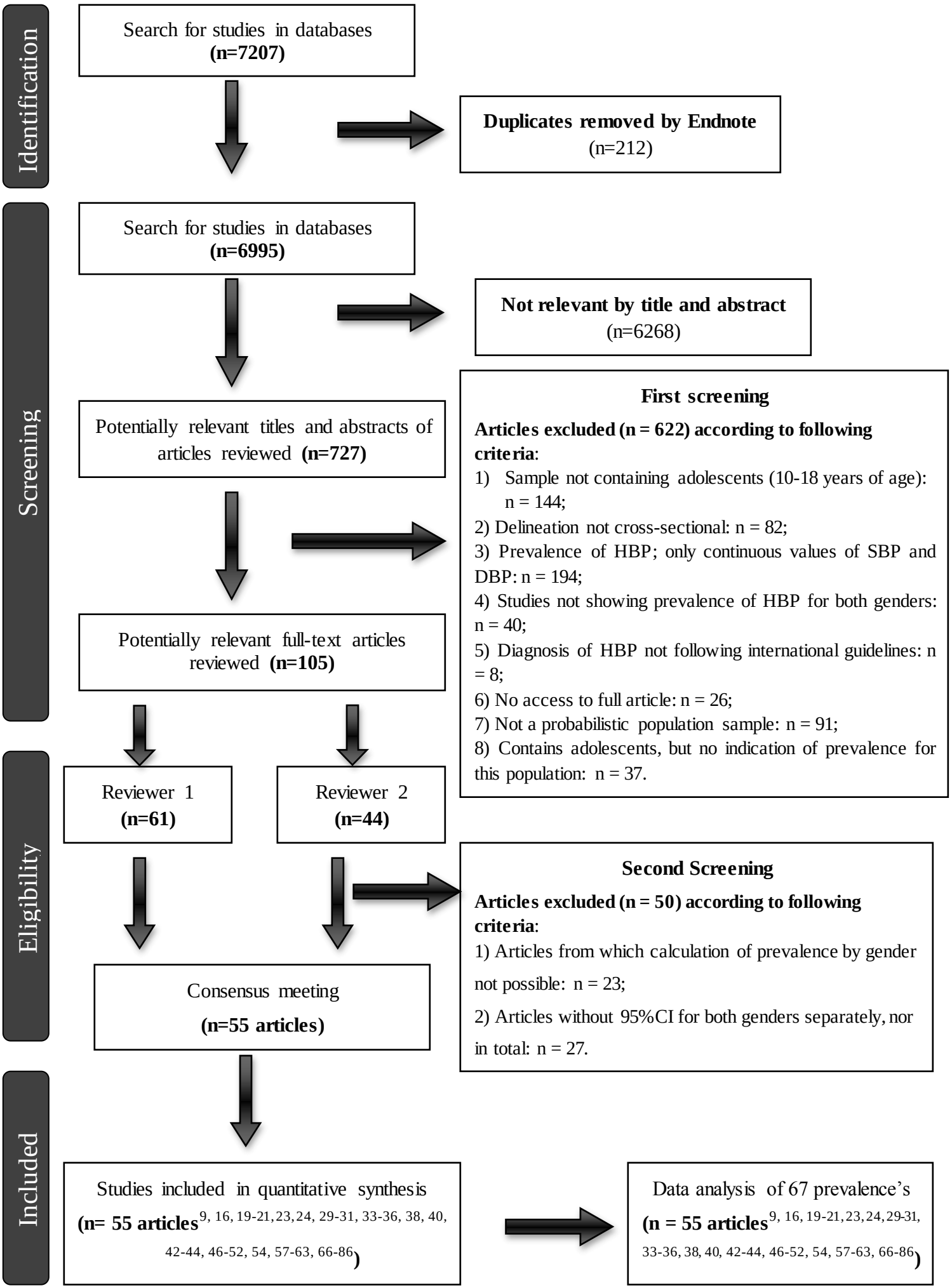
RESULTS

Literature search

The literature search yielded 727 titles of potentially relevant articles (see Fig. 1 for selection procedure flow diagram). Of these, 55 articles were eligible according to the inclusion criteria established for this review [9, 16, 19-21, 23, 24, 29-31, 33-36, 38, 40, 42-44, 46-52, 54, 56-62, 65-85]. The supplement file presents a description of the 55 articles with the relevant inclusion criteria including: lead author, year of publication, country where the study was performed, year of survey, total number of participants in the study, number of adolescents, proportion of girls, age range, study population, method of measurement, overall and gender-based prevalence, and the respective 95%CI.

Among the study that used automatic digital monitors for measuring BP, 77% used Omron blood pressure device (Omron Healthcare Inc., Tokyo, Japan); 22.3% did not describe which model and 0.7% used the Space Labs device.

Five articles evaluated the secular trend of prevalence, two of which were from USA. The continents with the highest numbers of studies included in this review were Asia and Latin America (n=18 in each), and only 1 study from Oceania was identified. Of the populations studied, 55.6% were from high-school samples; 75.5% of studies used sphygmomanometer to measure BP; 63.5% of surveys were conducted in low- and middle-income countries (supplementary file). Total of 122,053 adolescents included in this review (61,049 girls).


Identification
Search for studies in databases
(n=7207)
Duplicates removed by Endnote
(n=212)
Screening
Search for studies in databases
(n=6995)
Not relevant by title and abstract
(n=6268)
Potentially relevant titles and abstracts of articles reviewed (n=727)
First screening
Articles excluded (n = 622) according to following criteria:
1) Sample not containing adolescents (10-18 years of age): n = 144;
2) Delineation not cross-sectional: n = 82;
3) Prevalence of HBP; only continuous values of SBP and DBP: n = 194;
4) Studies not showing prevalence of HBP for both genders: n = 40;
5) Diagnosis of HBP not following international guidelines: n = 8;
6) No access to full article: n = 26;
7) Not a probabilistic population sample: n = 91;
8) Contains adolescents, but no indication of prevalence for this population: n = 37.
Potentially relevant full-text articles reviewed (n=105)
Eligibility
Reviewer 1
(n=61)
Reviewer 2
(n=44)
Second Screening
Articles excluded (n = 50) according to following criteria:
1) Articles from which calculation of prevalence by gender not possible: n = 23;
2) Articles without 95%CI for both genders separately, nor in total: n = 27.
Consensus meeting
(n=55 articles)
Included
Studies included in quantitative synthesis
(n= 55 articles 9, 16, 19-21, 23, 24, 29-31, 33-36, 38, 40, 42-44, 46-52, 54, 57-63, 66-86)
Data analysis of 67 prevalence's
(n = 55 articles 9, 16, 19-21, 23, 24, 29-31, 33-36, 38, 40, 42-44, 46-52, 54, 57-63, 66-86)

Prevalence

In the overall sample, the pooled-prevalence estimated by random effects was of HBP was 11.2% (Table 1); 13% for boys and 9.6% for girls ($p<0.01$) and the prevalence was higher among boys and the study conducted in Oceania (Table 3). The analyses revealed significant heterogeneity across studies for the overall population samples, as well as segregated according to gender ($p<0.001$, for all analyses).

Table 1 summarises the associations between HBP prevalence and characteristics of the study in the overall sample, as well as segregated according to gender. In the overall sample, the significant association of geographical location lost significance in the adjusted model. The year of survey was not significantly associated with the prevalence of HBP while, conversely, the characteristic of countries and method of measuring BP retained their significant associations.

Table 2 depicts the HBP prevalence in the girls in relation to the methodological characteristics. Those studies from Africa showed higher prevalence while the lowest were those studies from Latin America. We found no significant associations between prevalence of HBP in girls and methodological characteristics.

Table 3 shows the prevalence in the boys in relation to methodological characteristics. The highest prevalence was observed in Oceania and the lowest in the studies conducted in Middle East/Latin America, and North America.

Among boys, all the variables were associated with heterogeneity in the distributions of HBP in the univariate model, but only the year in which the survey was conducted, the geographical location, and the method of measuring BP maintain the significance in the adjusted model.

Table 1: Association between prevalence of high blood pressure with methodological covariates for total (n= 122,053) of sample of the studies.

Level**	Independent Variables	N of studies	Pooled Prevalence of HBP (95%CI)	Total of sample	
				Univariate Model *p*-value	Metaregression (Multivariate Model) * *p*-value
1	**Year of survey**			0.138	
	1988 - 1998	9	11.8 (7.7 - 12.4)		Referent
	1999 - 2004	26	8.3 (5.7 - 18.7)		0.098
	2005 - 2009	20	8.5 (5.3 - 16.4)		0.050
2	**Geographic location**			**0.001**	
	North America	14	7.3 (2.8 - 8.8)		Referent
	Europe	4	16.2 (10.3 - 27.1)		0.222
	Asia	15	12.4 (7.7 - 19.6)		0.177
	Latin America	14	6.2 (3.1 - 10.6)		0.720
	Oceania	1	24.6 (23.2 – 26.0)		0.692
	Middle East	4	5.3 (4 - 16.5)		0.971
	Africa	3	25.5 (10.1 - 39.1)		0.479
	Characteristic of countries			**0.001**	
	Higher income	19	8.3 (3.2 - 13.8)		Referent
	Low- and middle-income	36	9.8 (5.7 - 17.8)		0.646
	Study population			**0.004**	
	School	26	9.3 (4.7 - 17.5)		Referent
	Community	29	8.3 (6.9 - 13.8)		0.942
3	**Method of measured BP** ‡			**0.041**	
	Sphygmomanometer	34	8.9 (5.7 - 16.2)		Referent
	Automatic digital monitor	13	12.2 (5.3 - 21.5)		**0.015**
	Between-study variance assessed by moment-based estimate (tau 2= 5.307).				

HBP= high blood pressure; 95%CI= confidence interval of 95%.

* The adjusted analysis was conducted following a theoretical conceptual model that had been previously formulated in three levels: 1) year of survey (1988 – 1998. 1999 – 2004. and 2005 – 2009); 2) geographic location (North America. Europe. Asia. Latin America. Oceania. Middle East. and Africa); characteristic of countries (higher or low- and middle-income); and study population (community or school); 3) method of measured BP (sphygmomanometer or automatic digital monitor).

** The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with p >0.2 were not included in the subsequent adjusted models. Statistically significant associations are in **bold** type.

‡ 8 articles is not described the method of measured blood pressure.

Table 2: Association between prevalence of high blood pressure with methodological covariates for girls (n= 61,049) of the studies.

Level**	Independent Variables	N of studies	Pooled Prevalence of HBP (95%CI)	Univariate Model *p*-value	Girls Metaregression (Multivariate Model) * *p*-value
1	**Year of survey**			0.972	
	1988 - 1998	9	9.0 (5.9 - 11.5)		Referent
	1999 - 2004	26	7.8 (3.4 - 13.8)		0.111
	2005 - 2009	20	7 (3.1 - 14.1)		0.450
2	**Geographic location**			0.214	
	North America	14	5.85 (2.9 - 8.7)		Referent
	Europe	4	11.2 (7.8 - 15.2)		0.484
	Asia	15	11.5 (6.7 - 18.7)		0.536
	Latin America	14	4.8 (2.8 - 9.2)		0.913
	Oceania	1	24.7 (22.7 - 26.8)		0.717
	Middle East	4	5.0 (2.3 - 13.6)		0.751
	Africa	3	29. (23.8 - 33.3)		0.552
	Characteristic of countries			0.679	
	Higher income	19	7.8 (3.4 - 10.5)		Referent
	Low- and middle-income	36	9.1 (3.3 - 13.7)		0.755
	Study population			0.746	
	School	26	8.6 (3.4 - 12.5)		Referent
	Community	29	7.8 (3.4 - 13.8)		0.995
3	**Method of measured BP** ‡			0.897	
	Sphygmomanometer	34	8.6 (3.7 – 13)		Referent
	Automatic digital monitor	13	10 (5.5 - 23.8)		0.894
	Between-study variance assessed by moment-based estimate (tau 2= 2.349).				

HBP= high blood pressure.

* The adjusted analysis was conducted following a theoretical conceptual model that had been previously formulated in three levels: 1) year of survey (1988 – 1998. 1999 – 2004. and 2005 – 2009); 2) geographic location (North America. Europe. Asia. Latin America. Oceania. Middle East. and Africa); characteristic of countries (higher or low- and middle-income); and study population (community or school); 3) method of measured BP (sphygmomanometer or automatic digital monitor).

** The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with p >0.2 were not included in the subsequent adjusted models. Statistically significant associations are in **bold** type.

‡ 8 articles is not described the method of measured blood pressure.

Table 3: Association between prevalence of high blood pressure with methodological covariates for boys (n= 61,004) of the studies.

Level**	Independent Variables	N of studies	Pooled Prevalence of HBP (95%CI)	Boys Univariate Model *p*-value	Boys Metaregression (Multivariate Model) * *p*-value
1	**Year of survey**			**< 0.001**	
	1988 - 1998	9	13.3 (8.7 - 14.6)		Referent
	1999 - 2004	26	10.3 (6.3 - 18.8)		**0.011**
	2005 - 2009	20	9.4 (5.3 - 25.1)		**0.004**
2	**Geographic location**			**0.004**	
	North America	14	7.1 (3.3 - 10.8)		Referent
	Europe	4	17.5 (12.6 - 26.8)		0.334
	Asia	15	13.9 (10.4 - 22.8)		0.664
	Latin America	14	7.4 (4.4 - 13.5)		0.891
	Oceania	1	24.7 (22.8 - 26.7)		0.607
	Middle East	4	6.4 (3.4 – 20.0)		0.551
	Africa	3	24.0 (9 - 46.1)		0.617
	Characteristic of countries			**0.003**	
	Higher income	19	9.0 (4 – 18.0)		Referent
	Low- and middle-income	36	11.5 (6.4 - 22.7)		**0.007**
	Study population			**0.001**	
	School	26	9.4 (5.1 - 22.8)		Referent
	Community	29	10.8 (6.9 - 16.2)		0.557
3	**Method of measured BP** ‡			**< 0.001**	
	Sphygmomanometer	34	9.7 (6.1 - 19.4)		Referent
	Automatic digital monitor	13	14.4 (5.1 - 24.7)		**<0.001**
	Between-study variance assessed by moment-based estimate (tau 2=1.305).				

HBP= high blood pressure.

* The adjusted analysis was conducted following a theoretical conceptual model that had been previously formulated in three levels: 1) year of survey (1988 – 1998. 1999 – 2004. and 2005 – 2009); 2) geographic location (North America. Europe. Asia. Latin America. Oceania. Middle East. and Africa); characteristic of countries (higher or low- and middle-income); and study population (community or school); 3) method of measured BP (sphygmomanometer or automatic digital monitor).

** The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with p >0.2 were not included in the subsequent adjusted models. Statistically significant associations are in **bold** type.

‡ 8 articles is not described the method of measured blood pressure.

DISCUSSION

We conducted a comprehensive systematic review of studies addressing the prevalence of HBP in adolescents, and we used meta-regression to examine the possible

sources of heterogeneity in the data presented in the articles. The prevalence of HBP was higher among boys, and the heterogeneity across studies was due to methodological differences, especially method of measuring BP. Further, the prevalence of HBP was higher among studies from low- and middle-income countries in boys. To the best of our knowledge, this is the first article analysing these associations in adolescents, and is the most extensive systematic review on this subject, to date.

Contrary to expectations, the prevalence of HBP was inversely related to the year of the survey. Because the prevalence of obesity has been increasing [4] we would expect to observe an increase in the prevalence of HBP, since there is a strong association between obesity and hypertension [10]. Freedman et al. [10] also found that the prevalence of obesity increased but no increase in BP was observed. The authors emphasized that a possible explanation is the improvement of maternal and child health [86, 87] and increased prevalence of breastfeeding alone [88] observed over the past two decades. These factors, which are inversely associated with adolescent BP levels [89], can be responsible for the decrease in HBP prevalence.

Boys had higher pooled prevalence than girls. There are 2 possible explanations for our finding: 1) studies showed that boys has a higher accumulation of visceral fat [90] and intra-abdominal fat [91] than girls, and visceral fat has been associated with higher sympathetic activity [92, 93]. This activation is a key mechanism underlying the effect of intra-abdominal fat accumulation on the development of hypertension [94]. For example, increased sympathetic flow may increase sodium re-absorption and subsequent increased peripheral vascular resistance resulting in increased blood pressure [95]. Also, this increased sympathetic activation can be caused by increased testosterone concentrations in males. Testosterone, acting as a mediator of the androgen receptor gene function [96], has been associated not only with increased visceral fat but

also with greater vasomotor sympathetic tone and blood pressure in adolescent boys, compared to girls [96]. However, pubertal stage is not included in the diagnostic criteria of HBP, and it can be a limitation, since the analysis can not adjust for this variable is not included in the articles described.

2) The girls have a higher prevalence of healthy behaviour patterns (healthy eating habits [97]; avoidance of tobacco smoking [98]; less alcohol abuse [99]; lower levels of sedentary behaviour [7]) than boys, and these healthy life-style choices are associated with lower levels of blood pressure and HBP prevalence [100-102].

Of considerable note is that the type of device used to measure BP was associated with heterogeneity in the prevalence of HBP. The pooled prevalence was higher in articles using the automatic "digital" monitors. However, all reported that the monitors used had been validated for measurement of BP in adolescents, according criteria by European Hypertension Society and American Academy of Pediatrics for differences between averages of the measures mercury column and tested monitor for a device to be validated, should be $\leq$ 5mmHg and that the standard deviation of the differences of the averages is not larger than 8 mmHg. The differences in the prevalence can introduce differential or non-differential misclassification effects (errors due to disease status or exposure) and may cause underestimation or overestimation of the true prevalence [103]. Our findings suggest that automatic monitors should not be used for diagnosis of hypertension, but may be used only as an initial assessment of current status of cardiovascular health of the adolescents and, should the teenager present with HBP, additional analysis with more accurate instruments must be performed. The logistics in epidemiological studies often preclude the measurement of BP with the gold-standard; eg. ambulatory blood pressure monitoring or repeated office BP measurements. The technique is more difficult to master and is not cost-effective on an

epidemiological scale. However, cost-effectiveness becomes evident [104] if HBP diagnosis in adults; and the screening of the HBP in the adolescent can lead to better and more prompt treatment and so increase life expectancy of the adolescent, because HBP this age group is asymptomatic.

On the other hand, recently Thompson et al.[105] showed that there is no direct evidence that screening for hypertension in children and adolescents reduces adverse cardiovascular outcomes in adults. Are needed new research's to improve diagnosis and risk stratification of children with elevated blood pressure and to quantify risks and benefits of interventions, because on this review we demonstrated higher prevalence of HBP.

Another important factor could be influence of the classification of the HBP is the race and ethnicity, because the growth speed is influenced by these factors [106], but the guidelines (American Academy of Pediatrics [12] and European Society of Hypertension [13]) highlights that newly revised CDC growth charts (www.cdc.gov/growthcharts) should be used for the height percentile classification.

We observed higher HBP prevalence in low- and middle-income countries. Previous studies conducted in these countries reported that the hypertension was associated with low socioeconomic status [83, 107]. However, the nutritional burden is shifting from deficiency to excess energy imbalance in these countries, while awareness of the problem is increasing in developed countries and, as such, the prevalence in higher income countries is becoming stabilized, albeit not as-yet normalised [108, 109]. Hence, strategies for changing lifestyles are necessary; the objectives being to decrease the prevalence and to increase early treatment of HBP.

CONCLUSIONS

Our systematic review indicated that HBP prevalence is high among adolescents; higher in boys and adolescents from low- and middle-income countries. The method of measurement plays an important role in HBP prevalence distribution in the overall sample, and especially in boys, but not in girls. Public health programs that aim to reduce HBP should focus primarily on adolescents from low- and middle-income countries.

Author Contributions

Augusto César F. de Moraes: Dr. de Moraes designed the data collection instruments, coordinated and supervised data collection, carried out the initial analyses and the interpreted the data critically, drafted the initial manuscript and reviewed the manuscript, and approved the final manuscript as submitted.

Maria Beatriz Lacerda: Miss. Lacerda conceptualized and designed the study, drafted the initial manuscript, data collection, carried out the initial analyses and the interpreted the data critically and approved the final manuscript as submitted.

Luis Alberto Moreno: Dr. Moreno drafted the initial manuscript and reviewed the manuscript, and approved the final manuscript as submitted.

Bernardo L Horta: Dr. Horta carried out the initial, final analyses and the interpreted the data critically and approved the final manuscript as submitted.

Heráclito Barbosa Carvalho: **Dr. Carvalho** designed the data collection instruments, coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

COMPETING INTEREST

The remaining authors state no conflict of interest.

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FIGURE LEGENDS

Figure 1: Flowchart of search strategy and results.
HBP= High blood pressure; SBP= systolic blood pressure; DBP= diastolic blood pressure.

Supplement file 1: Descriptive analysis of the studies reviewed

First Author	Year published	Country	Year of survey	Total study; N	Adolescents: N	Age range	Proportion of girls	Study population	Method of measured utilized
Ucar B	2000	Turkey	.	4026	2882	11 to 18	47.8	School	Sphygmomanometer
Martínez CA	2001	Argentina	.	2115	2115	11 to 18	57.3	School	Sphygmomanometer
Addor V	2003	Switzerland	1996	6935	2895	10 to 19	52.7	School	Sphygmomanometer
Peñuela RM	2003	Colombia	.	2611	1617	10 to 18	52.4	School	Sphygmomanometer
Duncan GE	2004	USA	1999	991	991	12 to 19	.	Community	Sphygmomanometer
Moura AA	2004	Brazil	2000	1253	898	11 to 14	56.3	School	Sphygmomanometer
Kelishadi R	2005	Iran	2001	2000	2000	11 to 18	50.0	School	Sphygmomanometer
Lawlor DA	2005	Australia	.	3613	3613	14	48.2	Community	Digital monitor
Ramos E	2005	Portugal	2003	2023	2023	13 to 14	52.0	School	Sphygmomanometer
Esmaillzadeh A	2006	Iran	.	3036	3036	10 to 19	46.0	Community	Sphygmomanometer
Jago R	2006	USA	2003	1717	1717	15	56.0	School	Digital monitor
Castillo EH	2006	Mexico	2004	1366	967	10 to 14	56.6	School	Sphygmomanometer
Monego ET	2006	Brazil	2001	3169	3169	11 to 14	49.5	Community	?
Pollex RL	2006	Canada	1993	700	185	15 to 18	62.0	School	Sphygmomanometer
Rodrigues AN	2006	Brazil	2003	380	380	10 to 14	53.4	School	Sphygmomanometer
Yamamoto Kimura L	2006	Mexico	1996	3121	3121	12 to 16	55.0	School	Sphygmomanometer
Harding S	2007	England	.	6407	6407	11 to 13	47.0	School	Digital monitor
McNiece KL	2007	USA	2003	6790	6790	11 to 17	49.0	School	Digital monitor
Ng VW	2007	China	2003	2102	2102	12 to 19	54.4	School	Digital monitor
Rosa ML	2007	Brazil	2003	456	456	12 to 17	55.5	School	Digital monitor
Ryu SY	2007	Korea	.	1393	1393	12 to 13	52.5	School	Sphygmomanometer
Singh R	2007	India	.	1083	1083	12 to17	47.0	School	?
Juárez JG	2008	Mexico	.	1846	1846	12 to 16	58.3	School	Sphygmomanometer
Lozada M	2008	Venezuela	2006	88	88	12 to 17	50.0	School	Sphygmomanometer

Nur N	2008	Turkey	2004	1020	1020	14 to19	42.0	School	Sphygmomanometer
Pedrozo WR	2008	Argentina	2005	532	532	11 to 20	60.0	School	Sphygmomanometer
Seo SJ	2008	South Korea	2001	3431	3431	10 to 19	47.0	Community	Sphygmomanometer
Pan Y	2008	USA	1999	4204	4204	12 to 19	49.0	Community	Sphygmomanometer
Romanzini M	2008	Brazil	2005	644	644	15 to 18	61.6	School	Sphygmomanometer
Agyemang C	2009	Suriname	2007	855	855	12 to 17	54.5	School	Digital monitor
Azizi F	2009	Iran	.	4558	1708	11 to 18	53.0	Community	Sphygmomanometer
Cândido APC	2009	Brazil	2006	780	487	10 to 14	51.5	School	Digital monitor
Daratha KB	2009	USA	1999	613	613	12 to 19	47.7	Community	Sphygmomanometer
			2001	892	892	12 to 19	48.7	Community	Sphygmomanometer
			2003	857	857	12 to 19	47.5	Community	Sphygmomanometer
			2005	814	814	12 to 19	47.6	Community	Sphygmomanometer
Johnson WD	2009	USA	2001	2456	2456	12 to 19	47.5	Community	Sphygmomanometer
Lambert M	2009	Canada	1999	1322	1322	13 to 16	54.4	School	Digital monitor
Budak N	2009	Turkey	.	790	790	12 to 19	55.8	School	Sphygmomanometer
Bibiloni MM	2009	Spain	2007	362	362	12 to 17	60.5	School	Digital monitor
Ostchega Y	2009	USA	1988	4033	1798	13 to 17	53.0	Community	Sphygmomanometer
			1999	4846	2862	13 to 17	50.0	Community	Sphygmomanometer
			2006	4427	2619	13 to 17	49.0	Community	Sphygmomanometer
Park MJ	2009	Korea	1998	1792	1792	10 to 19	49.5	Community	?
			2001	1431	1431	10 to 19	48.1	Community	?
			2005	941	941	10 to 19	47.6	Community	?
Ella NAA	2010	Egypt	2004	4250	4250	11 to 15	47.5	School	?
Ejike CE	2010	Nigeria	2007	843	843	13 to 18	40.0	School	Digital monitor
Feliciano AJE	2010	Colombia	2005	249	249	15 to 20	41.4	School	Sphygmomanometer
Beck CC	2010	Brazil	2006	660	660	14 to 19	51.9	School	Sphygmomanometer

Lee YJ	2010	Korea	2005	12001	928	10 to 18	47.0	Community	Sphygmomanometer
Muller	2010	Germany	2003	17641	7698	11 to 17	49.0	Community	?
Riemenschneider F									
Moreira C	2010	Portugal	.	517	517	15 to 18	57.0	School	?
Nguyen TH	2010	Vietnam	2007	617	617	13 to 16	54.0	School	Digital monitor
Schwandt P	2010	Germany	2008	3647	3647	10 to 15	46.9	Community	?
Schwandt P	2010	Iran	2008	6375	6375	10 to 15	55.4	Community	?
Liang YJ	2011	China	1991	1936	865	13 to 17	50.0	School	?
			1993	1710	711	13 to 17	49.0	School	Digital monitor
			1997	1888	744	13 to 17	49.0	School	Sphygmomanometer
			2000	1602	748	13 to 17	47.0	School	Sphygmomanometer
			2004	1111	558	13 to 17	47.0	Community	Sphygmomanometer
Lin FH	2011	Taiwan	1996	1354	1354	12 to 14	50.0	Community	?
			2006	1203	1203	12 to 14	51.0	School	?
Perez-Fernandez GA	2012	Argentina	2002	1545	1545	12 to 15	49,3	School	Sphygmomanometer
Aounallah-Skhiri H	2012	Tunisia	2005	2870	2870	15 to 19	54.9	Community	Sphygmomanometer
Durrani AM	2012	India	2007	701	701	12 to 16	48.2	School	Sphygmomanometer
Ochoa-Aviles	2012	Ecuador	2009	766	766	10 to 16	49.8	School	Sphygmomanometer

? = Information not available in the published article.

3. ORIGINALS ARTICLES

3.1 POTENTIAL BIASES IN THE CLASSIFICATION, ANALYSIS AND INTERPRETATIONS IN CROSS-SECTIONAL STUDY – PLUBLISHED IN BMC PEDIATRICS

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ABSTRACT

Background: Resting heart rate reflects sympathetic nerve activity. A significant association between resting heart rate (HR) and all causes of cardiovascular mortality has been reported by some epidemiologic studies. Despite suggestive evidence, resting heart rate (RHR) has not been formally explored as a prognostic factor and potential therapeutic outcome and, therefore, is not generally accepted in adolescents. **Discussion:** the core of the debate is the methodological aspects used in "Resting heart rate: its correlations and potential for screening metabolic dysfunctions in adolescents"; the points are: cutoff used for cluster RHR, two different statistical models used to analyze the same set of variables, one for continuous data, and another for categorical data; interpretation of p-value <0.05, sampling process involving two random stages, analysis of design effect and the parameters of screening tests. **Summary:** aspects that

must be taken into account for evaluation of a screening test to measure the potential for discrimination for a common variable (population with outcome vs. no outcome population), the main indicators are: sensitivity, specificity, accuracy, positive predictive value and negative predictive value. The measures of argumentation equality (CI) or difference (p-valor) are important to validate these indicators but do not indicate quality of screening.

Keywords: resting heart rate; screening test; p-value; high glucose; high triglycerides

Background

Recently, Fernandes et al. published an article aimed at analyzing the potential effects of screening and resting heart rate (RHR) on cardiometabolic risk in adolescents [1] in this respected journal. We read the manuscript with great interest, since RHR reflects sympathetic nerve activity [2, 3], and it is an easily accessible clinical measurement. A significant association between resting HR and all-causes of cardiovascular mortality has been reported in some epidemiological studies [2, 4-6].

After studying the article, we decided to take the opportunity to propose a healthy debate on the methodological aspects used by Fernandes et al. [1]. With this debate, we hope to contribute to the enrichment of the reader, especially with regard to statistical analysis and interpretation of results.

The aim of this article is to present a critical appraisal of methodological aspects of the article "Resting heart rate: its correlations and potential for screening metabolic dysfunctions in adolescents" presented by BMC *pediatrics*.

Discussion

First, with regard to the manuscript methodology, what drew our attention was the cutoff used for cluster RHR. We see that the authors used cutoffs developed by the group of the first author (Fernandes RA) [7]. These cutoff points were developed by percentile distribution of a sample composed only of children and adolescent males and the study published in this journal is composed only of adolescents of both sexes. This decision introduced classification bias into the study, though it was not recognized as a study limitation: children are biologically different than adolescents because they have not gone through puberty, and there are important and significant differences between the sexes concerning the cardiovascular system [8].

Boys had higher pooled prevalence than girls [9, 10]. There are possible explanations for differences between the sexes: 1) the boys had a higher accumulation of visceral fat and intra-abdominal fat than girls [11], and visceral fat has been associated with higher sympathetic activity [12, 13] This activation is a key mechanism underlying the effect of intra-abdominal fat accumulation on the development of hypertension [14]. For example, increased sympathetic flow may increase sodium re-absorption and subsequent increased peripheral vascular resistance resulting in increased blood pressure [14]. Also, this increased sympathetic activation can be caused by increased testosterone concentrations in males. Testosterone, acting as a mediator of the androgen receptor gene function [15], has been associated not only with increased visceral fat but also with greater vasomotor sympathetic tone and blood pressure in adolescent boys, compared to girls [16]. Therefore, we believe that the cutoffs used are not appropriate for the above and highlight the need for the scientific community to develop better diagnostic criteria and methodological quality appropriate for each sex and age of this important indicator of the cardiovascular system.

According to the title of the article, the authors' objective was to analyze the impact of RHR for screening metabolic dysfunctions and also to identify its significance in adolescents.. For this, they used two different statistical models in order to analyze the same set of variables, one for continuous data, and another for categorical data. We found this odd, since assumptions for statistical models are quite distinct (binary logistic regression model vs. linear regression model). So we raise the following questions: *"Were the linear models used because no association was found with categorical variables? Why were the two models used? Why analyze variables with continuous data and then analyze these variables with categorical data, sequentially?"* We performed these questions, because according the objectives; the authors wanted determine the correlation between RHR and metabolic dysfunctions and also the potential power of screening the RHR. What is not clear is the use of logistic regression to meet those aims. In some instances we recommended that the authors state why they have used these tests and provide a reference for a definitive description for readers [17].

With regard to OR estimates using binary logistic regression, the literature shows that the use of OR (estimated with logistic regression) as a measure of effect in the cross-sectional studies has limitations: OR overestimates RP/RR according to increases of prevalence/incidence of outcome; between 5% and 10% OR has good approximation with RP/RR, after that the risk value is very distorted and it serves more to show the association direction (risk or protection) and not its magnitude; this topic was widely discussed in the nineties by experts [18-20], and confirms that OR overestimates the magnitude of the associations between exposures and outcomes, particularly in high prevalence [21, 22].The mathematical model for logistic regression was developed in the 1970s and 1980s to analyze case-control studies and used as a

proxy for relative risk [23, 24], where it is not possible to estimate prevalence, another important methodological factor neglected by the authors.

The authors say they used a sampling process involving two random stages (schools in the first stage and individual classes in the second stage), but give no further details of this process, for example, whether the complex sample has good accuracy. When using complex samples the design effect (*deff*) helps to estimate how accurate the sample was [25-27]. When the sampling process is not accurate the analyses need to be adjusted for the complexity of the sample, and the lack of this setting also impacts the associations [28]. Therefore, the impact of risk factors estimated by the logistic models, even without statistical significance, may not be exactly the absence shown by adjusting the primary sampling unit.

We found the use of RHR to screen for alterations in glucose and triglycerides interesting but, according to the data presented, we believe that there is no evidence for this. Accuracy (AUC) for high glucose was 0.611 (95% CI 0.534–0.688) and high triglycerides, 0.618 (95% CI 0.531–0.705), both with p-values <0.05, but with low discrimination power—note the lower confidence bound in some cases is very close to 0.50 (random event). In other words, if we consider random variations within the CI bounds of AUC, determining the presence or absence of high glucose and high triglycerides will be as precise as playing a game of heads or tails. With regard to the accuracy of results, Swets [31] suggested operational cut-off points: the test can be non-informative/test equal to chance (0.5AUC<0.7); moderately accurate (0.7>AUC≤0.9); highly accurate (0.9>AUC<1.0); and perfect discriminatory tests (AUC=1.0).

Nowadays a “p-value <0.05” or significant association is commonly employed to illustrate the importance of latest scientific finding. We emphasize, however, that statistical significance is neither a necessary nor a sufficient condition for proving a

scientific result [29]. P-values are often used to emphasize the certainty of data, but they are only a passive read-out of a statistical test and do not take into account how well an experiment was designed, for example [30]. Goodman [31], in his 'The P Value Fallacy" explains about the apparent inconsistency in much medical research, where by studies are designed according to a Neyman-Pearson statistical approach (eg. based on formal decision making and long-run evaluation of the inferential procedures), fixing statistical parameters as significance level and power, but are then analyzed by using a Fisherian point of view (eg. computing p-values and making inference based on its value, in comparison to common thresholds).

We must remember that the screening is conceptually defined as tests performed on apparently healthy people to identify those at an increased risk of a disease or disorder [32]. According to the literature, for screening to be accurate, a good screening test must have high sensitivity (few false-negative results) and a high specificity (few false-positive results) [33] and even very good tests have poor positive predictive value when applied to low-prevalence populations [34].

We would like to emphasize that Fernandes et al. [1] have provided an important scientific contribution with their study on RHR, and that criticism is an integral part of scientific progress. As the pediatrician John Locke said, "…every step the mind takes in its progress towards knowledge makes some discovery, which is not only new, but the best too, for the time at least."

Summary

The main indicators that must be taken into account for evaluation of a screening test to measure the potential for discrimination for a common variable (population with outcome vs. no outcome population) are: sensitivity, specificity, accuracy, positive

predictive value and negative predictive value. The measures of argumentation equality (CI) or difference (p-valor) are important to validate these indicators but do not indicate quality of screening.

We believe the statistical methodologies employed in support of science should consider the objectives of the paper, type of data available (with the least possible transformations) and statistical assumptions in order to answer scientific hypotheses. The interpretation of statistical data has to be made very carefully, otherwise science loses its footing and becomes a relentless pursuit of the "p-value <0.05".

Competing interests

The remaining authors state no conflict of interest.

Abbreviations

RHR: resting heart rate; HR: heart rate; p-value: descriptive level; deff: design effect; CI: confidence interval; AUC: accuracy.

Authors' contributions

ACFM and AJFC made substantial contributions to the conception and interpretation of the material; ACFM, HBC, AJFC, LAM were involved in drafting the manuscript and revising it critically for important intellectual content and approval of the version to be published.

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3.3 PREVALENCE OF CARDIOVASCULAR RISK FACTORS AMONG LATIN AMERICAN ADOLESCENTS: A MULTILEVEL ANALYSIS – PLUBLISHED IN JOURNAL OF HUMAN HYPERTENSION

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ABSTRACT

High blood pressure (HBP) and obesity is a well-established major risk factor for stroke and coronary heart disease. However, the literatures are scarce about these information's in adolescents from low-and-middle income countries. This school-based survey was carried out among students from Maringá (Brazil) and Buenos Aires (Argentina) selected random sampling. We studied 991 Brazilian adolescents (54.5% girls) age range 14 to 18 years. In Argentina we studied 933 adolescents (45.9% female) age range 11 to 17 years. The outcomes of this study are: general obesity, abdominal obesity and HBP. The associated factors analyzed were: gender, age and health behaviours. The prevalence of obesity was 5.8% in Brazil and 2.8% in Argentina, the prevalence of abdominal obesity 32.7% in Brazil and 11.1% in Argentina, HBP 14.9% in Brazil and 13.5% in Argentina. The multilevel analysis showed that older adolescents (>14 years old) have a little likelihood of being overweight, while male adolescents are more likely to be obese and have HBP. For the abdominal obesity in both indicators were not associated with the independent variables. The prevalence of cardiovascular

risk factors is high in Latin American adolescents independent of each country, and was associated with male gender.

Key-words: cardiovascular health; pediatrics; population; risk factors; low-and-middle countries; multicenter study.

INTRODUCTION

Cardiovascular diseases are the main sources of disease burden worldwide, and constitute a major public health problem in many countries[1]. Several studies have demonstrated that general obesity (measured by body mass index [BMI]), abdominal obesity and high blood pressure (HBP) in adolescence, are a good predictor for cardiovascular disease in adulthood.

In epidemiological studies, anthropometry has been considered an efficient method for diagnosis of obesity[2, 3]. The measurement of waist circumference (WC) represents a good marker of abdominal obesity. The other anthropometric indicator of abdominal fatness is waist to height ratio (WHtR); this is an easily measurable anthropometric index and predicts cardiovascular disease, diabetes and other metabolic risk factors [4]. The advantage of measuring WHtR is that a single value could be useful in different ethnic, age and sex groups[5], while WC requires population-specific boundary values[6]. Abdominal obesity, evaluated through the WC, plays a central role in the metabolic syndrome (MS), an entity that predisposes affected people to the development of diabetes and cardiovascular disease, and is associated with insulin resistance[7 8].

On the other hand, HBP is an established major risk factor for stroke and coronary heart disease.[9] The literature highlights, particularly in developing countries[1], increasing prevalence of childhood obesity in the last decades, which may influence the prevalence of HBP in adolescents.

Abdominal obesity and HBP are components of MS according to the criterion of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) [10]. Nevertheless the high prevalence of abdominal obesity and HBP, the analysis of the association of these outcomes with modifiable risk are scarce in adolescents from developing countries. It is therefore essential to try to identify and analyze the most significant variables involved in the development of MS in this population.

Reproducing the same results in different population groups with different characteristics would increase their biological plausibility and provide a higher level of scientific evidence. For this reason, we analyzed two cross-sectional studies conducted with adolescents: one developed in Argentina and other Brazil. The objective of this study was to evaluate the prevalence of cardiovascular risk factors among Latin American adolescents from two countries.

SUBJECTS AND METHODS

In this study we analyzed two cross-sectional studies previously carried out in the city of Maringá, located in the northwest of Paraná state (PR), Southern Brazil, which has a population of approximately 330,000 (51,428 adolescents, 50.1% female) and in Buenos Aires, capital city of Argentina, which has a population of 2.891.000 (53% female). In Brasil, a formal request to conduct this survey was sent to and subsequently accepted by the school boards of several schools in the city, those adolescents that met the inclusion criteria were admitted in the study. In Argentina, from a sample of 1023 high school adolescents who underwent a medical mandatory examination to be admitted in the High School of Buenos Aires University, those that met the inclusion criteria were included. All the participants were from the City of Buenos Aires or its suburbs, belonged to middle class and 96.2% of them were white Caucasians. Recruitment of students took place from 5 to 30 May 2008. The study

included 991 adolescents (540 females and 451 male, 14.0–17.5 yr) from Maringá and 943 (429 females and 514 males; 11–14 yr) from Buenos Aires. The complete methodology of this study has been described earlier [11, 12].

In both cities, adolescents were investigated on a broad battery of cardiovascular risk factors and health behavior parameters. Trained doctors interviewed them and participants completed a questionnaire dealing with habits, family, and personal background. Weight, height, WC, and blood pressure (BP) measurement were registered.

The protocol was approved by the Human Research Review Committees of the centers involved.

Outcomes

Blood Pressure Measurements

In both cities, BP measurements were performed following the recommendations that have been formulated for adolescents' populations [13]. The BP in both studies was measured twice after weight and height measurements. Subjects seated in a separate and quiet room for 10 min with their back supported and feet on the ground. Two BP readings were taken at a 10-min interval. The lower level of the two measures was used.

Systolic BP and Diastolic BP were measured in both countries using the oscillometric monitor OMRON HEM 742. This monitor was clinically and epidemiologically validated for adolescents for the Brazilian Research Group [14]. For the data collection in Argentina it was measured using the conventional method, approved by the British Hypertension Society[15].

Anthropometrics Measurements

BMI; was calculated as weight (kg)/height (m^2). The height was measured to the nearest 0.1cm and the body mass to the nearest 0.1kg, with a wood stadiometer and a calibrated portable digital scale, respectively (wearing light clothes and no shoes). Nutritional status, based on adolescents' BMI, was classified according to cutoff points for sex and age in three categories: normal weight; overweight and obese[16].

WC; was measured in both studies at the midpoint between the lowest rib cage and the top of the iliac crest with a non-elastic tape to the nearest 0.1 cm. Based on the evaluation of WC we classified the abdominal obesity (yes or no) according to cutoff points for sex and age[17].

WHtR; was calculated by dividing the waist size by the height, both in cm, and categorized into: <0.5= no abdominal obesity and ≥ 0.5= abdominal obesity[18].

Independent variables

The independent variables investigated were: gender, age (categorized in: ≤ 14 years old and ≥ 15 years old), smoking (yes: one or more cigarettes per day in the last 30 days, or no), alcohol consumption (yes: ingest of at least an alcoholic beverage once in the last 30 days or no), screen sedentary time (≥ 4h/day or < 4h/day) and sports participating (yes: trained any sport at least three times per week in the last 30 days, or no).

Statistical Analysis

The descriptive analyses were presented as means (quantitative variables), percentages (qualitative variables) and confidence intervals of 95% (CI95%). Multilevel Poisson regression model with robust variance adjustment, which is recommended for high prevalence outcomes[19], with a CI95% calculated for the prevalence ratio (PR) using fixed effects intercept were fitted to analyze the relationship between each outcome and independent variables[20], considering two levels of data organization: (i)

the individual behaviors; (ii) potential confounders (not shown). The context variable used was the school.

The p-values $\leq$ 0.20 were adopted in the univariate analysis[21] (as necessary to include variables in the multivariate analysis and then it was entered through the hierarchical model method following the levels above). Significance was adopted when p-values were <0.05 or when there was more than 10% modification in PR of any variable already in the model.

The multilevel analyzes were performed with two objectives: 1st) to test the associations between BP and two separate measures of individual behaviors; 2nd) to test the extent to which country specific characteristics and context variables mediate the associations between outcomes and independent variables.

The statistical software package Stata version 12.0 (Stata Corp., college Station, TX, USA) was used for all statistical calculations. All analyses were adjusted for the clustered nature of the sample using the Stata "svy" set of commands.

RESULTS

The prevalence of independent variables is shown in Table 1. Brazilian adolescents were older and the percentage of girls was higher. Argentinean adolescents presented lower prevalence of smoking and alcohol consumption; on the other hand Argentinean adolescents practice more sports than Brazilian, but spend more sedentary time.

In both countries we found high prevalence of cardiovascular risk factors, Figure 1 shows the prevalence of each risk factor for girls and Figure 2 shows the prevalence of each risk factor for boys. Brazilian adolescents of both sexes showed higher prevalence (p <0.001) of abdominal obesity (measured by WC) than Argentinean adolescents. On

the other hand, the prevalence of overweight (measured by BMI) was higher in Argentina than in Brazil, in both sexes.

The adjusted analysis showed that the older adolescents (> 14 years old) were less likely to have overweight (PR = 0.81, CI 95%= 0.76 - 0.83), while boys were more likely to have obesity (PR = 1.95, CI 95%= 1.41 - 2.67) and HBP (PR = 1.08, CI 95%= 1.08 - 1.74). The lifestyle variables were not significantly associated with any cardiovascular risk factor.

Table 1: The prevalence of independent variables.

Variable		Country	
		Brazil (n= 991)	Argentina (n=933)
Sex			
	Boys	45,5	54,5
	Girls	54,5	45,5
Age group			
	≤ 14 years old	8.9	2.2
	≥ 15 years old	91.2	97.8
Screen sedentary time (≥ 4h/day)			
	No	18,3	1.1
	Yes	81,7	98.9
Sports participating			
	No	56.9	40.3
	Yes	43.1	59.7
Smoking			
	No	94,2	99.3
	Yes	5,8	0.7
Alcohol consumption			
	No	67.8	99.3
	Yes	32.2	0.7

Sports participating= trained any sports at least three time per week in the last 30 days.
Smoking= smoked one or more cigarettes per day in the last 30 days.
Alcohol consumption= ingest an alcoholic beverage at least once in the last 30 days.

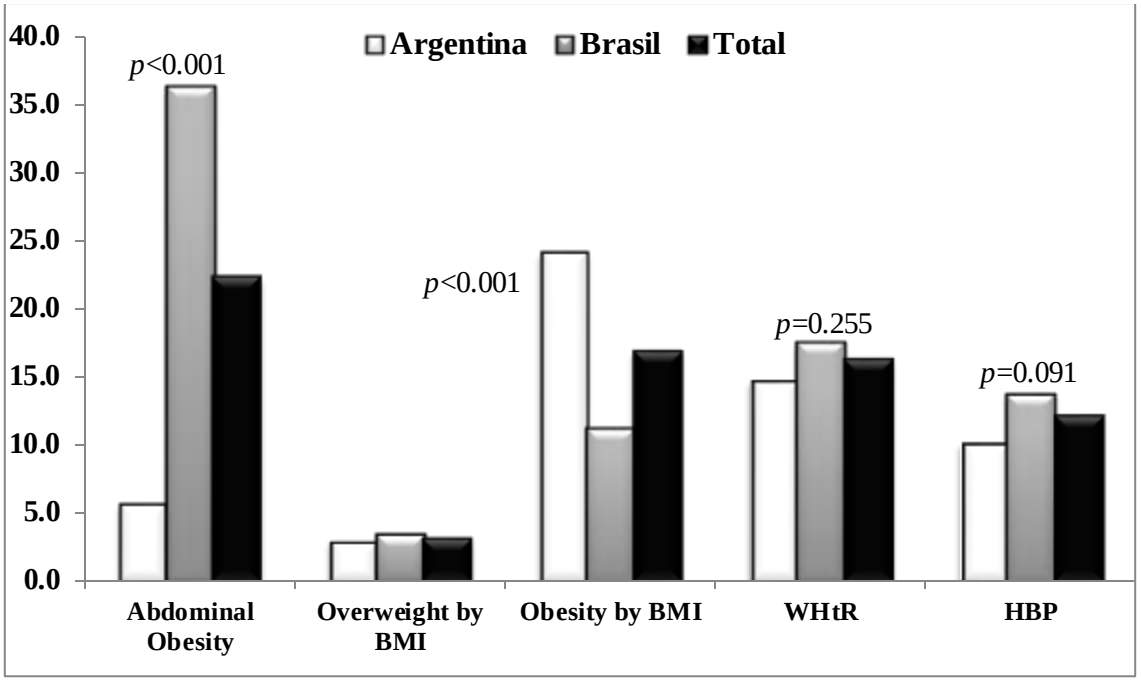

Figure 1: Prevalence of cardiovascular risk factors according country for girls.

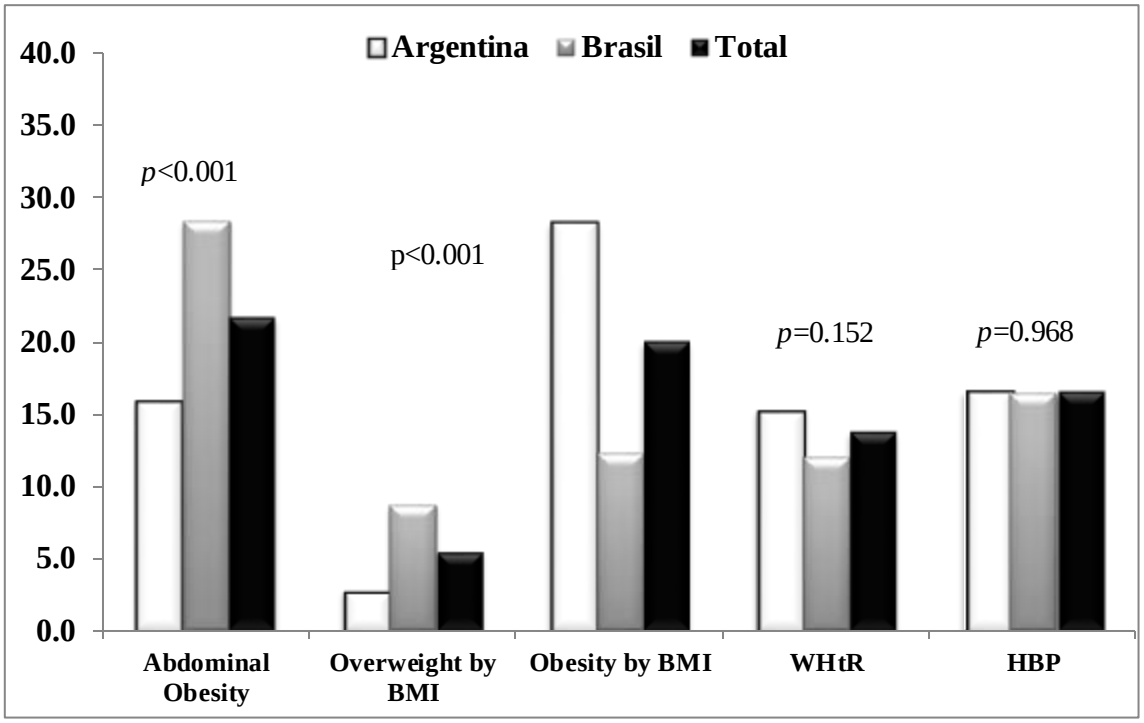

Figure 2: Prevalence of cardiovascular risk factors according country for boys.

DISCUSSION

The prevalence of four cardiovascular risk factors in adolescents from two observational studies has been explored and results suggest that the prevalence is high in both countries, but different between both countries, particularly for abdominal obesity and overweight. Boys presented more prevalence of HBP and girls showed more prevalence of obesity. These results, observed in two different studies carried out in adolescents', strengthen the conclusions and *is closely related with one of Hill's principles, consistency, that highlights: "different types of study populations and circumstances the results should be similar"* [22].

Obesity is a good indicator of unhealthy lifestyles, characterized by overeating and lack of physical activity. Obesity in childhood often follows into adulthood and is a major cardiovascular risk factor including diabetes, hypertension, dyslipidemia and coronary artery disease demonstrated by several epidemiological studies[23].

The fat distribution is also an important factor; excess of visceral or central fat is associated with an increased risk of cardiovascular disease[24]. Waist circumference is a surrogate marker of abdominal adiposity composed by subcutaneous and intra-abdominal adipose tissue. Although computed tomography and magnetic resonance imaging are "Gold Standard" methods for determining the quantity and quality of adiposity[25], the use of BMI, which provides information about the volume and body mass, and WC, which provides information about fat distribution, are recommended, given their easier measurement. Overall BMI and WC are highly correlated as demonstrated in some studies[4, 17]. However, in our study no coincidence has been observed. Brazilian adolescents showed higher prevalence of abdominal obesity (measured by WC) than Argentinean adolescents and the prevalence of overweight (measured by BMI) was higher in Argentina than in Brazil, in both sexes.

The WHtR index does not differ in specificity and sensitivity from classic anthropometric parameters (WC and BMI), moreover, it is easier to apply in clinical practice because of not requiring reference tables either being independent of age, sex, pubertal status and ethnicity. Regarding this index, we observed no significant differences in both groups, but, we found a relationship between central obesity and WHtR in girls, not seen in boys.

The results suggest that prevalence of these risk factors is high in both countries, but the prevalence is different between them in reference to the abdominal obesity and overweight. Boys presented more prevalence of HBP and girls showed more prevalence of obesity. These results, observed in two different studies carried out in adolescents', strengthen the conclusions.

The finding of a high prevalence of the outcomes analyzed in this study corroborate the data available in the literature, according to the National Center for Health Statistics (NCHS) reported by Ogden et al.[26], one in five American children are overweight. In Europe, Jiménez-Pavón et al. found that 15% of the adolescents had less favourable systolic blood pressure, [27]. These results suggest that cardiovascular risk factors have high prevalence in adolescents worldwide [28].

Even when there are limited data linking cardiovascular risk in adolescents we have recently demonstrated increased levels of apoB, the main protein component of LDL, in our group of adolescents. We also found that TG/HDL ratio was increased, and correlates with BMI and WC in our whole population. The ratio TG/HDL seems to be a simple and useful method to identify insulin resistant patients and also predict cardiovascular disease [12].

One constraint of the present study is its cross-sectional design, so causality can therefore not be established. Another important limitation is the evaluation of

modifiable behaviors, which was self-reported. However, it would be difficult to use more accurate methods, such as direct observation, since logistics are complicated and expensive in a population-based epidemiological study. Finally, the difference of age range between the adolescents of both countries, as well as the different geographic origin of the samples, were evaluated through the use of a multilevel adjusted analysis which is one of the main strengths of our study.

In the context of promoting healthy behaviors among adolescents has been observed that interventions which has carried out at school had positive effects when combined with printed educational materials and changes in the school [29]. Therefore, given the high prevalence of cardiovascular risk factors reported in the present study, interventions should be carried out in order to reduce the prevalence of abdominal obesity-associated risk behaviors, preferably through programs integrated into the curriculum of the school and monitored by parents and teachers.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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What is already known on this subject?

High blood pressure (HBP) is a well-established major risk factor for stroke and coronary heart disease.

Several studies have reported high prevalence of risk factors for cardiovascular disease in adolescents from low-and-middle income countries.

What does this study add?

The prevalence of cardiovascular risk factors is high in Latin American adolescents independent of each country.

The male adolescents are more likely to be obese and have HBP than girls.

3.4 INDEPENDENT AND COMBINED EFFECTS OF PHYSICAL ACTIVITY AND SEDENTARY BEHAVIOR ON BLOOD PRESSURE IN ADOLESCENTS: GENDER DIFFERENCES IN TWO CROSS-SECTIONAL STUDIES – PLUBLISHED IN PLOS ONE

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ABSTRACT

Objectives: To examine the independent and combined association of physical activity (PA) and sedentary behavior (SB) on both systolic (SBP) and diastolic blood pressure (DBP) in adolescents from two observational studies.

Methods: Participants from two cross-sectional studies, one conducted in Europe (n= 3,308; HELENA study) and the other in Brazil (n=991; BRACAH study), were selected by complex sampling. Systolic and diastolic blood pressure (outcomes), PA and SB,

both independently and combined, and potential confounders were analyzed. Associations were examined by multilevel linear regression.

Results: Performing the recommended amount of PA (≥60min/d) attenuated the effect of SB on DBP in BRACAH study girls and in boys from both studies. In contrast, PA did not attenuate the effects of SB on the SBP of girls in the HELENA study. The combination of less than recommended levels of PA with 2-4 h/d of sedentary behavior was found to be associated with increased SBP in boys from both studies.

Conclusions: Meeting current PA recommendations could mediate the association between SB and DBP in both sexes. In boys, the joint effect of low levels of PA and excessive sedentary activity increases SBP levels. Longitudinal studies are required to confirm these findings.

INTRODUCTION

Chronic non-communicable diseases (NCDs) are the main source of disease burden worldwide and are thus a major public health problem [1]. Among NCDs, hypertension has been shown to have the highest prevalence in adults [2], and studies have shown that blood pressure (BP) levels in childhood and adolescence greatly impact the development of hypertension in adulthood [3].

Among the factors that may influence blood pressure levels (e.g. genetics, intrauterine development, socioeconomic status, tobacco use, waist circumference, obesity), patterns of physical activity (PA) and sedentary behavior (SB) have been shown to have inverse [4] and direct associations,[5,6] respectively, with blood pressure in adolescents.

Although the effects of PA and SB on BP have mainly been examined in isolation, there are studies suggesting that these behaviors have an aggregate effect on

adolescents [7,8]; however, few studies have quantified the association between joint PA/SB levels and blood pressure in adolescents [7,8]. On the other hand, PA/SB levels are associated with sociodemographic and economic variables. The influence of sociodemographic factors on PA/SB bas been described in a review [9]. There is no consensus in the literature regarding socioeconomic variables as determinants of these behaviours since such differences may be attributed to the demographic context and characteristics of the populations studied rather than the individual [10,11]. For this reason, we have included results from a multi-national European study and another one conducted in South America (Brazil) in this report.

Reproducing the same results in different population groups with different characteristics would increase their biological plausibility and provide a higher level of scientific evidence. For this reason, we tested the hypothesis, separately, in two cross-sectional studies conducted with adolescents: Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) and Brazilian Cardiovascular Adolescent Health (BRACAH).

Thus, we hypothesized that higher levels of PA would attenuate the adverse effect of high SB levels on BP and that the combined effect of low PA and high SB levels may contribute to increased BP levels and that these effects may be different depending on where adolescents live.

METHODS

The HELENA study was based on data from a random sample of European adolescents who were tested on a wide range of nutrition and health-related parameters. The data were collected in 2006 and 2007 in ten cities from nine European countries. A detailed description of the HELENA sampling and recruitment methodology, harmonization processes, data collection, analysis strategies and quality control

activities has been published elsewhere [12]. After receiving complete information about the aims and methods of the study, all parents/guardians signed an informed consent form and the adolescents agreed to participate in the study. The protocol was approved by the Human Research Review Committees of the centers involved.

Data from the BRACAH study were collected in 2007 in the city of Maringá, PR, Brazil, population approximately 330,000 (51,428 adolescents, 50.1% female). The adolescents were selected by random sample and evaluated on a broad range of cardiovascular risk factors and various health behavior parameters. The complete sample size methodology of this study has been described previously [13]. A formal request to conduct this survey was submitted to and accepted by the boards of several public and private schools. This study was also approved by the Ethics Committee on Research Involving Human Participants of the University Center of Maringá and authorized by the Ethics Committee on Research Projects of the University of São Paulo in accordance with Brazilian laws.

For the current study, we selected adolescents from HELENA and BRACAH with complete data regarding gender, age, systolic BP (SBP), diastolic BP (DBP) (outcomes), PA levels, SB, socioeconomic status, parental education, regular tobacco consumption, body mass index and waist circumference. These variables are described in detail below.

A total of 3,308 adolescents from the HELENA study (12.5–17.5 years old) and 991 adolescents from the BRACAH study (14.0–17.5 years old) met all the inclusion criteria and were included in the analyses.

Blood Pressure Measurements

In both studies, BP measurements were performed following the recommendations for adolescent populations [14]. In both studies BP was measured

twice after weight and height measurements were taken. The subjects were seated in a separate, quiet room for 10 min with their backs supported and feet on the ground. Two BP readings were taken with a 10 min interval of quiet rest. The lower of the two measurements was used.

Systolic and Diastolic BP were measured by the arm blood pressure oscillometric monitor device OMRON® M3 (HEM 742) in the BRACAH study and the OMRON® M6 (HEM 70001) in the HELENA study. The OMRON® M3 (HEM 742) has been clinically and epidemiologically validated for adolescents by the Brazilian Research Group[15]. The OMRON® M6 (HEM 70001) has been approved by the British Hypertension Society [16]. These data collection procedures have been described in an earlier study [17].

Independent variables

The PA and SB levels were considered independent variables and measured by means of questionnaires in both studies. The questionnaire model used for PA measurements in both studies was developed to assess PA levels (moderate-to-vigorous levels) in adolescents [18].

In the HELENA study, PA was also measured with accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) for seven consecutive days, with a minimum of 8 hours recording/day for at least 3 days [19]. The time sampling interval (epoch) was set to 15 seconds. Inactive, moderate and vigorous PA was defined as <100, 2000-3999 and ≥4000 counts per minute, respectively. The cutoffs selected were similar to those used in previous studies [20,21]. In both methodologies (questionnaire and accelerometry), and following current PA guidelines, [22,23] subjects were classified as active when they accumulated at least 60 min/d of moderate-to-vigorous PA.

Sedentary behavior levels were assessed with a structured questionnaire, including questions on time habitually spent in front of the television, the computer and/or playing video games. In both studies, the questionnaire used questions such as "*During weekdays, how many hours do you usually spend watching television*?" - "*During weekdays, how many hours do you usually spend on computers? - "During weekdays, how many hours do you usually spend playing video games?"* Sedentary behavior was totaled and classified into the following categories: 0-2 h/d; >2 – 4 h/d; ≥4 h/d according to Dunstan et al. [24]. The same questions were asked for weekend days and this questionnaire was used with adolescents from both studies as a realiability, validity and translated tool [25-27].

We also established six clusters of PA according to PA recommendations [22,23] and SB according to Dunstan et al. [24] for use with both measurement methods, which are described below.

Questionnaire:

1. < 60 min/d of PA + > 4 h/d of SB;
2. < 60 min/d of PA + 2 - 4 h/d of SB;
3. < 60 min/d of PA + < 2 h/d of SB;
4. ≥ 60 min/d of PA + > 4 h/d of SB;
5. ≥ 60 min/d of PA + 2 - 4 h/d of SB;
6. ≥ 60 min/d of PA + < 2 h/d of SB;

Accelerometer (using PA recommendations and tertiles of sedentary time):

1. < 60 min/d of PA of PA + 3rd tertile of SB;
2. < 60 min/d of PA + 2nd tertile of SB;
3. < 60 min/d of PA + 1st tertile of SB;
4. ≥ 60 min/d of PA + 3rd tertile of SB;

5. ≥ 60 min/d of PA + 2nd tertile of SB;
6. ≥ 60 min/d of PA + 1st tertile of SB;

Potential confounders

The potential confounders for this study were:

- Country (HELENA only):
- Age (years):
- Socioeconomic status: based on the family's household goods. In the HELENA study, the same definitions were used in previous HELENA studies [28,29]. In the BRACAH study, the Brazil Criterion of Economic Classification [30] was employed. Three levels were used to classify socioeconomic status: low, medium and high.
- Parental education: determined with a self-reported questionnaire and classified into four levels: elementary education, lower secondary education, upper secondary education and university degree.
- Regular tobacco smoking: defined as the regular consumption of at least one cigarette per day for a minimum of one month [31];
- Body mass index (BMI): calculated as weight (kg)/height(m^2). BMI was used as a continuous variable in the analysis. Wearing light clothes and no shoes, the adolescents' height was measured to the nearest 0.1cm with a wood stadiometer and their body mass to the nearest 0.1kg with a calibrated portable digital scale.
- Waist circumference: measured in both studies at the midpoint between the lowest point of the rib cage and the top of the iliac crest next to skin with a non-elastic measuring tape to the nearest 0.1 cm.

Statistical Analysis

The descriptive analyses were presented as means (quantitative variables), percentages (qualitative variables) and 95% confidence intervals (CI95%). Multilevel linear regression models using fixed effects intercept were fitted to analyze the relationship between each BP level and independent variables [32,33], considering two levels of data organization: (i) individual behaviors and (ii) potential confounders (not shown) [34]. The context variable used was the school. Homoscedasticity was graphically assessed in all regression models to meet the analysis criteria. *p*-values of $\leq$0.20 were adopted in the univariate analysis [34] since they were necessary to include variables in the multivariate analysis and then the hierarchical model method according to the above-mentioned levels. *P*-values <0.05 or those representing >10% modification in the β of any variable already in the model were considered significant.

The multilevel analyses were performed with two objectives: 1st) to test the associations between BP and two separate measures of individual behavior; 2nd) to test the extent to which country-specific characteristics and contextual variables mediate the associations between SBP and DBP levels and PA and SB.

Stata 12 (Stata Corp., College Station, TX, USA) was used for all statistical calculations. All analyses were adjusted for the clustered nature of the sample using the "svy" set of commands and stratified by gender, since interactions between sex and the studied variables were observed (p<0.001).

For adolescents from the HELENA study (boys =1,106; girls =960) we conducted a comparative analysis between the PA and SB levels found with the questionnaires and the PA measures found with and without the use of accelerometers. No significant differences were found for either sex (p=0.406 for boys and p=0.714 for girls).

RESULTS

Subject characteristics, sociodemographic/socioeconomic variables, BP levels and PA and SB levels (independent and combined) are shown in a supplementary file. Table 1 shows the β coefficients from multilevel linear regression for DBP in girls. Table 2 shows the association between PA and SB patterns and DBP for boys. There was a positive association between those adolescents who did not meet the PA guidelines and DBP in the HELENA study. However, these associations were not significant after adjusted analysis (Table 2).

A positive and significant association was found for HELENA study girls, after adjustment for confounding variables, between ≥ 60 min/d of PA + 3rd tertile of SB and systolic BP (Table 3). The association between PA and SB patterns and SBP for boys is presented in Table 4. No independent variables showed significant association after adjustment for confounding variables.

Figure 1 shows β coefficients and their respective 95%CI, evaluating the association between blood pressure levels and clusters of PA and SB measured by questionnaire for each study according to sex (A for boys; B for girls). After conducting the adjusted analysis, the PA and SB cluster levels: <60 min/d + <2 h/d **β= -2.17 (95%CI= -2.81 - -1.53)**; ≥ 60 min/d +> 4 h/d **β= -3.02 (95%CI= -5.14 - -0.90)**; and ≥ 60 min/d + 2 - 4 h/d **β= -6.05 (95%CI= -10.16 - -1.94)** remained significantly and negatively associated with DBP in the girls from the BRACAH study.

Table 1: Multiple linear regression analysis evaluating the association between diastolic blood pressure according to independent variables for each study, in girls. Beta coefficient and their respective confidence intervals 95% (95% CI).

Independents Variables	**Null model**		**Unadjusted**		**Adjusted***	
	HELENA	**BRACAH**	**HELENA**	**BRACAH**	**HELENA**	**BRACAH**
	ß (95% CI)	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**
Fixed Effects Constant	68.5 (68.1 - 68.9)	67.1 (66.2 - 68.0)			56.6 (51.2 – 61.9)	78.9 (69.1 – 88.8)
Physical activity by questionnaire			p=0.894	p=0.011	p=0.737	p=0.123
≥ 60 min/d			Ref	Ref	Ref	Ref
< 60 min/d			0.08 (-1.06 - 1.21)	**2.56 (0.59 -4.53)**	-0.18 (-1.58 – 1.23)	1.68 (-0.46 – 3.82)
Sedentary behavior by questionnaire			p=0.066	p<0.001	p=0.54	p=0.501
< 2 h/d			Ref	Ref	Ref	Ref
2 - 4 h/d			0.41 (-0.57 - 1.39)	**10.49 (4.83 - 16.15)**	-0.02 (-1.18 – 1.14)	3.01 (-3.13 – 9.15)
> 4 h/d			1.10 (-0.06 - 2.26)	**13.31 (8.15 - 18.47)**	0.37 (-1.03 – 1.78)	2.83 (-2.75 – 8.41)
Physical activity by accelerometers**			p=0.159		p= 0.281	
< 60 min/d			Ref		Ref	
≥ 60 min/d			0.79 (-0.31 - 1.89)		0.72 (-0.52 – 2.04)	
MPA by accelerometers (min/d)			p= 0.499		p= 0.765	
			0.45 (-0.86 – 1.76)		-0.01 (-0.05 – 0.03)	
VPA by accelerometers (min/d)			p= 0.824		p= 0.163	
			-0.01 (-0.05 – 0.04)		-0.04 (-0.09 – 0.02)	
MVPA by accelerometers (min/d)			p= 0.41		p= 0.342	
			-0.04 (-0.10 – 0.01)		-0.01 (-0.04 – 0.02)	

SB by accelerometers (min/d)			p= 0.707 -0.01 (-0.02 – 0.01)	p= 0.544 0.01 (-0.01 – 0.01)	
Cluster PA and SB by accelerometers*			p=0.265	p=0.415	
< 60 min/d + 3° tercil			Ref	Ref	
< 60 min/d + 3rd tertile			-0.05 (-1.65 - 1.56)	-0.15 (-1.79 - 1.49)	
< 60 min/d + 2nd tertile			-0.26 (-2.07 - 1.55)	-0.35 (-2.19 - 1.50)	
< 60 min/d + 1st tertile			-0.04 (-2.53 - 2.45)	-0.16 (-2.81 - 2.48)	
≥ 60 min/d + 3rd tertile			-1.24 (-3.41 - 0.92)	-1.52 (-3.79 - 0.74)	
≥ 60 min/d + 2nd tertile			-0.80 (-2.95 - 1.36)	-0.29 (-2.56 - 1.98)	
Intraclass correlation coeficient	0.09	0.02		0.09	0.06
Standard deviation context	2.74	1.62		3.88	2.68
Standard deviation individual	8.74	10.41		9.88	10.96
Akaike Information Criterion	11,929.8	4,043.2		11,084.5	4,116.5

*This analysis was adjusted for potential confounders: *age, socioeconomic status, parental education, regular tobacco smoking, body mass index and waist circumference.*

**622 girls were excluded because they did not meet the inclusion criteria.

MPA= moderate physical activity.

VPA= vigorous physical activity.

MVPA= moderate and vigorous physical activity.

PA= physical activity.

SB= sedentary behavior.

Significant associations are in bold.

Table 2: Multiple linear regression analysis evaluating the association between diastolic blood pressure according to independent variables for each study, in boys. Beta coefficient and their respective confidence intervals 95% (95% CI).

Independents Variables	**Null model**		**Unadjusted**		**Adjusted***	
	HELENA	**BRACAH**	**HELENA**	**BRACAH**	**HELENA**	**BRACAH**
	ß (95% CI)	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**
Fixed Effects Constant	67.8 (67.4 - 68.3)	68.9 (68.1 - 69.9)			58.7 (52.9 – 64.4)	44.7 (35.9 – 53.4)
Physical activity by questionnaire			p=0.002	p=0.178	p= 0.012	p= 0.178
≥ 60 min/d			Ref	Ref	Ref	Ref
< 60 min/d			**2.02 (0.73 - 3.30)**	1.41 (-0.65 - 3.46)	**2.01 (0.43 – 3.59)**	1.43 (-0.65 – 3.51)
Sedentary behavior by questionnaire			p=0.312	p=0.575	p= 0.477	p= 0.671
< 2 h/d			Ref	Ref	Ref	Ref
2 - 4 h/d			**1.29 (0.06 - 2.52)**	-0.03 (-4.68 - 4.63)	1.80 (-0.34 – 3.26)	0.57 (-4.15 – 5.30)
> 4 h/d			0.83 (-0.40 2.52)	0.62 (-3.76 - 5.00)	0.81 (-0.67 – 2.29)	0.87 (-3.56 – 5.30)
Physical activity by accelerometers**			p= 0.575		p= 0.475	
< 60 min/d			Ref		Ref	
≥ 60 min/d			0.37 (-0.94 – 1.70)		0.50 (-0.55 – 0.95)	
MPA by accelerometers (min/d)			p= 0.767		p= 0.952	
			-0.01 (-0.05 – 0.04)		0.01 (-0.04 – 0.05)	
VPA by accelerometers (min/d)			p= 0.487		p= 0.647	
			-0.02 (-0.06 – 0.03)		-0.01 (-0.06 – 0.03)	
MVPA by accelerometers (min/d)			p= 0.566		p= 0.832	
			-0.01 (-0.03 – 0.02)		-0.01 (-0.03 – 0.02)	
SB by accelerometers (min/d)			p= 0.729		p= 0.942	
			0.01 (-0.01 – 0.01)		0.01 (-0.01 – 0.02)	

Cluster PA and SB by accelerometers*			p= 0.681	p=0.455
< 60 min/d + 3° tercil			Ref	Ref
< 60 min/d + 3rd tertile			- (0.29 (2.73 - 215)	-0.82 (-3.34 - 1.96)
< 60 min/d + 2nd tertile			-0.08 (-2.51 - 2.34)	-0.29 (-2.77 - 2.19)
< 60 min/d + 1st tertile			0.48 (-1.91 - 2.86)	0.29 (-2.16 - 2.76)
≥ 60 min/d + 3rd tertile			-1.99 (-4.17 -0.18)	-2.19 (-4.42 - 0.03)
≥ 60 min/d + 2nd tertile			0.17 (-1.82 - 2.16)	-0.15 (-2.27 - 1.97)
Intraclass correlation coeficient	0.10	0.07		0.13	0.05
Standard deviation context	3.46	2.54		3.96	2.09
Standard deviation individual	10.53	9.51		10.27	9.33
Akaike Information Criterion	11,533.8	3286.2		10,324.3	3,042.6

*This analysis was adjusted for potential confounders: *age, socioeconomic status, parental education, regular tobacco smoking, body mass index and waist circumference.*

**420 boys were excluded because they met the inclusion criteria.

MPA= moderate physical activity.

VPA= vigorous physical activity.

MVPA= moderate and vigorous physical activity.

PA= physical activity.

SB= sedentary behavior.

Significant associations are in bold.

Table 3: Multiple linear regression analysis evaluating the association between systolic blood pressure according to independent variables for each study, in girls. Beta coefficient and their respective confidence intervals 95% (95% CI).

Independents Variables	**Null model**		**Unadjusted**		**Adjusted***	
	HELENA	**BRACAH**	**HELENA**	**BRACAH**	**HELENA**	**BRACAH**
	ß (95% CI)	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**
Fixed Effects Constant	117.6 (115.7 – 119.6)	107.0 (101.1 - 108.0)			90.4 (83.4 – 97.5)	77.2 (66.9 – 87.5)
Physical activity by questionnaire			p=0.844	p=0.054	p= 0.443	p=0.099
≥ 60 min/d			Ref	Ref	Ref	Ref
< 60 min/d			-0.15 (-1.62 - 1.33)	2.11 (-0.04 - 4.27)	-0.64 (-2.38 – 1.11)	1.63 (-0.50 – 3.77)
Sedentary behavior by questionnaire			p=0.130	p=0.402	p= 0.963	p= 0.501
< 2 h/d			Ref	Ref	Ref	Ref
2 - 4 h/d			0.43 (-0.83 - 1.70)	4.05 (-2.30 - 10.39)	-0.20(-1.63 – 1.24)	3.47 (-2.65 – 9.61)
> 4 h/d			1.53 (0.04 - 3.03)	3.95 (-1.83 - 9.73)	0.05 (-2.39 – 1.82)	2.98 (-2.59 – 8.55)
Physical activity by accelerometers**			p= 0.635		p= 0.818	
≥ 60 min/d			Ref		Ref	
< 60 min/d			0.40 (-2.08 – 1.27)		0.20 (-1.99 – 1.53)	
MPA by accelerometers (min/d)			p= 0.921		p= 0.706	
			0.02 (-0.01 – 0.06)		-0.01 (-0.07 – 0.05)	
VPA by accelerometers (min/d)			p= 0.16		p= 0.176	
			-0.05 (-0.12 – 0.02)		-0.05 (-0.13 – 0.02)	
MPA by accelerometers (min/d)			p= 0.484		p= 0.364	
			-0.01 (-0.05 – 0.02)		-0.02 (-0.06 – 0.02)	
SB by accelerometers (min/d)			p= 0.328		p= 0.331	
			0.01 (-0.01 – 0.01)		0.01 (-0.01 – 0.01)	
Cluster PA and SB by accelerometers*			p=0.876		p=0.979	
< 60 min/d + 3rd tertile			Ref		Ref	
< 60 min/d + 2nd tertile			0.35 (-1.68 - 3.38)		0.14 (-1.86 - 2.13)	

< 60 min/d + 1st tertile			0.48 (1.81 -2.76)	-0.75 (-3.00 - 1.49)	
≥ 60 min/d + 3rd tertile			**3.59 (0.44 - 6.74)**	**3.61 (0.39 - 6.82)**	
≥ 60 min/d + 2nd tertile			-0.69 (-3.43 - 2.05)	-1.07 (-3.83 - 1.68)	
≥ 60 min/d + 1st tertile			-0.01 (-2.74 - 2.72)	0.22 (-2.53 - 2.98)	
Intraclass correlation coeficient	0.007	0.06		0.10	0.05
Standard deviation context	3.42	2.87		4.03	2.57
Standard deviation individual	12.75	11.4		11.88	10.92
Akaike Information Criterion	13.442.2	4123.9		10,978.2	4,115.1

*This analysis was adjusted for potential confounders: *age, socioeconomic status, parental education, regular tobacco smoking, body mass index and waist circumference.*

**622 girls were excluded because they did not meet the inclusion criteria.

MPA= moderate physical activity.

VPA= vigorous physical activity.

MVPA= moderate and vigorous physical activity.

PA= physical activity.

SB= sedentary behavior.

Significant associations are in bold.

Table 4: Multiple linear regression analysis evaluating the association between systolic blood pressure according to independent variables for each study, in boys. Beta coefficient and their respective confidence intervals 95% (95% CI).

Independents Variables	**Null model**		**Unadjusted**		**Adjusted***	
	HELENA	**BRACAH**	**HELENA**	**BRACAH**	**HELENA**	**BRACAH**
	ß (95% CI)	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**	**ß (95% CI)**
Fixed Effects Constant	124.81 (124.10 - 125.51)	119.64 (118.42 - 120.86)			84.4 (76.1 – 92.7)	
Physical activity by questionnaire			p= 0.005	p= 0.331	p= 0.066	
≥ 60 min/d			Ref	Ref	Ref	
< 60 min/d			**2.92 (0.90 - 4.96)**	**1.41 (-1.44 - 4.27)**	2.08 (-0.14 – 4.29)	
Sedentary behavior by questionnaire			p= 0.435	p= 0.811	p= 0.361	
< 2 h/d			Ref	Ref	Ref	
2 - 4 h/d			1.18 (-0.77 - 3.14)	-1.32 (-7.77 - 5.14)	1.02 (-1.01 - 3.05)	-0.96 (-7.75 – 5.84)
> 4 h/d			0.37 (-1.59 - 2.33)	-1.84 (-7.93 - 4.24)	-0.79 (-2.87 – 1.30)	-2.04 (-8.42 – 4.34)
Physical activity by accelerometers**			p= 0.399		p= 0.762	
≥ 60 min/d			Ref		Ref	
< 60 min/d			0.74 (-0.99 - 2.47)		-0.30 (-2.21 – 1.62)	
MPA by accelerometers (min/d)			p= 0.895		p= 0.859	
			0.13 (-1.78 – 2.04)		-0.01 (-0.05 – 0.07)	
VPA by accelerometers (min/d)			p= 0.133		p= 0.929	
			-0.05 (-0.11 – 0.01)		-0.01 (-0.06 – 0.07)	
MPA by accelerometers (min/d)			p= 0.646		p= 0.936	
			-0.01 (-0.05 – 0.03)		-0.01 (-0.04 – 0.04)	
SB by accelerometers (min/d)			p= 0.069		p= 0.337	

			0.01 (-0.01 – 0.02)	0.01 (-0.01 – 0.02)	
Cluster PA and SB by accelerometers*			p=0.673	p=0.465	
< 60 min/d + 3rd tertile			Ref	Ref	
< 60 min/d + 2nd tertile			-0.58 (-4.09 - 2.94)	-2.16 (-5.49 - 1.16)	
< 60 min/d + 1st tertile			0.36 (-3.14 - 3.85)	-0.09 (-3.37 - 3.18)	
≥ 60 min/d + 3rd tertile			0.88 (-2.56 - 4.31)	0.58 (-2.65 - 3.81)	
≥ 60 min/d + 2nd tertile			0.08 (-3.06 - 3.21)	0.30 (-2.65 - 3.24)	
≥ 60 min/d + 1st tertile			-0.88 (-3.75 - 1.98)	-0.06 (-2.86 - 2.74)	
Intraclass correlation coeficient	0.07	0.06		0.10	0.04
Standard deviation context	3.69	3.2		4.58	2.84
Standard deviation individual	13.98	13.2		13.91	13.40
Akaike Information Criterion	12,191.3	3,560.7		10,520.4	3,347.0

*This analysis was adjusted for potential confounders: *age, socioeconomic status, parental education, regular tobacco smoking, body mass index and waist circumference.*

**420 boys were excluded because they met the inclusion criteria.

MPA= moderate physical activity.

VPA= vigorous physical activity.

MVPA= moderate and vigorous physical activity.

PA= physical activity.

SB= sedentary behavior.

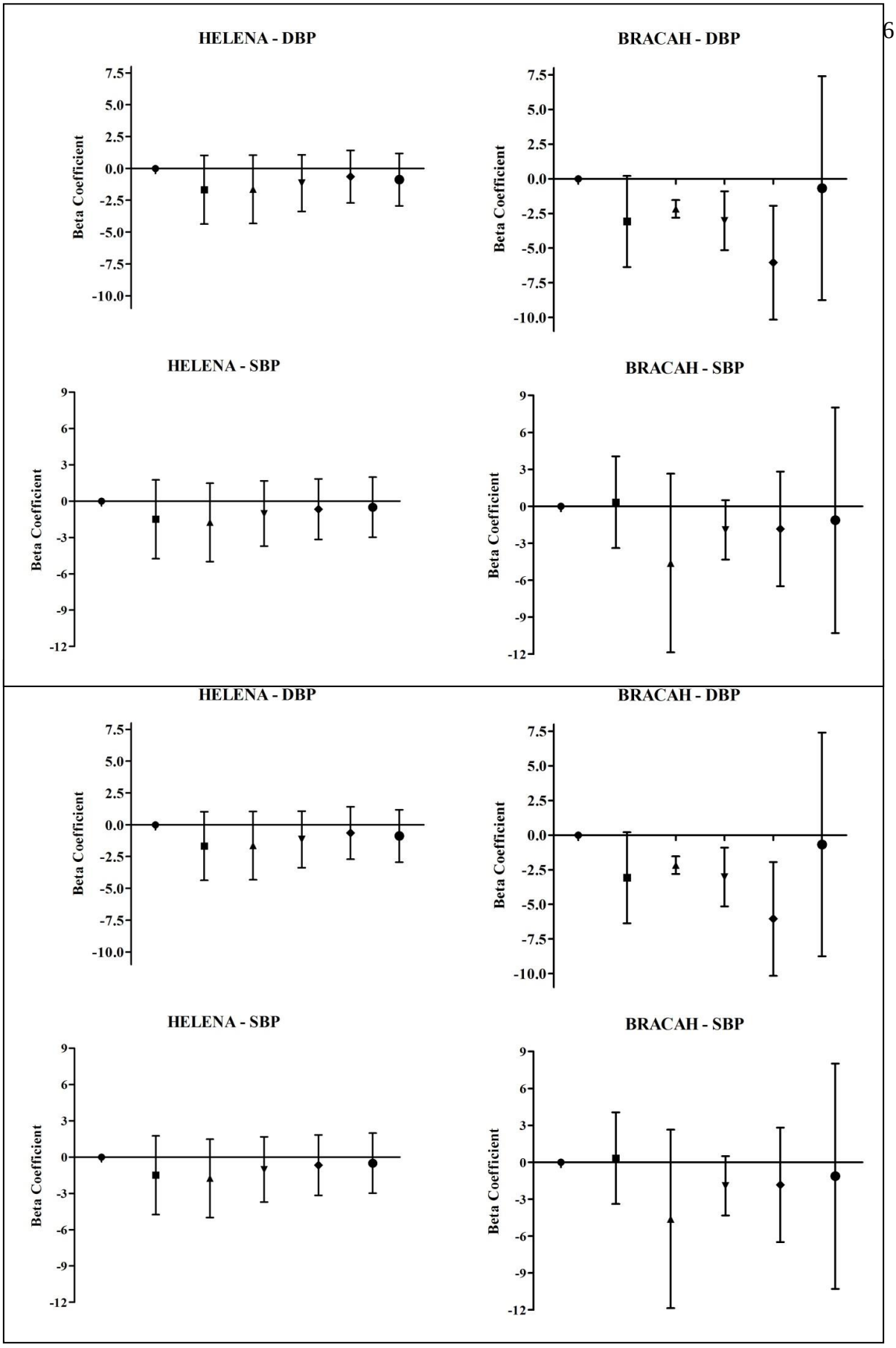
Significant associations are in bold.

In both studies, a significant association between diastolic BP, PA and SB cluster was observed in boys. In the HELENA study, the significant associations were observed in these categories in the PA and SB clusters: ≥ 60 min/d +> 4 h/d **β= -2.31 (95%CI= -4.23 - -0.39);** and ≥ 60 min/d + < 2 h/d **β= -1.21 (95%CI=-3.11 - -0.71).** While in the BRACAH study, a significant association was observed in this category: ≥ 60 min/d + 2 - 4 h/d **β= -4.33 (95%CI= -7.90 - -0.76)**. Moreover, the "<60 min/d + 2-4 h/d" cluster was directly associated with systolic BP in both studies, with the largest effect presented by HELENA study adolescents (Figure 1).

DISCUSSION

The effect of PA and SB levels, both independently and combined, on the BP of adolescents from two observational studies was explored. The results suggest that meeting PA recommendations could mediate the effect of SB on diastolic BP in both genders in the BRACAH study and in boys from HELENA study; on the other hand, low PA plus excessive SB was associated with increases in systolic BP in boys. These findings were consistent in two different epidemiological studies conducted on adolescents, which strengthens the conclusions. Including data from two different studies adds consistency to our report as some of the results were similar in different populations (Hill's principles) [35].

Differences between studies might be explained by 1) associations between behaviors analysed and determinants of BP (culture, income, environment, among others) and 2) susceptibility of individuals to different social environments and cultures [36,37]. The power remained greater than 90% in both studies, greater than 80% in the sex-specific analyses in both studies.


HELENA - DBP
BRACAH - DBP
HELENA - SBP
BRACAH - SBP
Beta Coefficient
7.5
5.0
2.5
0.0
-2.5
-5.0
-7.5
-10.0
9
6
3
0
-3
-6
-9
-12
HELENA - DBP
BRACAH - DBP
HELENA - SBP
BRACAH - SBP
Beta Coefficient
< 60 min/d + > 4 h/d
< 60 min/d + 2 - 4 h/d
< 60 min/d + < 2 h/d
≥ 60 min/d + > 4 h/d
≥ 60 min/d + 2 - 4 h/d
≥ 60 min/d + < 2 h/d

Our results differ from those of Ekelund et al. (2006), who found no differences in BP levels in the PA and SB cluster measured by accelerometers. On the other hand, our results corroborate studies that have evaluated the effects of PA and SB separately on BP levels [38,39]. Several mechanisms can explain the positive effects PA induces on BP levels. There is strong evidence that the sheer stress caused by regular PA has a powerful effect on the release of vasodilator factors produced by the vascular endothelium [40], such as nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) [41].

We observed that boys with low PA and high SB levels showed higher levels of systolic BP. This finding agrees with results from others studies in which adolescents with low PA and high SB presented low levels of cardiorespiratory fitness [42]. Low levels of PA, however, are also associated with other cardiovascular risk factors [43,44]. In a recent review study, Pedersen and Febbraio [45] describe how SB (i.e., reduced muscle contractions) leads to an altered myokine response in skeletal muscle. Consequently, these alterations promote increased pro-inflammatory adipokines that may contribute to the development of endothelial dysfunction in the cardiovascular system (i.e., increased synthesis of interleukin-6 and pathological processes of atherosclerosis); these dysfunctions may progressively develop into hypertension. Nevertheless, there are several such possible physiological mechanisms by which PA and SB may contribute to increased BP, and more research is needed to analyze the pathophysiological processes of increased BP due to insufficient PA combined with SB.

Furthermore, in our European female sample, we found that adequate PA levels do not attenuate the effect of high levels of SB on systolic blood pressure, probably because: 1) females have reduced PA levels and 2) estrogens have a more powerful influence on BP levels than PA during adolescence [46].

Accelerometers allow the study of activity patterns, and can establish the dose-response relationship between activity and health outcomes. On the other hand, self-reported PA data may not accurately reflect activity patterns due to recall bias and/or social desirability bias [47]. Moreover, studying sedentary behavior can be exceptionally challenging. Questionnaires that use a single sedentary activity (like TV viewing) may be considered a somewhat one-dimensional way of estimating a rather broad spectrum of sedentary activities, and this approach does not estimate the broad range of sedentary behaviors that adolescents have. However, PA questionnaires have advantages over accelerometers, such as low cost and PA domain information.

Our results are of importance since the behavioral patterns under consideration during adolescence tend to continue into adulthood [48] and high levels of sedentary behavior in adults increase the risk of mortality from cardiovascular diseases [49,50].

Since there were some methodological differences between the HELENA and the BRACAH studies (e.g., age range; accelerometers; geographic region) data from both studies were analyzed separately, but we used the multilevel analysis in order to control the influence of contextual (country-specific) variables, since several studies have shown that they influence PA and SB patterns [51,52].

A limitation of this study is its cross-sectional design; consequently, causality cannot be established. Moreover, it was not possible to adjust the analysis for other potentially BP-associated factors in either of the two samples, such as genetics or intrauterine development, but we developed an adjusted analysis for large potential confounders. On the other hand, the diverse geographic origin of the samples, the use of objective measures to assess PA and SB and multilevel adjusted analysis are some of the main strengths of our study.

CONCLUSIONS

According to our results, meeting current PA recommendations could mediate the association between SB and DBP in both sexes. In boys, the joint effect of low levels of PA and excessive sedentary activity increases SBP levels. These results suggest that regular PA should be promoted and SB discouraged in adolescent populations to prevent elevated blood pressure and its consequences in adulthood.

ACKNOWLEDGMENTS

We gratefully acknowledge all participating adolescents and their parents for their collaboration.

FIGURE LEGENDS

Figure 1: Beta coefficient and confidence intervals of 95% used to evaluate the association between blood pressure levels and clusters of physical activity and sedentary behavior measured by questionnaire in each study, **(A)** boys and **(B)** girls.

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3.5 ATTRIBUTABLE FRACTIONS OF THE ASSOCIATION BETWEEN FAMILY SOCIOECONOMIC FACTORS AND BLOOD PRESSURE IN ADOLESCENTS: RESULTS FROM TWO OBSERVATIONAL STUDIES – SUBMITTED IN BLOOD PRESSURE

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The authors of this paper have no conflict of interest to declare.

List of abbreviations

SBP= systolic blood pressure
DBP= diastolic blood pressure
FSFs= family socioeconomic status
HELENA study= Healthy Lifestyle in Europe by Nutrition in Adolescence
BRACAH study= Brazilian Cardiovascular Adolescent Health Study
NCDs= non-communicable diseases
BP= Blood Pressure
SES= Socioeconomic status
BMI= Body mass index
WC= Waist circumference
PA= Physical activity
95%CI= 95% confidence intervals

ABSTRACT

We aimed to estimate the attributable fraction of systolic (SBP) and diastolic blood pressure (DBP) that can be explained by the family socioeconomic status (FSFs) in adolescents from two observational studies. Participants were recruited by multi-stage random cluster in two cross-sectional studies performed in Europe (n= 3,308; HELENA study) and Brazil (n=991; BRACAH study). SBP and DBP were measured and FSFs were self-reported in both studies: socioeconomic status and parental education. Associations of SBP and DBP with FSFs were examined by multilevel linear regression through two different models (hierarchical and fully adjusted). The generalized attributable fractions of the FSFs were estimated by comparison between models. The higher generalized attributable fraction was observed in boys on the SBP; 13.2% to 22.4%. In girls, we found lower generalized attributable fractions, in Brazilians girls between 10.8% to 12.1%; and European girls between 3.1% to 3.8% on the DBP. We found no significant association between the FSFs and blood pressure levels in girls regardless of the study. Our findings showed that FSFs proportion has important influence on BP levels in adolescents. These results suggest that future interventions aiming to improve adolescents' BP levels should consider parents' educational level.

Key-words: Adolescents; cardiovascular system; epidemiology; social factors; multilevel modeling.

INTRODUCTION

Chronic non-communicable diseases (NCDs) are the main sources of disease burden worldwide, therefore they consist a major public health problem (Beaglehole & Horton, 2010). NCDs are highly prevalent even during adolescence (de Moraes et al., 2011) and previous studies showed that having high blood pressure (BP) levels in

childhood and adolescence is a risk factor for developing hypertension in adulthood (Lauer & Clarke, 1989).

Out of all the factors that may influence NCDs (e.g., genetics, intrauterine development, physical activity, sedentary behavior, tobacco, total and abdominal obesity), the family socioeconomic factors (FSFs) are both directly and indirectly associated with the health of adolescents, largely indirectly through health behaviors (Fernández-Alvira et al., 2013) and studies have shown that adolescents of lower socioeconomic status (SES) have a higher prevalence of NCDs (Fernandes, Christofaro, Cardoso, et al., 2011).

However, most studies aiming to analyze the association of FSFs and NCDs in adolescents have focused on obesity (Fernandes, Christofaro, Cardoso, et al., 2011; Pampel, Denney, & Krueger, 2012) or a cluster of cardiovascular risk factors (Ochoa-Avilés et al., 2012). Literature is scarce on the association between FSFs and BP levels in adolescents.

Thus, we hypothesized that FSFs contribute significantly to the variation seen on systolic (SBP) and diastolic blood pressure (DBP) in adolescents from two observational studies. We tested this hypothesis in two cross-sectional studies conducted in adolescents: the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study and the Brazilian Cardiovascular Adolescent Health Study (BRACAH).

METHODS

The objective of the HELENA study was to obtain data from a sample of European adolescents on a broad battery of nutrition and health-related parameters. Data collection took place during 2006 and 2007 in ten cities from nine European countries. A detailed description of the HELENA sampling and recruitment methodology, data

collection and quality control activities has been published elsewhere (Moreno, De Henauw, et al., 2008; Moreno, González-Gross, et al., 2008). After receiving complete information about the aims and methods of the study, all the parents/guardians signed a consent form, and adolescents gave assent to participate in the study. The protocol was approved by the human research review committees of the centers involved (Béghin et al., 2008).

The BRACAH study was carried out in the city of Maringá, located in the northwest of Paraná state (PR), southern Brazil, which has a population of approximately 330,000 (51,428 adolescents, 50.1% female). Adolescents were investigated on a broad battery of cardiovascular risk factors and various health behavior parameters. The methodology of this study has been described previously (Moraes, Delaporte, Molena-Fernandes, & Falcão, 2011). A formal request to conduct this survey was sent to and subsequently accepted by the school boards of several schools of the city. This study was also approved by the Ethics Committee for Research involving Human Participants of the University Center of Maringá and authorized by the Ethics Committee for Research Projects of the University of São Paulo in accordance with Brazilian law.

For the current study, we included adolescents from the HELENA and the BRACAH studies with valid data on gender, age, SBP, DBP (outcomes), socioeconomic status, parental education, regular tobacco consumption, physical activity, sedentary behavior, body mass index and waist circumference. These variables are described in detail below.

A total of 3,308 adolescents from the HELENA study (12.5–17.5 years old) and 991 adolescents from the BRACAH study (14.0–17.5 years old) met all the inclusion criteria and were included in the analyses.

The response rate of questionnaires was high, more than 85% in the HELENA study; and 83% in the BRACAH study.

Outcome

Blood Pressure (BP)

In both studies, BP measurements were performed following the recommendations for adolescent populations (NHBPEP, 2004). Blood pressure levels in both studies were measured twice after weight and height measurements were taken. The subjects were seated in a separate, quiet room for 10 min with their backs supported and feet on the floor. Two BP readings were taken at a 10 min interval of quiet rest. The lowest of the two measurements was used.

Systolic and Diastolic BP were measured using the OMRON® M6 (HEM 70001) oscillometric monitor device in the HELENA study and the OMRON® M3 (HEM 742) device in the BRACAH study. The OMRON® M3 (HEM 742) has been clinically and epidemiologically validated for adolescents by the Brazilian Research Group (Christofaro et al., 2009). The OMRON® M6 (HEM 70001) has been approved by the British Hypertension Society (Topouchian, El Assaad, Orobinskaia, El Feghali, & Asmar, 2006). These data collection procedures have been described previously (Iliescu et al., 2008).

Independent variables

The family socioeconomic factors analyzed were:

Socioeconomic status (SES): based on family financial situation, but the questionnaires applied in the two studies were different because of the differences between the countries. In both studies the SES was classified into three levels: low, medium and high.

In the HELENA study, we used the same definition as previous studies in HELENA (Gracia-Marco et al., 2012; Jiménez Pavón et al., 2010). The SES scale is composed of four questions and for each response a score was given as following: "Do you have your own bedroom?" (No=0, Yes=1); "How many cars are there in your family?" (None=0, 1=1, 2=2, >2=3); "How many computers are there in your home?" (None=0, 1=1, 2=2, ≥3=3); "Do you have Internet access at home?" (No=0, Yes=1). We computed the total score by summing the answers from all the questions (range, 0–8), we grouped these scores into three levels: low (0 –2), medium (3–5) and high (6–8). In the BRACAH study, the Brazil Criterion of Economic Classification was employed. These criteria consider parents' education level, presence/absence and number of domestic appliances, vehicles and rooms in the adolescent's home. Through a specific score (range 0–46) attributed by the questionnaire, the family was classified into one of seven categories (A1 [the wealthiest], A2, B1, B2, C, D and E [the poorest]) (ABEP, 2006), in BRACAH we grouped these categories into three levels as well: low (D and E), medium (B2 and C) and high (A1, A2 and B1).

Parental education: calculated using a self-reported questionnaire in both studies, and classified into four levels: lower education, lower secondary education, higher secondary education and university degree.

Potential confounders

The potential confounders which were considered in this study were:

- Country (only in the HELENA study);
- Age (years) calculated from birthday and medical examination day;
- Physical activity (PA): measured by questionnaire in both studies. It was adapted for the assessment of PA levels (moderate and vigorous levels)

among adolescents (Hagströmer et al., 2008). Active subjects were classified when they reached at least 60 min/d of moderate and vigorous PA according to PA recommendations (Strong et al., 2005).

- Sedentary behavior: measured by a structured questionnaire, including questions on the average amount of time spent in front of the television (TV), computer and/or video games. This questionnaire is a reliable tool for use with adolescents (de Moraes, Adami, & Falcão, 2012; Rey-Lopez et al., 2011). For example, the question "*On weekdays, how many hours do you usually watch TV*?" assessed the time spent in front of the TV Monday through Friday. For both studies the time in front of TV, computer and video games were classified into: 0-2 h/d; >2 – 4 h/d; ≥4 h/d.
- Regular tobacco smoking: defined as the regular consumption of at least one cigarette per day in the last month (Malcon, Menezes, & Chatkin, 2003);
- Body mass index: calculated as weight (kg)/height^2 (m^2). The continuous variable of BMI was used in the analysis. Height was measured to the nearest 0.1 cm and body mass to the nearest 0.1 kg, with a wooden stadiometer and a calibrated portable digital scale, respectively (wearing light clothes and no shoes).
- Waist circumference: measured in both studies at the midpoint between the lowest rib and the top of the iliac crest with a non-elastic tape to the nearest 0.1 cm (Nagy et al., 2008).

Statistical Analysis

Descriptive analyses were presented as means (quantitative variables) and percentages (qualitative variables) and 95% confidence intervals (95%CI). The attributable fractions were estimated by multilevel linear regression models using fixed

effects for intercepts were fitted to analyze the relationship between each BP level (continuous values) and independent variables (Snijders & Bosker, 1999). The context variable used was the school. Moreover, homoscedasticity was graphically assessed (not showed) in all regression models to meet the criteria of this analysis.

For multivariate analysis, we used two different models: the hierarchical and the fully adjusted model. The hierarchical models only included the FSFs variables and this analyses (Victora, Huttly, Fuchs, & Olinto, 1997) were adjusted for age. The hierarchical models including the FSF variables (each variable in a separate model, eg: one model for SES, another two models for each parental education) were not adjusted for the potential confounders (individual behaviors, BMI or WC). The objective was to obtain crude associations between FSFs and BP levels.

The subsequent fully adjusted multivariate models, assessed associations adjusted for all variables: age, FSFs (each variable in a separate model) and potential confounders (individual behaviors, BMI or WC). The p-values ≤ 0.20 were adopted in the univariate analysis (Victora, et al., 1997). Significance was considered when p-values were <0.05 or when there was more than 10% modification in β due to any variable already in the model.

The comparison of the hierarchical and the fully adjusted models were made in order to estimate the generalized attributable fraction of FSFs associated with BP levels. For the calculation of the attributable fractions, we adapted the formula proposed by Menvielle et al. (Menvielle, Luce, Goldberg, & Leclerc, 2004) (logic equation is maintained) to continuous data (ßh – ßf)/(ßh – 1), where ßf refers to the ß for SES and parental education in the fully adjusted model and ßh refers to the ß in the hierarchical model. We carried out the comparisons between the models, with the aim of estimating the proportion of the effect of the FSFs on BP levels, being this proportion already

"adjusted" for the confounders' variables, since the R^2 from the ful models, were adjusted for all variables.

The statistical software package Stata version 12.0 (Stata Corp., College Station, TX, USA) was used for all statistical calculations. All analyses were adjusted for the clustered nature of the sample using the "svy" set of commands and stratified by gender, since interactions between sex and the studied variables were observed ($p<0.001$).

RESULTS

Subject characteristics, FSF variables, BP levels and potential confounders are shown in Table 1. Figure 1 shows the proportion of BP explained by FSFs for boys (A) and girls (B). For boys the proportion of explanation of the association between BP and FSF levels was higher in the BRACAH study than in the HELENA study, with higher percentages in the SBP. The proportions explained by the association between SBP and FSFs among studies regarding girls are similar, whereas for DBP the proportion is higher in the Brazilian study.

The supplementary file presents the β coefficients from both regression models for BP levels in girls and boys of both studies; we did not find any significant associations between FSFs and BP levels regardless of the study. In both models the education of parents (father and mother) was inversely associated with SBP in boys from the HELENA study. However, the family's SES was negatively associated only in the hierarchical model in the HELENA study.

Table 1: Characteristics of the samples from the HELENA and BRACAH studies.

Variables	Boys		Girls	
	HELENA (n=1,580)	**BRACAH (n=451)**	**HELENA (n=1,728)**	**BRACAH (n= 540)**
	mean or % (95% CI)	**mean or % (95% CI)**	**mean or % (95% CI)**	**mean or % (95% CI)**
Age (years)	**14.8 (14.7 - 14.9)**	**16.4 (16.3 - 16.5)**	**14.7 (14.6 - 14.8)**	**16.2 (16.2 - 16.3)**
Familial Socioeconomic Factors				
Education father				
Lower education	6.8 (5.5 - 8.2)	6.4 (4.1 - 8.7)	8.1 (6.7 - 9.5)	8.1 (5.7 - 10.4)
Lower secondary education	29.6 (27.1 -32.0)	25.2 (-21.1 - 29.3)	29.5 (27.5 - 32.2)	26.7 (22.9 - 30.5)
Higher secondary education	27.6 (25.2 - 30.0)	47.0 (42.2 - 51.7)	27.3 (25.1 - 29.7)	45.5 (41.2 - 49.8)
University degree	36.0 (33.4 - 38.6)	21.3 (17.5 - 25.2)	34.7 (32.2 - 37.2)	19.8 (16.3 - 23.2)
Education mother				
Lower education	7.5 (6.1 - 8.9)	6.4 (4.1 - 8.7)	8.0 (6.6 - 9.4)	8.4 (6.1 - 10.8)
Lower secondary education	26.3 (23.9 - 28.7)	25.0 (20.9 - 29.1)	24.9 (22.7 - 27.2)	24.2 (20.5 - 27.9)
Higher secondary education	31.2 (28.7 - 33.7)	42.7 (38.0 - 47.3)	31.8 (29.4 - 34.2)	43.0 (38.7 - 47.3)
University degree	35.0 (32.5 - 37.6)	25.9 (21.8 - 30.0)	35.2 (32.8 - 37.7)	24.4 (20.7 - 28.1)
Socioeconomic status				
High	**33.5 (31.0 - 36.1)**	**14.4 (11.1- 17.8)**	**31.0 (28.6 - 33.4)**	**14.2 (11.2 - 17.2)**
Medium	**56.5 (53.9 - 59.2)**	**81.7 (78.0 - 85.3)**	**56.1 (53.5 - 58.7)**	**78.5 (75.0 - 82.0)**
Low	**10.0 (8.3 - 11.6)**	**3.9 (2.1 - 5.7)**	**12.9 (11.1 - 14.6)**	**7.3 (5.1 - 9.5)**
Systolic Blood Pressure (mmHg)	**126.4 (125.5 - 127.1)**	**119.6 (118.4 - 120.9)**	**117.3 (116.7 - 117.9)**	**107.0 (106.0 - 108.0)**
Diastolic Blood Pressure (mmHg)	68.5 (68.0 - 69.0)	69.0 (68.1 - 69.9)	69.3 (68.8 - 69.8)	67.1 (66.2 - 68.0)
Physical activity by questionnaire				
< 60 min/d MVPA	**14.4 (12.5 - 16.3)**	**68.6 (64.2 - 72.9)**	**16.0 (14.1 - 17.9)**	**68.9 (64.9 - 72.9)**
≥ 60 min/d MVPA	**85.6 (83.7 - 87.5)**	**31.4 (27.1 - 35.8)**	**84.0 (82.1 - 85.9)**	**31.1 (27.1 - 35.1)**
Sedentary behavior by questionnaire*				

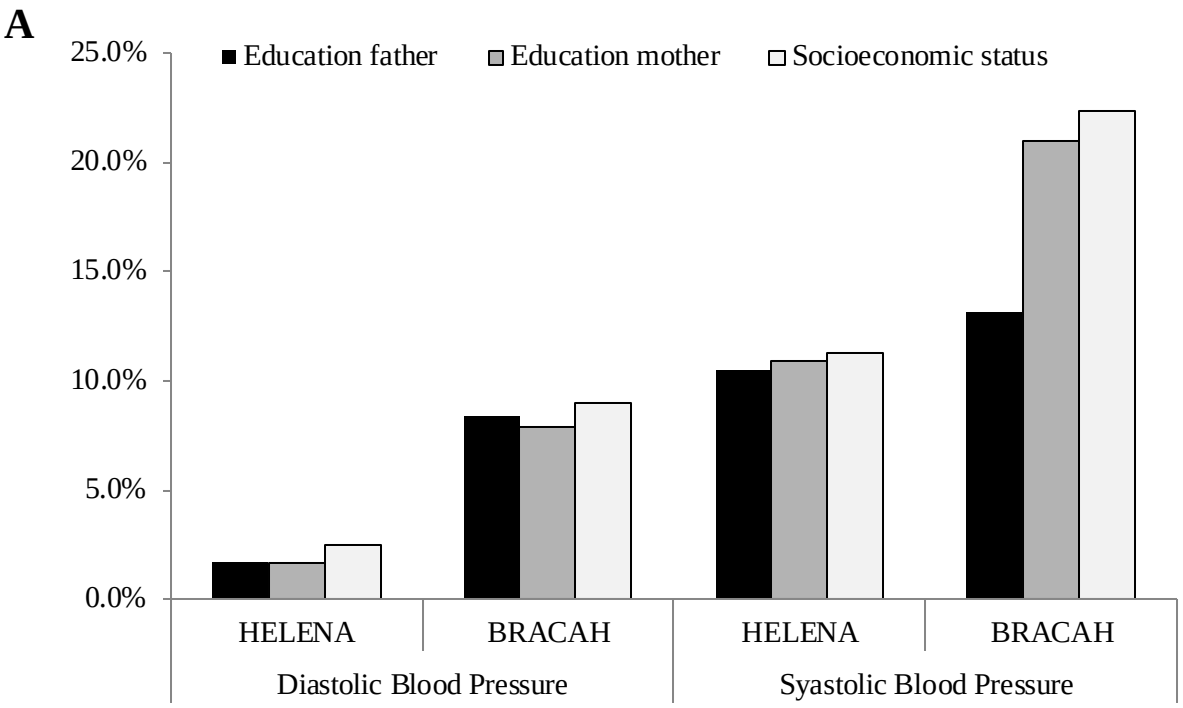
	> 4 h/d	**38.8 (36.2 - 41.5)**	**75.0 (70.9 - 79.1)**	**20.4 (18.3 - 22.5)**	**84.5 (81.3 - 87.6)**
	2 - 4 h/d	**39.1 (36.4 - 41.7)**	**20.6 (16.8 - 24.4)**	**36.3 (33.8 - 38.8)**	**12.5 (9.6 - 15.3)**
	< 2 h/d	**22.1 (19.9 - 24.4)**	**4.4 (2.4 - 6.3)**	**43.3 (40.7 - 45.8)**	**3.1 (1.6 - 4.6)**
Regular smoking**					
	Yes	**12.2 (10.5 - 14.0)**	**6.4 (4.1 - 8.7)**	**12.8 (11.1 - 14.5)**	**5.2 (3.3 - 7.1)**
	No	**87.8 (86.0 - 89.5)**	**93.6 (91.3 - 95.9)**	**87.2 (85.5 - 88.9)**	**94.8 (92.3 - 96.7)**
Waist Circumference (cm)		74.4 (73.9 - 74.9)	80.4 (79.5 - 81.4)	70.2 (69.8 - 70.6)	77.3 (76.5 - 78.1)
Body Mass Index (kg/m2)		21.5 (21.3 - 21.7)	21.8 (21.5 - 22.2)	21.3 (21.1 - 21.5)	21.2 (21.0 - 21.6)

MVPA: Moderate to vigorous physical activity.

* Television watching, internet and video games.

** More than 1 cigarette per day in the last month.

Significant differences between the HELENA and BRACAH ($p<0.05$) are in bold; for the ordinal variables (socioeconomic status and sedentary behavior) are a p-value for trend.



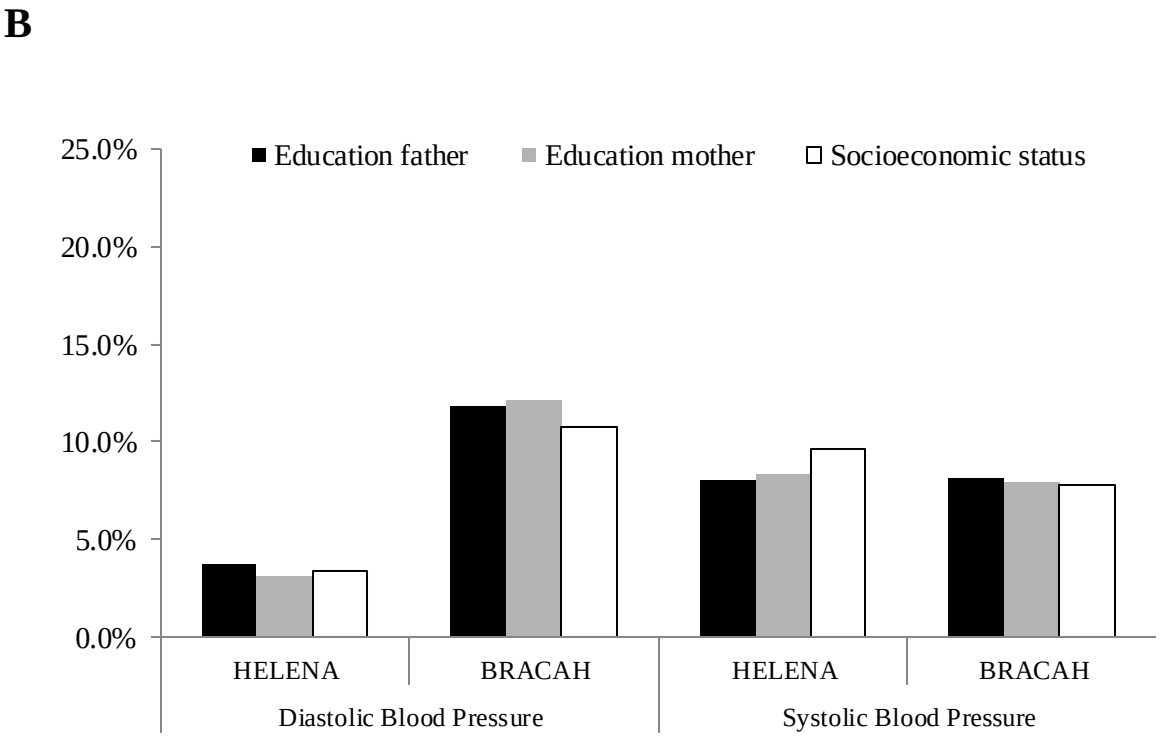


Figure 1: The proportion that familial social factors explain the variance between blood pressure levels for boys (A) and girls (B).

DISCUSSION

To our knowledge, this is the first article analyzing FSFs and levels of BP in adolescents from two different observational studies; HELENA study (European sample) and BRACAH study (Brazilian sample). Only parental education was found to be associated with SBP in boys from the HELENA study; on the other hand, we did not find any significant associations for girls in either study. Results from two different studies carried out in adolescents strengthen the conclusions.

Parents' education has been previously observed to be directly associated with various aspects of adolescent health in developed and developing countries (Viner et al., 2012); however, in our article we found an inverse association only for boys from the HELENA study. Part of these results may be explained because parents with less education might receive less health care for their sons (Fletcher, Steinberg, & Williams-Wheeler, 2004). Another plausible explanation for this association is that adolescents of lower socioeconomic levels have lower levels of physical fitness (Jiménez Pavón, et al., 2010) and also lower levels of physical activity (Gracia-Marco et al., 2010); and these variables are directly associated with high levels of BP (Tsioufis et al., 2011). In our analysis we adjusted for physical activity and sedentary behavior, because adolescents from lower socioeconomic levels have a higher prevalence of the cluster risk factors for high BP.

We found an association between the independent variables and the outcome only in boys and not in girls from the HELENA study. One possible explanation may be that parents encourage girls to take care of their own health and use health services more so than boys (Berra et al., 2009; Palacio-Vieira et al., 2012), while boys are still more dependent on parents and the health of adults is also directly associated with the educational and socioeconomic level (Sato, 2012; Stringhini et al., 2011).

We conducted a further analysis, quantifying the generalized attributable fraction of FSFs in BP levels. In the BRACAH study these percentages are higher than in the HELENA study for both genders; therefore the family socioeconomic factors appear to have greater influence on the levels of BP in Brazilian adolescents than in Europeans. In countries with low-middle incomes FSFs are associated with obesity (Fernandes, Christofaro, Cardoso, et al., 2011), which is directly associated with high BP (Fernandes, Christofaro, Buonani, et al., 2011; Polderman et al., 2011). In adolescents from Brazil, these associations can be explained because the young people living in these countries are more vulnerable to the rapid economic and urban development (Viner, et al., 2012) that mainly affect healthy behaviors such as PA (de Farias Júnior et al., 2011).

An important aspect to consider is that the criteria used to define SES are different in these studies. The differences in results can be partly explained by such methodological aspects. Another factor that may influence the recorded prevalence of SES is the question of measurement accuracy. Differential or non-differential misclassification effects (error due to disease status or exposure) of SES prevalence are unpredictable, and may have caused the underestimation or overestimation of the true prevalence. In the context of this study, it is likely that the validity of criteria and tools used varied for each characteristic of the adolescents studied (Mertens, 1993). However, we believe this to be a small limitation, since the questionnaires measured family financial situations and were divided into three levels in both studies.

Since some methodological differences between the HELENA and the BRACAH studies were present (e.g., age range and geographic region) data from both studies were analyzed separately, but we used the multilevel analysis in order to control the influence of contextual (country-specific) variables, because several studies have

shown that influence on the outcome (Dengel, Hearst, Harmon, Forsyth, & Lytle, 2009; Griffiths et al., 2012).

A limitation of this study is its cross-sectional design; consequently, causality cannot be established. Moreover, it has not been possible to adjust the analysis for other factors potentially associated to BP and/or FSFs, such as, alcohol consumption, genetic and intrauterine development, in either of the samples. On the other hand, the large sample size, the diverse geographic origins of the samples and multilevel analyses help to strengthen our study.

IMPLICATIONS AND CONTRIBUITION

In our findings showed that FSFs proportion has important influence on levels of BP in adolescents, mainly in Brazilian adolescents. These results suggest that directing intervention at improving adolescent health should consider parents' educational levels in adolescent populations for the prevention of elevated BP and its implications for adult health.

COMPETING INTEREST

The authors state no conflict of interest.

AUTHORS' CONTRIBUTIONS

ACFM and HBC were the principal design researcher responsible for the data collection from BRACAH study; **LAM, MY, MS, AK, KW, LB and FG** were the principal design researcher responsible for the data collection from HELENA study; **ACFM, HCB and LAM** analyzed and performed data interpretation, as well as helping draft the manuscript; **All authors** was involved in revising the manuscript critically for important intellectual content. **ACFM** has primary responsibility for the final content. All authors read and approved the final manuscript.

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3.6 VITAMINS AND IRON BLOOD BIOMARKERS ARE ASSOCIATED WITH BLOOD PRESSURE LEVELS IN EUROPEAN ADOLESCENTS. THE HELENA STUDY – PLUBLISHED IN NUTRITION

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ABSTRACT

Background: Previous research showed that low concentration (in blood) of biomarkers during adolescence (i.e. iron status, retinol, vitamin B_6, vitamin B_{12} vitamin C and vitamin D) may be involved in the early stages of many chronic diseases development, like hypertension.

Methods: Participants from the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study (HELENA-CSS) (n = 1,089; 12.5–17.5 yr; 580 girls) were selected by complex sampling. Multilevel linear regression models examined the associations between vitamins and iron blood and BP, the analyses were stratified by sex and adjusted for contextual and individual potential confounders.

Results: In girls, we found a positive association between red blood cell folate concentration and systolic BP (SBP) (β= 3.19; CI 95%= 0.61 ; 5.77), although no association between the vitamins serum concentration biomarkers and diastolic BP (DBP) was found. In boys, retinol was positively associated with DBP (β= 3.84; CI 95%= 0.51 ; 7.17) and vitamin B_6 was positively associated with SBP (β= 3.82; CI 95%= 1.46 ; 6.18). In contrast, holo-transcobalamin was inversely associated with SBP (β= -3.74; CI 95%= -7.28 ; -0.21).

Conclusions: Levels of RBC-folate and vitamin B_6 in blood may impact BP in adolescents. In this context, the programs avoid high blood pressure levels should promote healthy eating behavior may need to focus on the promotion of vegetable proteins and foods rich in vitamin B_{12} i.e. white meat and eggs, which may help to blood pressure control in adolescents.

Key-words: iron; vitamins; blood pressure; adolescents; multicenter study.

INTRODUCTION

High blood pressure (HBP) is a well-established major risk factor for stroke and coronary heart disease [1]. Several studies have reported high prevalence of risk factors for cardiovascular disease in adolescents [2] and studies have shown that levels of blood pressure (BP) in childhood and adolescence are mediators of developing hypertension in adulthood [3].

Recently, Tzoulaki et al.[4] observed that dietary phosphorus, magnesium, iron, thiamin, folacin, and riboflavin were inversely associated with systolic BP in adults. In addition, dietary folacin and riboflavin were negatively associated with diastolic BP. Also in adolescents, nutritional biomarkers are associated with their health, and studies showed a low biomarkers serum concentrations during adolescence (i.e. iron status, retinol, vitamin B_6, vitamin B_{12} vitamin C and vitamin D). These biomarkers present anti-inflammatory and antioxidant effects, and their low serum concentrations maybe an important factor early stages in the pathogenesis of many chronic diseases [5].

Most studies aiming to analyze the association of biomarkers serum concentrations and non-communicable diseases have focused on obesity [6] or a cluster of cardiovascular risk factors [7]. However, data concerning the association between the concentration of vitamins and iron blood biomarkers and BP in adolescents are scarce, which is crucial due to the high prevalence of hypertension (and as a consequence of the risk for some diseases) in children and adolescents in the last decades [8].

We hypothesized that: *low levels of vitamins and low levels of iron biomarkers both related to higher levels of blood pressure in adolescents*? We tested this hypothesis in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study, which was conducted in European adolescents.

METHODS

Sample Size

The HELENA cross-sectional study aimed of obtaining data from a sample of European adolescents on a broad battery of nutritional and health-related parameters. Data collection took place within 2006 and 2007 in ten cities from nine European countries. Detailed descriptions of the HELENA sampling and recruitment methodology, inclusion criteria, data collection and quality control activities have been published elsewhere [9]. After receiving complete information about the aims and methods of the study, all parents/guardians signed a consent, and adolescents gave assent to participate in the study. The Human Research Review Committees of the centers involved approved the protocol. The age range considered valid for the HELENA study was 12.5–17.5 yr. All of the analyses conducted on the HELENA data are adjusted for a weighing factor to balance the sample according to the theoretical age distribution foreseen. A total of 3,528 adolescents (1,845 girls), were considered eligible for the HELENA analyses. In the HELENA protocol, it was established that blood samples were obtained randomly in one-third from population sample. To make maximum use of the data, all valid data on BP levels were included in this paper. Consequently, sample sizes vary for the different BP levels and biomarkers. A total of 1,089 adolescents (12.5–17.5 years old; 580 girls) from the HELENA study met all the inclusion criteria and were, therefore, included in the analyses. Distribution per city was: Stockholm (n=94), Dortmund (n=117), Ghent (n=119), Lille (n=82), Vienna (n=128), Pecs (n=136), Rome (n=115), Zaragoza (n=110), Athens (n=92), and Heraklion (n=96). We performed sensitivity analyzes in the sample by comparing the levels of BP and all confounders' variables among adolescents the 1,089 who had blood sample data, and the 2,439 who did not have blood samples data.

Sensitivity analyses were performed to compare blood pressure and confounding variables included in this study between those adolescents who had blood samples and those who had not. Results showed that there were no significant differences for any of the variables included in this study avoiding therefore, selection bias.

Outcome

Blood Pressure

Blood pressure measurements were performed following the recommendations for adolescent populations. Systolic and diastolic BP were measured with the use of the arm blood pressure oscillometric monitor device OMRON® model HEM 7001 that has been approved by the European Hypertension Society [10]. These data collection procedures have been described in an earlier study [11]. Briefly, the subjects were seated in a separate, quiet room for 10 min with their backs supported and feet on the ground. Inter-observer coefficients of variation were 2.1% and 3.6% for systolic and diastolic BP, respectively. Two BP readings were taken with a 10-min interval in-between and the lowest reading recorded, according European Hypertension Society [10].

Independent variables

The blood sampling procedure and sample logistics have been described in detail elsewhere [12]. Briefly, fasting blood samples were collected by venipuncture at school between 8 and 10 o'clock in the morning after a 10-h overnight fast. Samples for the different analyses were manipulated in situ, as described below, and transported according to the protocol to the central laboratory in Bonn, Department of Nutrition and Food Sciences, for analysis. Whole blood samples for the hemogram were sent directly

to the local laboratory of each country to be analyzed. A specific handling, transport, and traceability system for biological samples was developed for the HELENA study and it was already described, which has previously been González-Gross et al. [13]. Blood samples were obtained between October 2006 and June 2007 and in October 2007.

Iron status/blood characteristics assessment: Soluble transferrin receptor (sTfR) and serum ferritin were measured using ELISA (enzyme-linked immunosorbent assay) [14] in the Human Nutrition Laboratory of the National Research Institute on Food and Nutrition (Rome, Italy). A commercially available control sample from Bio-Rad Liquichek Immunology Control Level 3 (Bio-Rad, Milan, Italy) was used to obtain a calibration curve on each plate. Whole-blood samples for the red blood parameters (haemoglobin, red blood cell, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration and red cell distribution width) were sent directly to the local laboratory of each country to be analyzed.

Provitamin A (β-carotene), vitamin A (retinol), and vitamin E (α-tocopherol) measurements: β-Carotene, retinol, and α-tocopherol were analyzed by reversed-phase high-performance liquid chromatography using UV detection (RP-HPLC) (Sykam Gilching Germany) in serum. The vacutainer was centrifuged for 15 min at 3,500 rpm at 4°C. Standards (β-Carotene, retinol, α-tocopherol), hexane, and isopropanol were obtained from Sigma Aldrich (Germany) and had all HPLC grade. The variation of the method is =3% for all of the vitamins. The samples were stable over 24 h at room temperature coefficient of variation (CV) vitamin E = 4.6%; vitamin A= 3.2%.

Vitamin C measurement: For vitamin C measurements, the heparin tubes were put immediately on ice and centrifuged within 30 min (3,500 rpm for 15 min). For

stabilization, heparin plasma was precipitated with a 6% (wt/wt) perchloric acid solution spiked with metaphosphoric acid (1:1). The precipitated samples were transported at a stable temperature of 4–7°C within 24 h to the central laboratory and stored at 80°C until analysis. Plasma vitamin C was analyzed by RP-HPLC using UV detection (Sykam Gilching Germany). The CV of the method was 1.7%.

Vitamin B6, B12 (cobalamin and holo-transcobalamin), homocysteine and folate plasma and red blood cell (RBC) measurements: For the measurement of vitamin B_6 (pyridoxal 5'-phosphate), aliquots of EDTA whole blood were sent by cooled transport to the central laboratory and stored at -80°C until bunched analysis. Pyridoxal 5'-phosphate was measured by HPLC (Varian Deutschland, Darmstadt, Germany; CV = 1%) with a modified method of Kimura et al. [15]. For vitamin B_{12} status, cobalamin and holo-transcobalamin were determined. For folate status, plasma folate and RBC folate were determined. For the measurement of cobalamin and plasma folate, blood was collected in heparinized tubes, immediately placed on ice, and centrifuged within 30 min (3,500 rpm for 15 min). The supernatant fluid was transported at a stable temperature of 4–7°C to the central laboratory and stored there at 80°C until assayed. After hematocrit was measured in situ, EDTA whole blood was sent to the central laboratory for the RBC folate analysis. EDTA whole blood was diluted 1:5 with freshly prepared 0.1% ascorbic acid for cell lysis incubated for 60 min in the dark before storage at -80°C. Plasma cobalamin, and RBC folate were measured by competitive immunoassay (Immulite 2000, DPC Biermann, Bad Nauheim, Germany) (CV for plasma folate = 5.4%, RBC folate = 10.7%, cobalamin = 5.0%). Sera for measuring holo-transcobalamin were obtained by centrifuging blood collected in evacuated tubes without anticoagulant at 3,500 rpm for 15 min within 1 h. Once sent to the central laboratory, the sera were aliquoted and stored at -80°C until transport in dry ice to the

biochemical laboratory at the Universidad Politécnica de Madrid for analysis (Laboratorio número 242 de la Red de Laboratorios de la Comunidad de Madrid). Holo-transcobalamin was measured by micro particle enzyme immunoassay (Active B_{12} Axis-Shield, Dundee, Scotland, UK; CV = 5.1%) with the use of AxSym (Abbot Diagnostics, Abbott Park, IL).

25(OH)D measurement: For vitamin D status, plasma 25(OH)D was measured. Blood was collected in EDTA tubes and transported at room temperature to the central laboratory at IEL within 24 h. There it was centrifuged at 3,500 rpm for 15 min at 4°C, and the supernatant stored at -80°C until analysis. Plasma 25(OH)D was analyzed by ELISA using a kit (OCTEIA 25-Hydroxy Vitamin D) from Immunodiagnostic System (Germany) and measured with a Sunrise Photometer by TECAN (Germany). The IDS OCTEIA 25(OH)D kit is an enzyme immunoassay intended for the quantitative determination of 25(OH)D and other hydroxylated metabolites in human serum or plasma. The sensitivity of this method is 5 nmol/l 25(OH)D, and the variation is = 6%. The mean recovery of 25(OH)D is 101%. The CV for the method was = 1%.

Contextual variables

Seasonality: A variable was computed by recoding the original variable "blood drawing date" into "seasonality", as follows: winter (from 21st December to 20th March, coded as 1), autumn (from 21st September to 20th December, coded as 2), spring (from 21st March to 20th June, coded as 3), and summer (from 21st June to 20th September, coded as 4), as it was performed in previous studies [12]. As the HELENA study was performed during the academic year, few adolescents (n=25) were assessed in the first days of summer, and they were included along with those assessed during spring. Therefore, the final variable was composed of three groups: winter (coded as 1), autumn (coded as 2), and spring (coded as 3) [12].

Latitude of residence: The latitude of the study centers was also taken into account as a confounder in the analyses. The latitude of each city was obtained from http://maps.google.es/. Latitudes of the involved cities were as follows: Stockholm (59°33; North), Dortmund (51°51; North), Ghent (51°06; North), Lille (50°63; North), Vienna (48°21; North), Pecs (46°07; North), Rome (41°89; North), Zaragoza (41°66; North), Athens (37°98; North), and Heraklion (35°33; North). To make use of this data, latitudes were added to the database as numeric variables with two decimals (i.e., Stockholm = 59.55[12].

Potential individual confounders

All potential confounders fulfilled the requirement for confounding variable, and were associated with the outcomes and biomarkers ($p < 0.20$) [16].

Maternal education level: Using a self-reported questionnaire, and classified in four levels: lower education, lower secondary education, higher secondary education, university degree.

Age at menarche: A quantitative variable was computed as a measure of time (months) as follows: menarche (months) age (months) at the moment of blood drawing – age (months) at the moment of menarche. Those girls (n=104) who had no menarche before blood drawing were considered as "0" for the analyses, following previous studies[12].

Age (years): calculated from birthday and medical examination day;

Anthropometric measurements: were collected by trained researchers in a standardized format with participants barefoot and in underwear [17]. The height was measured to the nearest 0.1cm and the body mass to the nearest 0.1 kg, with a telescopic stadiometer and an electronic scale, respectively Body mass index (BMI) was calculated as weight (kg)/height (m^2).Waist circumference (WC) was measured at the midpoint

between the lowest rib cage and the top of the iliac crest with a non-elastic tape to the nearest 0.1 cm [17].

Physical activity (PA): measured with accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) for seven consecutive days, with a minimum of 8 hours recorded/day for at least 3 days [18]. The time sampling interval (epoch) was set at 15 seconds. Inactive, light, moderate and vigorous PA was defined as <1000, 1000-1999, 2000-3999 and ≥4000 counts per minute, respectively. Following current PA recommendations [19], subjects were categorized as active (if they accumulated at least 60 min/d of moderate to vigorous PA) and inactive (<60min/d of moderate to vigorous PA).

Serum lipid concentrations: triglycerides, total cholesterol and high-density lipoprotein cholesterol were measured using enzymatic methods (Dade Behring, Schwalbach, Germany) [13].

Statistical Analysis

The descriptive analyses are presented as mean or median (quantitative variables) and percentages (qualitative variables) and 95% confidence intervals (95%CI). The variables sTfR, β-Carotene, vitamin B6, cobalamin, holo-transcobalamin, plasma folate, RBC folate were log-transformed; and serum ferritin was square-root transformed. After transformations all variables showed a normal distribution.

We assessed the association between vitamins and iron blood biomarkers and BP levels using bivariate linear regression. We considered the continuous values of the systolic BP and diastolic BP as outcome variables. The vitamins and iron blood biomarker variables were included as principals independent variables. The mean of BP levels were analyzed according to each independent variable. The magnitude of these

associations was subsequently expressed as unadjusted and adjusted β-coefficients and their respective 95%CI. Multilevel linear regression models using fixed effects intercept were fitted to analyze the relationship between each BP levels and independent variables [20]. The context variable used was the school. Moreover, homoscedasticity were graphically assessed in all regression models to meet the criteria of this analysis.

The multilevel adjusted analysis was conducted following a theoretical conceptual model (supplementary file) that had been previously formulated in three levels (the association of the first two levels not shown): 1) contextual variables (seasonality, latitude of residence and school; 2) potential individual confounders (maternal education, age (years) age at menarche (in girls), body mass index, waist circumference, physical activity, serum lipid concentrations; and 3) vitamins and iron blood biomarkers; The biomarkers were divided in four groups: I) blood composition (hematocrite, MCV mean corpuscular volume., MCH mean corpuscular hemoglobin. and MCHC mean corpuscular hemoglobin concentration.) and iron status indicators soluble transferrin receptor (STfR) and serum ferritin.; II) provitamin A (α-carotene), vitamin A (retinol), and vitamin E (β- tocopherol), vitamin C; III) vitamin B_6, B_{12} (cobalamin and holo-transcobalamin); homocysteine; folate plasma and RBC.; and IV) vitamin D 25(OH)D. In this model, the variables were controlled for those in the same level and those in higher ones [16]. The p-values $\leq$ 0.20 were adopted in the univariate analysis [16] (as necessary to include variables in the multivariate analysis and then it was entered through the theoretical conceptual model method following the levels above). Significance was adopted at p-values were <0.05 or when there was more than 10% modification in β of any variable already in the model. Before performing the adjusted analyses, we checked if there were possible interactions between the variables of the vitamins and iron blood biomarkers in the same group. No interactions were

found and therefore, the variables were introduced individually to analyze the adjusted vitamins and iron blood biomarkers models.

The statistical software package Stata version 12.0 (Stata Corp., college Station, TX, USA) was used for all statistical calculations.

RESULTS

Subject characteristics of BP levels, serum concentration biomarkers and potential individual confounders are shown in Table 1. Boys had significantly higher values than girls: lipid profile, MCV, α-tocopherol, vitamin C and cobalamin. On the other hand, females had values higher than boys: hemoglobin, hematocrit, MCHC and homocysteine. Non-normally distributed variables are presented as medians and CI95%, other quantitative variables are presented as mean and categorical variables are presented as percentages.

Table 2 shows the association between BP levels and biomarkers serum concentration in girls. Our results showed a significant and positive association between RBC folate with SBP. In contrast, our results did not found significant associations between serum concentration biomarkers and DBP and SBP.

Table 3 shows the association between the BP levels and biomarkers serum concentration in boys. Our results showed a significant and positive association between retinol with DBP. In addition, vitamin B_6 was positively related to SBP, while holo-transcobalamin was negatively associated with SBP.

For both Table 2 and Table 3, the complete model presented better goodness of fit (significantly lower Akaike Information Criterion) when compared to the preceding partial models.

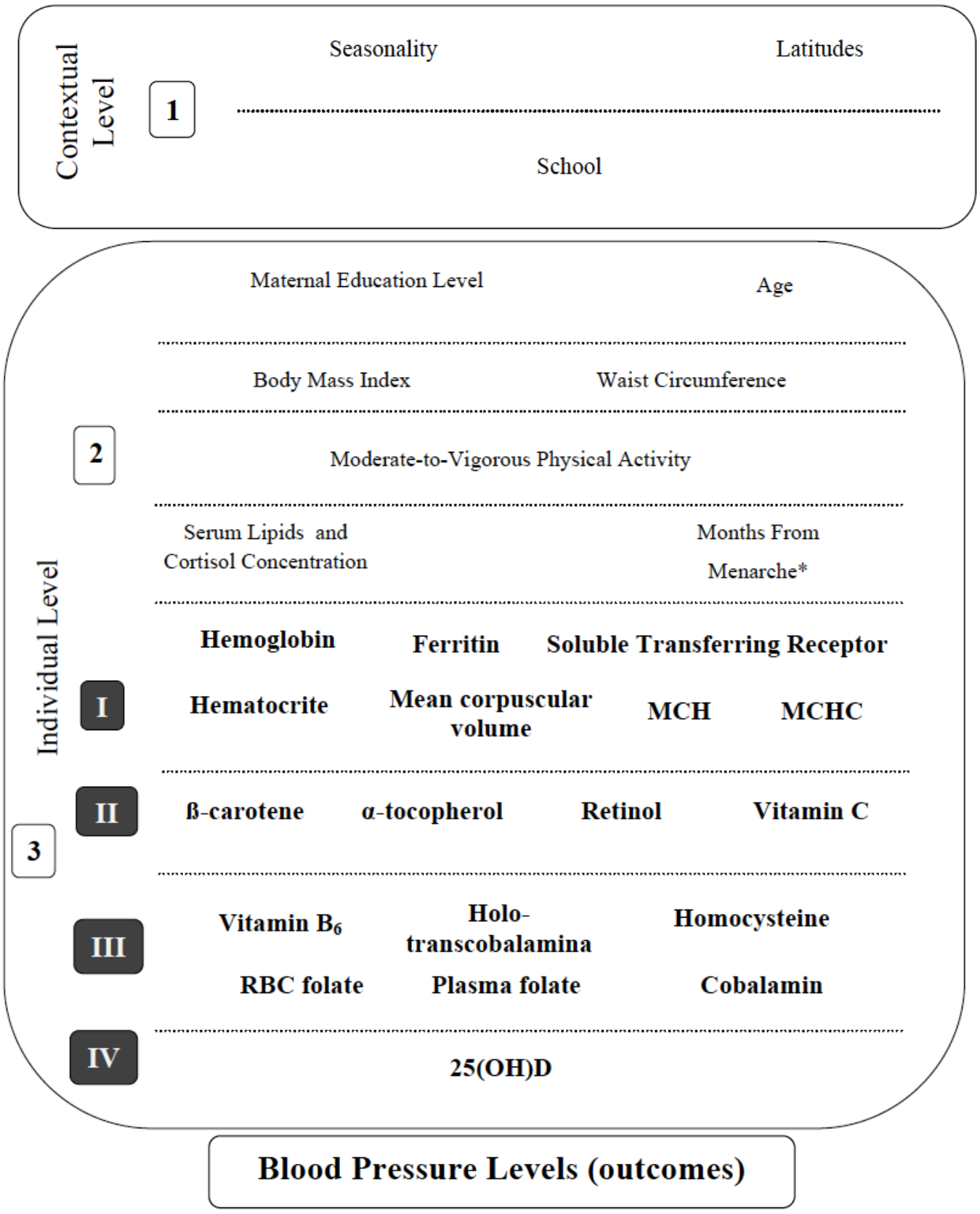

Figure 1: Theoretical conceptual model of the association between contextual and individual variables on adolescents' blood pressure levels. The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with $p > 0.2$ were not included in the subsequent adjustment models. *For girls.
The principals independents variables are in bold.
MCH= Mean corpuscular hemoglobin.
MCHC= Mean corpuscular hemoglobin concentration.

Table 1: Descriptive characteristics of the studied adolescents by sex.

Variables	Girls mean, median* or % (95% CI)	Boys mean, median* or % (95% CI)
Seasonality		
Winter	25.0 (21.4 - 28.5)	28.3 (24.4 - 32.3)
Autumn	32.8 (29.0 - 36.7)	31.3 (27.3 - 35.3)
Spring	42.2 (38.2 - 46.3)	40.4 (36.1 - 44.6)
Age (years)	14.8 (17.7 - 14.9)	14.8 (14.7 - 14.9)
Education mother		
Lower education	8.4 (6.0 - 10.7)	8.9 (6.3 - 11.4)
Lower secondary education	30.3 (26.4 - 34.2)	27.6 (23.6 - 31.7)
Higher secondary education	30.7 (26.8 - 34.6)	29.6 (25.4 - 33.8)
University degree	30.6 (26.8 - 34.6)	33.9 (25.4 - 33.8)
Physical activity by accelerometers*		
< 60 min/d	**72.3 (67.9 - 76.7)**	**39.3 (33.9 - 44.6)**
≥ 60 min/d	**27.7 (23.3 - 32.1)**	**60.7 (55.4 - 66.1)**
Waist Circumference, cm	**70.6 (70.0 - 71.3)**	**74.4 (73.6 - 75.2)**
Body Mass Index, kg/m2	21.3 (21.0 - 21.6)	21.4 (21.1 - 21.8)
Tryglicerides, mg/dl	**73.3 (70.3 - 76.4)**	**64.4 (61.6 - 67.2)**
HDLc, mg/dl	**60.0 (56.1 - 57.9)**	**53.0 (52.1 - 53.9)**
LDLc, mg/dl	**98.0 (95.9 - 100.0)**	**90.7 (88.6 - 92.8)**
Total cholesterol, mg/dl	**166.9 (164.6 - 169.2)**	**153.8 (151.6 - 156.1)**
Months from menarche	24.0 (22.4 - 25.6)	
Systolic Blood Pressure, mmHg	116.2 (115.3 - 117.1)	124.4 (123.1 - 125.8)
Diastolic Blood Pressure, mmHg	68.5 (67.8 - 69.3)	67.7 (66.9 - 68.4)
Iron status		
Hemoglobin, g/dl	**13.4 (13.3 - 13.5)**	**14.7 (14.6 - 14.8)**
sTfR, mg/l *	5.7 (4.7 - 7.0)	5.9 (4.5 - 7.1)
Serum ferritin, µg/l *	24.4 (18.2 - 50.0)	32.8 (14.1 - 38.7)
Hematocrite (%)	**39.9 (39.7 - 40.2)**	**42.9 (42.6 - 43.2)**
MCV (fl)	**84.8 (84.4 - 85.2)**	**83.4 (83.0 - 83.8)**
MCH (pg)	28.6 (28.4 - 28.7)	28.5 (28.3 - 28.7)
MCHC (g/dL)	**33.7 (33.6 - 33.8)**	**34.2 (34.1 - 34.3)**
Antioxidant vitamins		
Homocysteine, µmol/l	**39.9 (39.7 - 40.2)**	**42.9 (42.6 - 43.2)**
β-Carotene, ng/ml *	219.9 (130.8 - 279.2)	192.5 (148.6 - 311.3)
Retinol, ng/ml *	325.5 (277.0 - 402.2)	349.7 (282.7 - 411.5)
α-Tocopherol, µg/l	**10.2 (10.1 - 10.4)**	**9.5 (9.3 - 9.6)**
Vitamin C, mg/l	**10.6 (10.3 - 10.4)**	**10.0 (9.7 - 10.3)**
Hidrosoluble vitamins		
Vitamin B6, pmol/l *	46.5 (32.1 - 70.1)	53.7 (37.9 - 79.3)
Cobalamin, pmol/l *	**368.3 (358.6 - 384.8)**	**328.4 (318.0 - 340.1)**

Holo-transcobalamin, pmol/l *	57.7 (45.3–74.5)	58.7 (44.2–72.1)
Plasma folate, nmol/l *	16.0 (12.2–22.8)	16.2 (11.8–22.1)
RBC folate, nmol/l *	707.9 (568.5–952.9)	728.8 (544.4–931.6)
Vitamin D		
25(OH)D, nmol/l	59.2 (57.3 - 61.2)	57.3 (55.2 - 59.3)

HDLc= High-density lipoprotein cholesterol. LDLc= Low -density lipoprotein cholesterol.
TC= Total cholesterol. sTfR= Soluble transferrin receptor. MCV= Mean corpuscular volume.
MCH= Mean corpuscular hemoglobin. MCHC= Mean corpuscular hemoglobin concentration.
RBC= Red blood cells. 25(OH)D= 25-hydroxyvitamin D.
Significant associations between sexes ($p<0.05$) are in bold.

DISCUSSION

This study analyzed the association between vitamins and iron blood biomarkers and BP levels in a large sample of European adolescents. The main findings were: 1) vitamins and iron blood biomarkers associations with BP differ between girls and boys; 2) In girls, RBC folate was positively associated with SBP; 3) In boys, vitamins and iron blood biomarkers showed distinct associations with SBP and only retinol was associated with DBP; 4) the association between vitamins and iron blood biomarkers and BP levels was mediated by contextual variables. The results observed in this study justify the need to verify the effect of biomarkers on BP, since the literature is scarce in this topic.

To the best of our knowledge, this is the first study analyzing these associations in adolescents. We do not know the mechanisms of the association between blood vitamins and minerals and the blood pressure may suffer influences of sex differences, but is possible because the sex hormone levels have effect on the BP, the testosterone, acting as a mediator of the androgen receptor gene function [21], has been associated not only with increased visceral fat but also with greater vasomotor sympathetic tone and blood pressure in adolescent boys, compared to girls [21].

Table 2: Multiple linear regression analysis of both models evaluating the association between blood pressure levels and vitamins and iron blood biomarkers, in girls. Beta coefficient and their respective confidence intervals 95% (95% CI).

Conceptual Model Level**	Vitamins and iron blood biomarkers	Null Model	n	Diastolic Blood Pressure Unadjusted ß (95% CI)	p-value	Adjusted* ß (95% CI)	p-value
	Fixed Effects Constant	68.5 (67.8 ; 69.2)				47.0 (35.5 ; 58.4)	
I	**Iron status/Blood Characteristics**						
	Hemoglobin, g/dl		561	0.36 (-0.27 ; 0.98)	p=0.26	9.83 (-7.61 - 27.3)	p=0.269
	sTfR, mg/l[a]		526	0.02 (-1.74 ; 1.78)	p=0.984	-0.45 (-2.48 ; 1.57)	p=0.66
	Serum ferritin, µg/l[b]		530	0.15 (-0.25 ; 0.55)	p=0.458	0.07 (-0.38 ; 0.52)	p=0.754
	Hematocrite (%)		561	0.15 (-0.08 ; 0.39)	p=0.207	-3.15 (-8.96 ; 2.66)	p=0.287
	MCV (fl)		561	-0.06 (-0.20 ; 0.08)	p=0.424	0.27 (-1.39 ; 1.94)	p=0.747
	MCH (pg)		561	-0.12 (-0.45 ; 0.21)	p=0.488	-1.32 (-6.40 ; 3.76)	p=0.609
	MCHC (g/dL)		561	-0.03 (-0.77 ; 0.71)	p=0.936	-2.25 (-8.22 ; 4.32)	p=0.5
II	**Antioxidant vitamins**						
	β-Carotene, ng/ml[a]		500	0.31 (-1.04 ; 1.66)	p=0.652	0.63 (-1.07 ; 2.32)	p=0.469
	Retinol, ng/ml		495	**3.45 (0.80 ; 6.10)**	**p=0.011**	-0.15(-3.53 ; 3.22)	p=0.929
	α-Tocopherol, µg/l		496	**0.46 (0.10 ; 0.83)**	**p=0.013**	0.11 (-0.44 ; 0.65)	p=0.701
	Vitamin C, mg/l		548	0.03 (-0.19 ; 0.25)	p=0.785	0.06 (-0.20 ; 0.32)	p=0.636
III	**Hidrosoluble vitamins**						
	Homocysteine, µmol/l		552	0.05 (-0.25 ; 0.36)	p=0.727	0.08 (-0.45 ; 0.28)	p=0.650
	Vitamin B6, pmol/l[a]		523	0.84 (-0.43 ; 2.11)	p=0.193	1.18 (0.49 ; 2.61)	p=0.105
	Cobalamin, pmol/l[a]		546	-0.80 (-2.57 ; 0.98)	p=0.379	0.48 (-2.29 ; 2.97)	p=0.705
	Holo-transcobalamin, pmol/l[a]		545	0.77 (-0.98 ; 2.51)	p=0.389	-0.38 (-2.92 ; 2.27)	p=0.771
	Plasma folate, nmol/l[a]		546	0.14 (-1.40 ; 1.69)	p=0.859	-0.31 (-2.73 ; 2.11)	p=0.800
	RBC folate, nmol/l[a]		539	1.00 (-0.89 ; 2.89)	p=0.300	0.12 (-2.67 ; 2.91)	p=0.933
IV	**Vitamin D**						
	25(OH)D, nmol/l		531	0.01 (-0.03 ; 0.03)	p=0.993	-0.01 (-0.05 ; 0.02)	p=0.506
	Intraclass correlation coeficient	0.085				0.038	
	Standard deviation context	2.63				1.67	
	Standard deviation individual	8.61				8.40	

	Akaike Information Criterion	4094.9				3313.6	
Conceptual Model Level**	**Vitamins and iron blood biomarkers**	**Null Model**	**n**	**Systolic Blood Pressure**			
				Unadjusted		**Adjusted***	
				ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**
	Fixed Effects Constant	116.2 (115.3 ; 117.1)				66.4 (47.2 ; 85.7)	
I	**Iron status/Blood Characteristics**						
	Hemoglobin, g/dl		561	0.31 (-0.49 ; 1.11)	p=0.447	8.92 (-13.4 ; 31.24)	p=0.433
	sTfR, mg/l[a]		526	0.14 (-0.39 ; 0.67)	p=0.258	-0.09 (-2.66 ; 2.49)	p=0.945
	Serum ferritin, µg/l[b]		530	1.33 (-0.97 ; 3.62)	p=0.587	-0.03 (-0.62 ; 0.55)	p=0.918
	Hematocrite (%)		561	0.11 (-0.20 ; 0.41)	p=0.499	3.76 (-0.62 ; 0.55)	p=0.465
	MCV (fl)		561	-0.18 (-0.36 ; 0.01)	p=0.058	-1.38 (-3.49 ; 0.72)	p=0.197
	MCH (pg)		561	-0.29 (-0.73 ; 0.14)	p=0.188	3.13 (-3.29 ; 9.55)	p=0.338
	MCHC (g/dL)		561	0.17 (-0.79 ; 1.14)	p=0.722	-4.59 (-12.97 ; 3.79)	p=0.283
II	**Antioxidant vitamins**						
	β-Carotene, ng/ml[a]		500	0.75 (-0.99 ; 2.49)	p=0.395	1.13 (-1.04 ; 3.29)	p=0.306
	Retinol, ng/ml		495	**6.83 (3.49 ; 10.2)**	**p<0.001**	1.86 (-2.46 ; 6.19)	p=0.398
	α-Tocopherol, µg/l		496	0.37 (-0.10 ; 0.84)	p=0.126	-0.11 (-0.81 ; 0.59)	p=0.76
	Vitamin C, mg/l		548	-0.06 (-0.35 ; 0.22)	p=0.668	-0.04 (-0.39 ; 0.30)	p=0.81
III	**Hidrosoluble vitamins**						
	Homocysteine, µmol/l		552	0.14 (-0.25 ; 0.54)	p=0.468	0.08 (-0.37 ; 0.53)	p=0.713
	Vitamin B6, pmol/l[a]		523	1.40 (-0.21 ; 3.02)	p=0.088	0.75 (-0.95 ; 2.45)	p=0.388
	Cobalamin, pmol/l[a]		546	-1.26 (-3.54 ; 1.02)	p=0.278	-0.23 (-3.29 ; 2.84)	p=0.885
	Holo-transcobalamin, pmol/l[a]		545	0.53 (-1.75 ; 2.80)	p=0.649	-0.43 (-3.52 ; 2.66)	p=0.785
	Plasma folate, nmol/l[a]		546	0.31 (-1.67 ; 2.30)	p=0.757	0.72 (-2.14 ; 3.58)	p=0.622
	RBC folate, nmol/l[a]		539	2.31 (-0.12 ; 4.74)	p=0.062	**3.19 (0.61 ; 5.77)**	**p=0.015**
IV	**Vitamin D**						
	25(OH)D, nmol/l		531	-0.01 (-0.06 ; 0.03)	p=0.512	-0.01 (-0.05 ; 0.03)	p=0.651
	Intraclass correlation coeficient	0.164				0.129	
	Standard deviation context	4.95				4.00	
	Standard deviation individual	11.18				10.49	
	Akaike Information Criterion	4394.7				3761.9	

* The adjusted analysis was conducted following a theoretical conceptual model that had been previously formulated in three levels (the association of the first two levels not shown): 1) contextual variables (seasonality, latitude of residence and school; 2) potential individual confounders (maternal education, age (years) age at menarche (in girls), BMI [body, mass index], WC [waist circumference], physical activity, serum lipid concentrations; and 3) BSC; The biomarkers were divided in more four groups: I) blood composition (hematocrite, MCV [mean corpuscular volume], MCH [mean corpuscular hemoglobin] and MCHC [mean corpuscular hemoglobin concentration]) and iron status indicators [soluble transferrin receptor (STfR) and serum ferritin]; II) provitamin A (α-carotene), vitamin A (retinol), and vitamin E (β- tocopherol), vitamin C; III) vitamin B6, B12 (cobalamin and holo-transcobalamin); homocysteine; ; folate [plasma and red blood cell (RBC)]; and IV) vitamin D [25(OH)D].

** The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with p>0.2 were not included in the subsequent adjustment models.

a Log-transformed data.

b Square-root transformed data.

Significant associations are in bold.

Table 3: Multiple linear regression analysis of both models evaluating the association between blood pressure levels and vitamins and iron blood biomarkers, in boys. Beta coefficient and their respective confidence intervals 95% (95% CI).

Conceptual Model Level**	Vitamins and iron blood biomarkers	Null Model	n	Diastolic Blood Pressure			
				Unadjusted		Adjusted*	
				ß (IC 95%)	p-value	ß (IC 95%)	p-value
	Fixed Effects Constant	67.7 (66.9 ; 68.4)				37.8 (18.1 -;57.4)	
I	**Iron status/Blood Characteristics**						
	Hemoglobin, g/dl		496	**1.55 (0.92 ; 2.19)**	**p<0.001**	-3.43 (-17.0 ; 0.7)	p=0.619
	sTfR, mg/l[a]		459	-1.18 (-3.63 ; 1.28)	p=0.345	-0.35 (-2.77 ; 2.08)	p=0.779
	Serum ferritin, µg/l[b]		467	**0.65 (0.26 ; 1.04)**	**p=0.001**	0.27 (-0.16 ; 0.70)	p=0.22
	Hematocrite (%)		496	**0.66 (0.44 ; 0.87)**	**p<0.001**	1.66 (-2.93 ; 6.24)	p=0.478
	MCV (fl)		496	0.08 (-0.08 ; 0.24)	p=0.314	0.01 (-0.65 ; 0.68)	p=0.974
	MCH (pg)		496	-0.04 (-0.43 ; 0.35)	p=0.822	-0.15 (-2.07 ; 1.77)	p=0.877
	MCHC (g/dL)		496	**-1.17 (-1.99 ; -0.34)**	**p=0.006**	1.31 (-4.61 ; 7.23)	p=0.664
II	**Antioxidant vitamins**						
	β-Carotene, ng/ml[a]		443	**-1.37 (-2.69 ; -0.06)**	**p=0.041**	0.39 (-1.30 ; 2.08)	p=0.648
	Retinol, ng/ml		436	**7.07 (4.05 ; 10.08)**	**p<0.001**	**3.84 (0.51 ; 7.17)**	**p=0.024**
	α-Tocopherol, µg/l		448	0.15 (-0.28 ; 0.58)	p=0.488	0.04 (-0.55 ; 0.62)	p=0.897
	Vitamin C, mg/l		491	-0.23 (-0.47 ; 0.01)	p=0.055	-0.10 (-0.38 ; 0.17)	p=0.457
III	**Hidrosoluble vitamins**						
	Homocysteine, µmol/l		488	0.15 (-0.02 ; 0.33)	p=0.081	0.06 (-0.18 ; 0.29)	p=0.636
	Vitamin B6, pmol/l[a]		448	1.36 (-0.07 ; 2.79)	p=0.063	0.81 (-0.89 ; 2.51)	p=0.348
	Cobalamin, pmol/l[a]		489	-1.03 (-3.13 ; 1.07)	p=0.335	0.38 (-2.44 ; 3.20)	p=0.729
	Holo-transcobalamin, pmol/l[a]		459	-0.05 (-2.18 ; 2.08)	p=0.966	-1.45 (-4.41 ; 1.52)	p=0.338
	Plasma folate, nmol/l[a]		487	-1.11 (-2.75 ; 0.52)	p=0.182	-0.49 (-2.98 ; 1.99)	p=0.696
	RBC folate, nmol/l[a]		485	0.18 (-1.70 ; 2.07)	p=0.847	0.93 (-1.74 ; 3.60)	p=0.494
IV	**Vitamin D**						
	25(OH)D, nmol/l		465	-0.01 (-0.04 ; 0.04)	p=0.949	0.01 (-0.03 ; 0.05)	p=0.588
	Intraclass correlation coeficient	0.067				0.044	
	Standard deviation context	2.37				1.68	
	Standard deviation individual	8.86				7.86	

	Akaike Information Criterion	3587.2				2591.6	
Conceptual Model Level**	**Vitamins and iron blood biomarkers**	**Null Model**	**n**	**Systolic Blood Pressure**			
				Unadjusted		**Adjusted***	
				ß (IC 95%)	**p-value**	**ß (IC 95%)**	**p-value**
	Fixed Effects Constant	124.4 (123.1 ; 125.6)				93.4 (74.8 ; 111.9)	
I	**Iron status/Blood Characteristics**						
	Hemoglobin, g/dl		496	**3.01 (2.03 ; 3.98)**	**p<0.001**	-2.17 (23.0 ; 18.6)	p=0.837
	sTfR, mg/l[a]		459	-2.15 (-5.95 ; 1.66)	p=0.268	-1.53 (-5.20 ; 2.12)	p=0.41
	Serum ferritin, µg/l[b]		467	**0.78 (0.17 ; 1.40)**	**p=0.012**	-0.09 (-0.74 ; 0.55)	p=0.773
	Hematocrite (%)		496	**1.20 (0.87 ; 1.54)**	**p<0.001**	1.57 (-5.46 ; 8.59)	p=0.661
	MCV (fl)		496	0.15 (-0.10 ; 0.40)	p=0.238	0.42 (-0.59 ; 1.43)	p=0.416
	MCH (pg)		496	0.01 (-0.60 ; 0.62)	p=0.965	-1.03 (-3.94 ; 1.89)	p=0.489
	MCHC (g/dL)		496	**-1.54 (-2.84 ; 0.25)**	**p=0.019**	1.28 (-7.79 ; 10.4)	p=0.781
II	**Antioxidant vitamins**						
	β-Carotene, ng/ml[a]		443	-(1.75 (-3.81 ; 0.31)	p=0.095	0.37 (-2.15 ; 2.90)	p=0.77
	Retinol, ng/ml		436	**11.28 (6.55 ; 16.01)**	**p<0.001**	4.69 (-0.58 ; 9.97)	p=0.081
	α-Tocopherol, µg/l		448	-0.10 (-0.78 ; 0.57)	p=0.765	0.11 (-0.79 ; 1.02)	p=0.804
	Vitamin C, mg/l		491	0.30 (-0.07 ; 0.67)	p=0.116	0.28 (-0.15 ; 0.70)	p=0.205
III	**Hidrosoluble vitamins**						
	Homocysteine, µmol/l		488	0.26 (-0.20 ; 0.53)	p=0.069	0.01 (-0.27 ; 0.27)	p=0.982
	Vitamin B6, pmol/l[a]		448	**4.32 (2.08 ; 6.58)**	**p<0.001**	**3.82 (1.46 ; 6.18)**	**p=0.002**
	Cobalamin, pmol/l[a]		489	**-3.33 (-6.67 ; -0.001)**	**p=0.05**	0.63 (-3.62 ; 4.87)	p=0.772
	Holo-transcobalamin, pmol/l[a]		459	-2.87 (-6.21 ; 0.46)	p=0.091	**-3.74 (-7.28 ; -0.21)**	**p=0.038**
	Plasma folate, nmol/l[a]		487	0.13 (-2.51 ; 2.78)	p=0.924	-0.98 (-4.55 ; 2.58)	p=0.587
	RBC folate, nmol/l[a]		485	1.67 (-13.6 ; 4.71)	p=0.28)	2.57 (-1.49 ; 6.62)	p=0.214
IV	**Vitamin D**						
	25(OH)D, nmol/l		465	-0.05 (-0.10 ; 0.01)	p=0.129	-0.05 (-0.11 ; 0.02)	p=0.151
	Intraclass correlation coeficient	0.046				0.03	
	Standard deviation context	3.03				2.21	
	Standard deviation individual	13.86				12.26	
	Akaike Information Criterion	4033.6				2929.1	

* The adjusted analysis was conducted following a theoretical conceptual model that had been previously formulated in three levels (the association of the first two levels not shown): 1) contextual variables (seasonality, latitude of residence and school; 2) potential individual confounders (maternal education, age (years) age at menarche (in girls), BMI [body, mass index], WC [waist circumference], physical activity, serum lipid concentrations; and 3) BSC; The biomarkers were divided in more four groups: I) blood composition (hematocrite, MCV [mean corpuscular volume], MCH [mean corpuscular hemoglobin] and MCHC [mean corpuscular hemoglobin concentration]) and iron status indicators [soluble transferrin receptor (STfR) and serum ferritin]; II) provitamin A (α-carotene), vitamin A (retinol), and vitamin E (β- tocopherol), vitamin C; III) vitamin B6, B12 (cobalamin and holo-transcobalamin); homocysteine; ; folate [plasma and red blood cell (RBC)]; and IV) vitamin D [25(OH)D].

** The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with p>0.2 were not included in the subsequent adjustment models.

a Log-transformed data.

b Square-root transformed data.

Significant associations are in bold.

In girls, RBC folate was positively associated with SBP. One biological and plausible explanation for this association is that girls have shown a high prevalence of iron deficiency (13.8% lower than boys, or 21% of girls have iron defficiency) [22] and RBC folate levels decrease with low iron concentration. These biomarkers (RBC folate and iron concentration) are responsible for the transport of O^2 and, with a more efficient transport of O^2, systolic BP level decrease.

We found that a high vitamin B_6 concentration was positively associated with DBP in boys. Major sources of vitamin B_6 is red meat [23] and the consumption of red meat is associated with increased blood pressure and cardiovascular disease [24].

Our results also showed a positive association between retinol and SBP in boys. Previous studies have also demonstrated that a high concentration of retinol reduces cardiovascular risk [25]. Cross-sectional studies are susceptible to reverse causality. One possible explanation is that the boys participating in the HELENA study had higher concentrations of inflammatory markers than girls [26]. These inflammatory markers can inhibit the activation effect of retinol on mitochondria, which is the responsible for cellular respiration [27], and increased mitochondrial activity may be associated with increased blood flow O^2 by decreasing the BP.

Low vitamin D 25-hydroxyvitamin D (25(OH)D). serum concentration is known to be related to cardiometabolic disorders in adults; however, this associations with cardiovascular risk factors in adolescents are not well elucidated. Recently, Nam et al.[28] found that adolescents with low levels of vitamin D (<50 nmol/l) have an increased risk for developing metabolic syndrome. In our study we found no association between vitamin D serum concentrations and BP. One possible explanation is that 75% of adolescents participating in the HELENA study has lower concentrations of 25-hydroxyvitamin D (25 (OH) D)[29] than <50 nmol/l, nevertheless, in adulthood, low

concentrations of vitamin D can have an effect on the development of cardiovascular diseases [28].

Interestingly, high concentrations of holo-transcobalamin was negatively associated with SBP in boys. Holo-transcobalamin has mediating effects on the positive association between homocysteine and blood pressure [30]. According to Herrmann et al. [31], holo-transcobalamin reduces the concentration of homocysteine and this decrease SBP. However, this is a novel finding that needs further research focused on the understanding of the physiological mechanisms behind this association.

Our study is multicenter design and the diverse geographic origin of the sample, as well, the multilevel analysis with a complete set of potential confounders is some of the main strengths of the present study. The cross-sectional design of the study represents a limitation as causality cannot be established. In addition, it has not been possible to adjust the analysis for other factors potentially associated with BP, eg. genetic factors and intrauterine development.

CONCLUSIONS

Vitamins blood biomarkers (i.e. RBC folate, vitamin B_6 and retinol) were positively associated with SBP in boys, while vitamin B_{12} was negatively associated with DBP. These associations are independent of contextual variables. Longitudinal studies are required to confirm these findings. These results may highlights the importance of the consumption of vegetable proteins and foods rich in vitamin B_{12} (white meat and eggs) in programs aiming to promote healthy eating behavior.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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3.6 RESTING HEART RATE IS NOT A GOOD PREDICTOR OF A CLUSTERED CARDIOVASCULAR RISK SCORE IN ADOLESCENTS: THE HELENA STUDY – SUBMITTED IN PLOS ONE

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COMPETING INTEREST

The remaining authors state no conflict of interest.

Key-words: screening; cardiovascular diseases risk; ROC curve; adolescents; HELENA study.

ABSTRACT

Background: Resting heart rate (RHR) reflects sympathetic nerve activity a significant association between RHR and all-cause and cardiovascular mortality has been reported in some epidemiologic studies.

Methods: To analyze the predictive power and accuracy of RHR as a screening measure for individual and clustered cardiovascular risk in adolescents. The study comprised 769 European adolescents (376 boys) participating in the HELENA cross-sectional study (2006–2008) were included in this study. Measurements on systolic blood pressure, HOMA index, triglycerides, TC/HDL-c, VO_2máx and the sum of four skinfolds were obtained, and a clustered cardiovascular disease (CVD) risk index was computed. The receiver operating characteristics curve was applied to calculate the power and accuracy of RHR to predict individual and clustered CVD risk factors.

Results: RHR showed low accuracy for screening CVD risk factors in both sexes; (range 38.5% - 54.4% in boys and 45.5% - 54.3 % in girls). Low specificity's (15.6% - 19.7% in boys; 18.1% - 20.0% in girls) was also found. Nevertheless, the sensitivities were moderate-to-high (61.4% - 89.1% in boys; 72.9% - 90.3% in girls).

Conclusion: RHR is a poor predictor of individual CVD risk factors and of clustered CVD and the estimates based on RHR are not accurate. The use on RHR as an indicator of CVD risk in adolescents may produce a biased screening of cardiovascular health in both sexes.

Key-words: screening; cardiovascular diseases risk; ROC curve; adolescents; HELENA study.

INTRODUCTION

Resting heart rate (RHR) reflects sympathetic nerve activity [1,2], and it is an accessible clinical measurement. A significant association between resting HR and all-cause of cardiovascular mortality has been reported in some epidemiologic studies [1,3-5]. Based on epidemiologic data and inferences from clinical trials the results showed that RHR are undesirable in terms of cardiovascular disease. However, the importance of RHR as a prognostic factor and potential therapeutic outcome has not been formally explored, and therefore, despite suggestive evidence, is not generally accepted [6].

The main metabolic cardiovascular diseases (CVD) risk factors are dyslipidemia, glucose intolerance, hypertension and obesity, which are highly prevalent in young people [7]. From a methodological perspective, the use of a clustered cardio-metabolic risk score is recommended because it can compensate for day-to-day fluctuations observed when using the single risk factors [8]. Additionally, cardio-metabolic risk factors acquired in youth, as well as their health risks, tend to persist into adulthood [9]. Therefore, identifying good predictors for cardio-metabolic risk factors is necessary to assist in the development of actions designed to improve cardio-metabolic health in young populations.

Thus, we hypothesized that RHR is a good predictive power and accuracy of RHR as screening measure for individual and clustered CVD risk in adolescents. We tested this hypothesis on the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study (HELENA-CSS).

METHODS

Study population

The HELENA-CSS aimed to describe the lifestyle and nutritional status of European adolescents. Data collection took place between October 2006 and December 2007 in the following cities: Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria, and Zaragoza in Spain. Further information about the study design has been published elsewhere [10,11]. Participants were recruited at schools. To ensure that the heterogeneity of social background of the population would be represented, schools were randomly selected after stratification by school zone or district. In cases where the selected schools refused to participate, a second list of substitute schools had already been drawn up. Up to three classes from two grades were selected per school. A class was considered eligible if the participation rate was at least 70%. The general inclusion criteria for HELENA were age range of 12.5-17.5 years, not participating simultaneously in another clinical trial, and free of any acute infection lasting less than 1 week before inclusion [10].

From a sample of 3528 adolescents who met the HELENA general inclusion criteria, one third of the school classes were randomly selected in each centre for blood collection, resulting in a total of 1089 adolescents. For the purposes of the present study, adolescents with valid data for sedentary behaviour, accelerometry, cardiorespiratory fitness, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), insulin, glucose, systolic blood pressure and triceps, biceps, subscapular and supra-iliac skinfolds were finally included in the analysis (n=769, **Figure 1**). The study sample did not differ in sex distribution, mean age, mean body mass index (BMI) and mean values of cardiorespiratory fitness from the full HELENA sample (all $p>0.05$).

The study was performed following the ethical guidelines of the Declaration of Helsinki 1975 (as revised in 1983). The study was approved by the Research Ethics

Committee of each city involved. Written informed consents were obtained from both the adolescents and their parents.

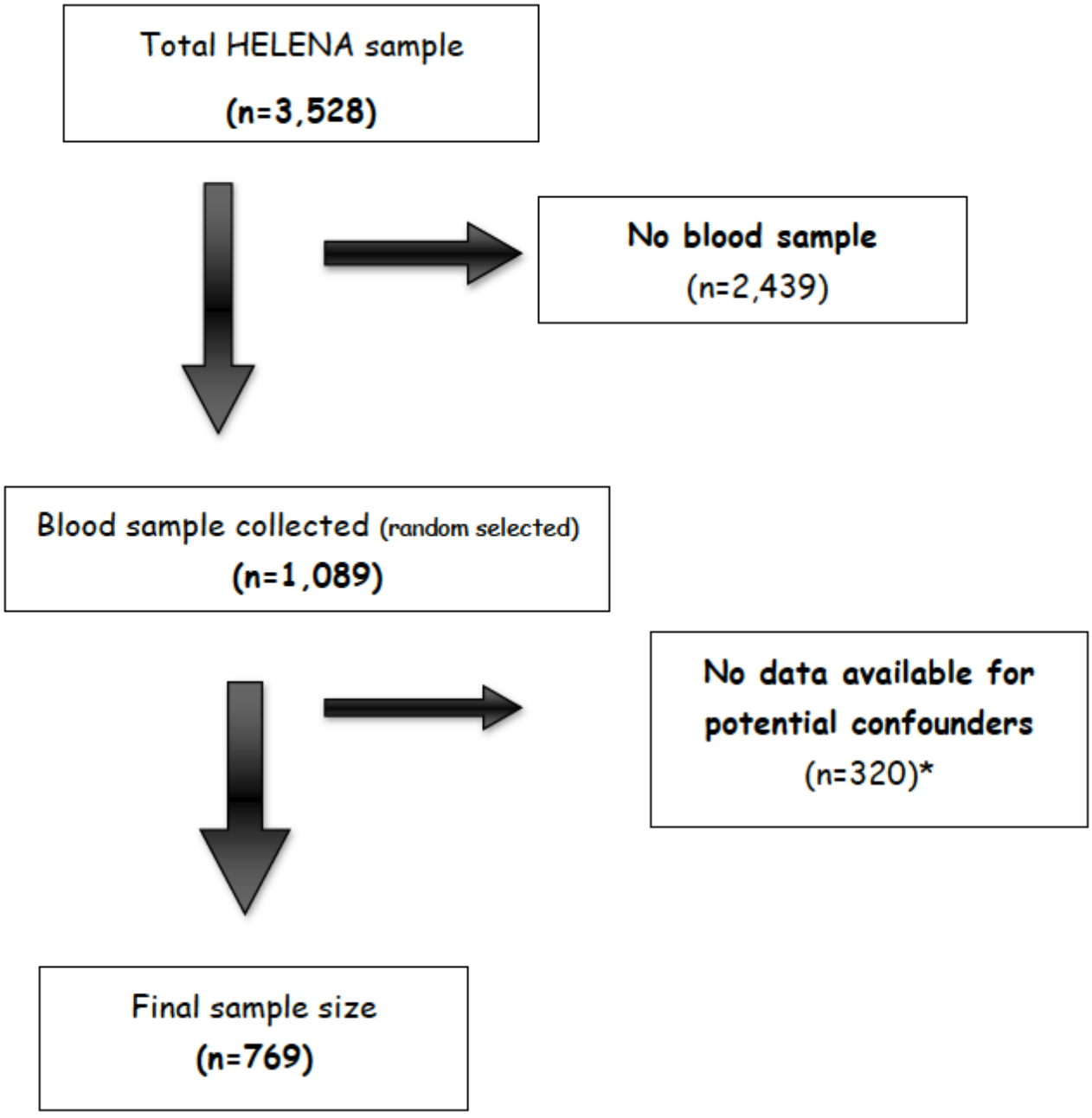

Figure 1: Final sample size flowchart. *Missing data for accelerometry datas.

Resting Heart Rate (RHR)

The RHR were measured in all centers using the same type of oscillometric monitor device OMRON® M6 (HEM 70001) which has been approved by the British Hypertension Society [12]. All devices were calibrated by measuring of the RHR and blood pressure following the procedure in the operations manual, we observed no significant differences between devices; measurements were taken twice (10 min apart) and the lowest value was retained, these data collection procedures have been described previously [13].

Physical examination

Waist circumference, height, weight and four skinfold thicknesses (on the left side from biceps, triceps, subscapular, supra-iliac) were measured following a standardized protocol [14]. The definition of obesity (including overweight) was based on international BMI cutoffs proposed by Cole et al. [14] from several different countries. Systolic and diastolic blood pressure measurements by the arm blood pressure oscillometric monitor device OMRON® M6 (HEM 70001) which has been approved by the British Hypertension Society [12]. Measurements were taken twice (10 min apart) and the lowest value was retained. These data collection procedures have been described previously[13].

Cardiorespiratory fitness

Participants ran between two lines 20 m apart, keeping the pace with audio signals. The initial speed was 8.5 km/h, and each minute speed was increased by 0.5 km/h. Participants had to run in a straight line and to pivot on the lines. The test finished when subjects stopped due to fatigue or when they failed to reach the end line

concurrent with the signals on two consecutive occasions. The last completed stage or half-stage was recorded. Finally, the maximal oxygen consumption (VO_2 max) in ml/kg/min was estimated by the Leger equation (Boys and girls: VO2max = 31.025 + (3.238 x S x 3.248 x A) + (0.1536 x S x A) (A the age; S the final speed) (S = 8 + 0.5 last stage completed) [15], [16]. Physical fitness levels were described in detail elsewhere [17].

Cardiovascular diseases risk factors

Blood samples were obtained for a third of the HELENA-CSS participants. Blood samples (24.3 ml) were collected by venipuncture at school between 8 and 10 o'clock in the morning after a 10-hour overnight fast. Centrifugation was performed at room temperature. Blood was collected in heparinized tubes, immediately placed on ice and centrifuged within 30 min (3,500 r.p.m. for 15 min) to avoid haemolysis. Immediately after centrifugation, the samples were stored and transported at 4-7°C (for a maximum of 14 h) to the central laboratory in Bonn (Germany) and stored there at –80°C until assayed. Triglycerides, TC, high-density lipoprotein cholesterol (HDL-c) and glucose were measured using enzymatic methods (Dade Behring, Schwalbach, Germany). Insulin levels were measured using an Immulite 200 analyser (DPC Bierman GmbH, Bad Nauheim, Germany). The homeostasis model assessment (HOMA) calculation was used as a measurement of insulin resistance (glycaemia X insulin/22.5) [18].

A clustered cardiovascular risk index was created from the following variables: systolic blood pressure, HOMA index, triglycerides, TC/HDL-c ratio, VO2max and the sum of four skinfolds. The standardized value of each variable was calculated as follows: (value-mean)/SD, separately for boys and girls and by 1-yr age groups. For

variables characterized by a lower metabolic risk with increasing values (VO2max), Z scores were multiplied by -1. To create the metabolic risk score, all the Z-scores were summed, where the lowest values are indicative of a better cardio-metabolic risk profile. Finally, all those subjects at or above age and gender specific cut-offs, subjects were classified as having metabolic risk when they accumulated ≥1SD, similar to previous studies [8,19].

Statistical Analysis

The descriptive analyses were presented as means (quantitative variables) and percentages (qualitative variables) and confidence intervals 95% (95% CI).

All cardiovascular risk factors variables were entered as fixed factors. Education of the mother, MVPA, waist circumference and months from menarche for girls were entered as covariates. Receiver operating characteristics (ROC) curve analysis was applied to calculate the relationship between clustered and individual cardiovascular risk factors (were used binary outcome) and RHR. ROC curve provides the whole spectrum of specificity/sensitivity values for all the possible cut-offs. The area under the curve (AUC) is determined from plotting sensitivity versus 1 – specificity of a test as the threshold varies over its entire range. Taking into account the suggested cut-off points, the test can be non-informative/test equal to chance less accurate (0.5AUC<0.7); moderately accurate (0.7>AUC≤0.9); highly accurate (0.9>AUC<1.0); and perfect discriminatory tests (AUC=1.0) [20]. In addition, ROC curve indexes of each cut-off point were calculated through the determination of positive and negative predictive values, overall misclassification rate, positive and negative likelihood ratios, and Youden Index [21].

The statistical software package Stata version 12.0 (Stata Corp., college Station, TX, USA) was used for all statistical calculations.

RESULTS

The proportion of boys had significantly performing physical activity the recommended amount of physical activity (≥60min/d) was higher than girls. Among CVD risk factors, males showed higher significant levels for SBP and TC/HDL, while girls had higher plasma concentrations of TC, HDL-c and triglycerides. Boys had also higher RHR than their female peers (**Table 1**).

The accuracy of prediction of RHR for the six factors individual CVD risk factors and for the cluster of CVD separately by sex. For all CVD risk factors, the RHR have a high sensitivity, low specificity and accuracy (area under of curve), regardless of sex (**Table 2**).

DISCUSSION

This study analyzed the predictive power and accuracy of RHR as a screening measure for individual and clustered CVD risk factors in a large sample of European adolescents. The main finding was that RHR is not a good predictor of CVD risk factors in this population, regardless of sex, age and level of physical activity. Our hypothesis is biologically plausible, since the onset of these factors in adolescence is strongly associated with increased risk of CVD in adulthood [9].

Table 1: Characteristics of the study population.

Variables	Girls (n= 393) mean or % (95%CI)	Boys (n= 376) mean or % (95%CI)
Age (years)	14.8 (14.7 - 14.9)	14.8 (14.7 - 14.9)
Tanner Stage (%)		
1 and 2 (pre-pubertal)	7.3 (5.0 – 9.6)	7.1 (4.7 – 9.5)
3 and 4 (pubertal)	65.6 (61.6 – 70.1)	64.4 (59.9 – 68.9)
5 (post-pubertal)	26.8 (59.9 – 68.9)	28.5 (24.3 – 32.8)
Education mother		
Lower education	8.4 (6.0 - 10.7)	8.9 (6.3 - 11.4)
Lower secondary education	30.3 (26.4 - 34.2)	27.6 (23.6 - 31.7)
Higher secondary education	30.7 (26.8 - 34.6)	29.6 (25.4 - 33.8)
University degree	30.6 (26.8 - 34.6)	33.9 (25.4 - 33.8)
MVPA		
< 60 min/d	**72.3 (67.9 - 76.7)**	**39.3 (33.9 - 44.6)**
≥ 60 min/d	**27.7 (23.3 - 32.1)**	**60.7 (55.4 - 66.1)**
Sedentary behavior by questionnaire		
> 4 h/d	**20.4 (18.3 - 22.5)**	**38.8 (36.2 - 41.5)**
2 - 4 h/d	**36.3 (33.8 - 38.8)**	**39.1 (36.4 - 41.7)**
< 2 h/d	**43.3 (40.7 - 45.8)**	**22.1 (19.9 - 24.4)**
Months from menarche	24.0 (22.4 - 25.6)	
Height (cm)	**162.3 (161.8 - 162.9)**	**169.3 (168.5 - 170.1)**
Weight (kg)	**56.7 (55.9 - 57.6)**	**61.0 (59.9 - 62.2)**
BMI (kg/m^2)	21.5 (21.2 - 21.8)	21.1 (20.8 - 21.5)
Obesity (%) by Cole	**3.0 (1.3 - 4.6)**	**5.5 (3.1 - 7.8)**
Waist circumference (cm)	**70.6 (70.0 - 71.3)**	**74.4 (73.6 - 75.2)**
VO2max (ml/kg/min)	84.8 (84.4 - 85.2)	83.4 (83.0 - 83.8)
Tryglicerides (mg/dl)	**73.3 (70.3 - 76.4)**	**64.4 (61.6 - 67.2)**
HDLc(mg/dl)	**60.0 (56.1 - 57.9)**	**53.0 (52.1 - 53.9)**
Total cholesterol(mg/dl)	**166.9 (164.6 - 169.2)**	**153.8 (151.6 - 156.1)**
TC/HDL-c	2.99 (2.93 - 3.04)	3.02 (2.96 - 3.09)
Systolic Blood Pressure (mmHg)	**116.2 (115.3 - 117.1)**	**124.4 (123.1 - 125.8)**
HOMA index	2.38 (2.20 - 2.56)	2.28 (2.12 - 2.43)
∑ Four skinfolds	53.6 (51.5 - 55.8)	52.2 (49.9 - 54.4)
Resting heart rate (bpm)	**78.9 (77.8 - 80.00)**	**80.6 (79.3 - 81.8)**
Metabolic risk (%)	15.3 (11.8 - 18.9)	15.6 (11.9 - 19.4)

95% CI: confidence interval of 95%; BMI: body mass index; MVPA: Moderate to vigorous physical activity; HDLc= High-density lipoprotein cholesterol; TC= Total cholesterol. Significance difference (p <0.05) between girls and boys are in bold.

Table 2. Accuracy of resting heart rate in screening of individual and clustered cardio-metabolic risk factors in adolescents from HELENA study.

Cardiovascular risk factors		Sensitivity	Specificity	Area Under the Curve ±SE	CI 95%		LR +	LR –	Youden Index
					Lower	Upper			
Clustered metabolic risk									
	Male	0.897	0.190	0.507 ±0.039	0.430	0.584	1.11	0.54	0.087
	Female	0.940	0.104	0.584 ±0.039	0.507	0.661	1.05	0.58	0.045
TC/HDL-c									
	Male	0.885	0.173	0.509 ±0.035	0.441	0.576	1.07	0.66	0.058
	Female	0.543	0.516	0.525 ±0.033	0.460	0.590	1.12	0.89	0.059
VO2max									
	Male	0.517	0.548	0.528 ±0.019	0.492	0.565	1.14	0.88	0.065
	Female	0.898	0.141	0.499 ±0.018	0.465	0.534	1.05	0.72	0.039
∑ Four skin folds									
	Male	0.505	0.543	0.518 ±0.017	0.486	0.551	1.11	0.91	0.052
	Female	0.900	0.136	0.498 ±0.016	0.467	0.529	1.04	0.74	0.037
HOMA index									
	Male	0.881	0.175	0.503 ±0.035	0.435	0.572	1.07	0.68	0.056
	Female	0.544	0.511	0.520 ±0.033	0.455	0.585	1.11	0.89	0.055
Systolic Blood Pressure									
	Male	0.511	0.548	0.521 ±0.017	0.488	0.553	1.13	0.89	0.059
	Female	0.901	0.135	0.496 ±0.016	0.465	0.527	1.04	0.73	0.037
Triglycerides									
	Male	0.885	0.173	0.509 ±0.035	0.441	0.576	1.07	0.66	0.058
	Female	0.542	0.516	0.525 ±0.033	0.460	0.590	1.12	0.89	0.059

CI 95% = confidence interval 95%; LR + = Positive likelihood ratios; LR – = Negative likelihood ratios; HDLc = High-density lipoprotein cholesterol; TC = total cholesterol.

Girls had lower RHR than boys (statistically significant), this difference can be partially explained by two reasons: 1) the girls have a higher VO2max and this increased aerobic capacity decreases RHR; 2) boys has a higher accumulation abdominal fat (measured by waist circumference) [22] than girls, and visceral fat has been associated with higher sympathetic activity [23,24]. This activation is a key mechanism underlying the effect of intra-abdominal fat accumulation on the development of hypertension [25].

Recently, another study analyzed the potential effects of screening and resting heart rate (RHR) on cardiometabolic risk in adolescents [26]. They found the use of RHR to screen for alterations in glucose and triglycerides interesting but, according to the data presented, we believe that there is no evidence for this. Accuracy (AUC) for high glucose was 0.611 (95% CI 0.534–0.688) and high triglycerides, 0.618 (95% CI 0.531–0.705), both with p-values <0.05, but with low discrimination power—note the lower confidence bound in some cases is very close to 0.50 (random event). In other words, if we consider random variations within the CI bounds of AUC, determining the presence or absence of high glucose and high triglycerides will be as precise as playing a game of heads or tails. With regard to the accuracy of results, Swets [20] suggested operational cut-off points: the test can be non-informative/test equal to chance (0.5AUC<0.7); moderately accurate (0.7>AUC≤0.9); highly accurate (0.9>AUC<1.0); and perfect discriminatory tests (AUC=1.0).

Although RHR has been recently showed to be a good predictor for CVD in adults [1], our findings do not confirm these results in adolescents. These differences may explained by the fact that the analyses carried out in adults considered as risk values into percentiles of the RHR [1,3-5] which is intrinsically associated with the distribution of the variable within the sample. In our study, we analyzed the predictive

value using a more accurate analysis (ROC curve) that the distribution in percentiles. Another important point is that the onset of CVD takes several years [27], and here only we compare with risk factors for diseases.

Fernandes et al. [28] found that higher RHR is associated with higher levels of SBP regardless of nutritional status in children, however the authors also used percentiles to classify the RHR. There is evidence that obese adolescents have higher levels of SBP [28], which might also be translated into having higher RHR. However, accurate measurement of RHR is difficult, and the biological parameter has no advantage over the use of other CVD risk factors, since RHR has been shown to have an accuracy of less than 55% for all the factors. Another important point which may explain the absence of good prediction is the fact that adolescents are in the process of biological maturation and maturation-related hormones influence the sympathetic activation [29] can be reflects vagal nerve activity that controls the RHR [1,2].

This study adds more evidence to the existing literature about predictors of cardio-metabolic risk in adolescents. As noted, "*we are biased by findings that are published and are thus blind to any studies that produce negative findings* [30]". The principal message from our study is that RHR is an important cardiovascular indicator and may play an important role in adolescents´ health, despite not being a good predictor of cardio-metabolic health during the adolescence.

Our study was developed in European adolescents, and one possible limitation is lack of generalizability of these findings to other non-European adolescent populations [31]. But we believe that this limitation is minimized by our being multicenter study conducted in 10 cities in 9 countries with different ethnic groups, and also because we studied two biological variables (cardiovascular risk and RHR), as did Doll & Hill in their major on cigarette consumption and cancer study [32], which was taken only in

UK doctors and was generalized to all ethnic groups and cultures of the world, for its outcome to be a biological variable.

The strengths of this study are that samples were collected in different countries using the same methodology, appropriate statistical analysis controlling for potential confounding factors were performed as well as the analysis of the efficiency of RHR such a predictor for different individual CVD risk factors and clustered CVD. On the other hand, diverse geographic origin of the sample and multilevel analysis are some of the main strengths of our analysis.

In this study there are some limitations such as its cross-sectional design; consequently, causality cannot be established. Moreover, it has not been possible to adjust the analysis for other factors potentially associated with BP, eg. genetic, intrauterine development and inflammatory indicators.

CONCLUSIONS

In conclusion that the RHR is a poor predictor for individual and clustered CVD risk factors. Furthermore, the estimates based on RHR are not accurate. According to our findings, the use of RHR as an indicator of cardiovascular risk in adolescents may result in a biased screening of cardiovascular health in both sexes.

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3.7 DIETARY PROTEIN AND AMINO ACIDS INTAKE AND ITS RELATIONSHIP WITH BLOOD PRESSURE IN ADOLESCENTS. THE HELENA STUDY – SUBMITTED IN EUROPEAN JOURNAL OF PUBLIC HEALTH

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ABSTRACT

Objective: To analyze the association between dietary protein and amino acids intake and systolic (SBP) and diastolic (DBP) blood pressure in European adolescents.

Methods: Participants were from the cross-sectional study performed in Europe, Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA study; n= 1605; 12.5–17.5 yr; 833 girls) selected by complex sampling. The associations between dietary protein and amino acids intake and SBP/DBP were examined by multilevel linear regression models (context variable by school); the analysis being stratified by sex. Cities, seasonality, age, socioeconomic level, parental education level, body mass index, waist circumference, Tanner stage, and physical activity were used as covariates.

Results: In boys, we found an inverse association between protein (animal and vegetable) intake and DBP; and a positive association between histidine and SBP. In girls, we observed a positive association among tryptophan, histidine with SBP and methionine with DBP. On the other hand, we observed an inverse association between tyrosine and both systolic and diastolic BP levels in girls.

Conclusions: The association between amino acids and BP levels is controversial and depends on the type of amino acids, and protein intake can help control the DBP in boys.

Key-words: dietary protein; dietary amino acids; blood pressure; adolescents; multicenter study

INTRODUCTION

Cardiovascular diseases (CVD) are the main sources of disease burden worldwide, thus being a major public health problem [1]. Elevated blood pressure is considered the risk factor with the highest attributable fractions for CVD mortality being 40.6% [2]. The prevalence of elevated blood pressure (BP) is already high during adolescence [3] and some studies have shown that levels of BP in childhood and adolescence are crucial for developing hypertension in adulthood [4].

Among all factors that may influence BP (e.g. genetics, intrauterine development, physical activity, sedentary behavior, tobacco, total and abdominal obesity) dietary protein intake seems to be associated with reduced BP in adults [5]. The underlying mechanism for a potential beneficial effect of protein and amino acids intake (AA) on BP has not been clarified yet. Dietary protein intake or protein fractions could improve insulin sensitivity and thereby BP [5]. On the other hand, AA like tyrosine and tryptophan could trigger a vasodilatory response in regions of the brain or blood vessel wall [6, 7]. However, the associations between AA and BP in adolescents are unclear.

Thus, we hypothesized that dietary protein and AA intake are negatively associated with levels of BP, and that the magnitude of the association is dependent of some individual (socioeconomic status, lifestyle variables and anthropometrics variables) and contextual characteristics (city and seasonality) where the research was conducted. The hypothesis was tested among European adolescents participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study.

METHODS

The HELENA study (n = 3,528) aimed to obtain data from a sample of European adolescents on a broad battery of nutrition and health-related parameters. Data collection took place during 2006 and 2007 in ten cities from nine European countries (Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pécs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain). A detailed description of the HELENA sampling and recruitment methodology, data collection and quality control activities has been published elsewhere [8, 9]. After receiving complete information about the aims and methods of the study, all the parents/guardians signed a consent, and adolescents gave assent to participate in the study. The Human Research Review Committees of each center approved the study protocol. All participants were recruited via schools and met the general HELENA inclusion criteria: age range 12.5-17.5 years, not participating in another clinical trial at the same time, and being free of any acute infection lasting less than one week before the inclusion.

Heraklion and Pécs were excluded from the 24- hour dietary recall (HDR) analyses because of lack of available information due to logistical reasons. Nevertheless, 2,084 adolescents had complete dietary data. Additionally, BP was assessed in all the study subjects. Therefore, a total of 1,605 adolescents from the HELENA study (12.5–17.5 years old; 833 girls) met all the inclusion criteria and were, therefore, included in the current analyses. We performed sensitivity analyzes in the sample by comparing the levels of BP among adolescents the 2,084 who had complete dietary data, and the 1,444 who did not have complete dietary data. We found no significant differences (p>0.05) in both levels of blood pressure in girls and boys (supplemental file).

Outcome

Blood Pressure (BP)

BP measurements were performed following the recommendations for adolescent populations [10] and were taken twice after weight and height measurements. The subjects were seated in a separate, quiet room for 10 min with their backs leant and feet on the ground. Two BP readings were taken at a 10-min interval of quiet rest. The average of two measurements was used. Participants who had taken any medication during the previous week or 24h were excluded from the analyses. The BP measurements were made concurrently with the dietary recall collections.

Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured by using the arm blood pressure oscillometric monitor device OMRON® M6 (HEM 70001) as approved by the British Hypertension Society [11]. Data collection procedures have been described in before [12].

Independent variables

A self-administered computer-based 24-HDR, HELENA-DIAT (Dietary Assessment Tool), was used for diet assessment. The software was based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) software which has been shown as an accurate tool to collect dietary data among adolescents [13, 14]. Adolescents registered all food and drinks consumed during the previous day according to six meal occasions during school time and assisted by fieldworkers. No data regarding Fridays and Saturdays was collected. Adolescents were asked to fill in the HELENA-DIAT twice on non-consecutive days and within a time span of two weeks.

Total protein and AA, including vegetal and animal protein intake were calculated using the German Food Code and Nutrition Date Base

(Bundeslebensmittelschlussel, version II.3.1, 2005). The usual food and nutrients intake were estimated by the multiple source method (MSM). The method considers the between- and within-person variability of the dietary data [15]. In detail, the AA included in the analyses were: alanine, arginine, asparaginic acid, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, and valine. AA and protein intake were both estimated in grams per day (g/d).

Contextual variables

City: Athens in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain were the cities involved.

Seasonality: A variable was computed by recoding the original variable "blood drawing date" into "seasonality", as follows: winter (from 21st December to 20th March, coded as 1), autumn (from 21st September to 20th December, coded as 2), spring (from 21st March to 20th June, coded as 3), and summer (from 21st June to 20th September, coded as 4), as performed in previous studies [16]. As the HELENA study was performed during the academic year, only a few adolescents (n=25) were assessed in the first days of summer They were included along with those assessed during spring. Therefore, the final variable was composed of three groups: winter (coded as 1), autumn (coded as 2), and spring (coded as 3) [16].

Potential individual confounders

Socioeconomic status (SES): it was based on familiar material conditions as defined previously [17, 18]. The SES scale is composed of four questions: Do you have

your own bedroom? (No=0, Yes=1); How many cars are there in your family? (None=0, 1=1, 2=2, >2=3); How many computers are there in your home? (None=0, 1=1, 2=2, ≥3=3); Do you have Internet access at home? (No=0, Yes=1.) We computed a final score by summing the answers from all the questions (range, 0–8). The SES was classified in three levels: low, medium and high. This questionnaire was validated in adolescents by Currie et al. [19].

Parental education: It was obtained by using a self-reported questionnaire, and was classified in four levels: lower education, lower secondary education, higher secondary education, university degree.

Pubertal development stage: Physical examination was performed by a physician aiming to classify the adolescents in one of the five stages of pubertal maturity defined by Tanner and Whitehouse [20]. The final variable was classified into three categories: pre-pubertal (stage 1 and 2); pubertal (stage 3 e 4) post-pubertal (stage 5).

Age (years): It was calculated from the adolescents' birthday and medical examination day.

Height: The height was measured to the nearest 0.1cm and the body weight to the nearest 0.1kg, with a stadiometer (wearing light clothes and no shoes)[21].

Waist circumference: It was measured at the midpoint between the lowest rib cage and the top of the iliac crest with a non-elastic tape to the nearest 0.1 cm. The intraobserver technical errors of measurement were between 0.53 and 1.75 cm for circumferences and interobserver reliability for circumferences were greater than 90% [21].

Physical activity (PA): An uniaxial accelerometer (Actigraph GT1M) was used to assess physical activity for 7 days, as described previously [22]. At least 3 days of recording with a minimum of 8 hours' registration per day was set as an inclusion

criterion. In this study, the interval of time (epoch) was set at 15 seconds. The cut-offs to define the intensity categories are similar to those used in previous studies [23]. Active subjects were classified when they accumulated at least 60 min/d of moderate and vigorous PA according to PA recommendations [24].

Tobacco smoking: defined as the regular consumption of at least one cigarette per day for a minimum of one month [25].

Total energy and sodium intake: Energy intake was estimated in calories per day (kcal/d) and sodium intake as micrograms per day (mg/d) following the same methodology used to calculated AA and protein intakes.

Statistical Analysis

The descriptive analyses were presented as means (quantitative variables) and percentages (qualitative variables) and confidence intervals 95% (95% CI). The means of BP levels were analyzed according to each independent variable.

We assessed the association between protein and AA intake (independent variables) and continuous BP levels (outcomes) by using bivariate linear regression. The magnitude of these associations was subsequently expressed in unadjusted and adjusted β-coefficients and their respective 95% CI. Multilevel linear regression models using mixed effects intercept were fitted to analyze the relationship between each BP levels and independent variables [26, 27]. The context variable used was the school. Moreover, homoscedasticity was graphically assessed in all regression models to meet the criteria of this analysis.

We analyzed the associations by means of three separate models: 1) total protein intake; 2) vegetal and animal protein intake; and 3) AA intake; but adopting the same theoretical model. Analyses were adjusted following a hierarchical model (figure 1) previously separated into six levels (the association of these levels not shown): 1) city,

seasonality; 2) parental education; age (years); 3) body mass index and waist circumference; pubertal development stage; 4) physical activity and tobacco smoking; 5) total energy and sodium intake; 6) AA intakes: *Aliphatic side chains* (alanine, glycine, isoleucine, leucine, valine); *Aromatic side chains* (phenylalanine, tryptophane, tyrosine); *Basic side chains* (arginine, histidine, lysine); *Acidic side chains* (asparaginic acid, glutamic acid); *Hydroxyl side chains* (serine, threonine); *Sulfur-containing side chains* (cysteine, methionine); *Cyclic side chain* (Proline); or *protein vegetal, protein animal and protein total*. In this model, variables were controlled for those in the same level but also for these in the higher one [28]. P-values $\leq$ 0.20 were adopted in the univariate analysis [28] (as necessary to include a variable in the multivariate analysis and, then, it was entered through the hierarchical model method following the levels above) or when there was more than 10% modification in β of any variable already in the model. Significance was controlled for multiple testing by Bonferroni method (Bonferroni method, $p<0.05$/number of tests; for protein for $p\leq 0.025$; amino acids intakes $p\leq 0.003$ for 2 sided) [29].

The statistical software package Stata version 12.0 (Stata Corp., college Station, TX, USA) was used for all statistical calculations.

RESULTS

Main subject characteristics are shown in the Table 1. Boys had higher physical activity levels, waist circumference, histidine and leucine intakes and SBP than their female peers, however, girls presented higher DBP and tyrosine intakes.

Table 2 presents the association between protein/AA intake and levels of BP in girls. The intakes of tryptophane ($p=0.002$) and histidine ($p=0.001$) were positively associated with SBP and methionine positively associated with DBP ($p<0.001$), whereas

an inverse association was observed for tyrosine with SBP ($p<0.01$) and DBP ($p=0.021$); and alanine was negatively associated with DBP ($p=0.001$).

Table 3 shows the associations between protein/AA intake and DBP in boys. Protein intake (animal, $p=0.022$ and vegetable $p=0.016$) was inversely associated with DBP and histidine was positively associated with SBP ($p<0.001$).

Table 1: Characteristics of the study population.

Variables	Girls mean or % (95% CI)	Boys mean or % (95% CI)
Seasonality		
Winter	21.2 (16.5 ; 25.9)	16.8 (12.0 ; 21.6)
Autumn	33.9 (28.5 ; 39.4)	34.8 (28.8 ; 40.9)
Spring	44.9 (39.1 ; 50.6)	48.3 (41.9 ; 54.7)
Age (years)	14.7 (14.6 ; 14.8)	14.8 (14.7 ; 14.9)
Education mother		
Lower education	9.6 (6.2 ; 13.0)	5.5 (2.6 ; 8.3)
Lower secondary education	25.7(20.7 ; 30.7)	31.5 (25.6 ; 37.4)
Higher secondary education	29.8 (24.5 ; 35.1)	26.5 (20.8 ; 32.1)
University degree	34.9 (29.4 ; 40.2)	36.6 (30.5 ; 42.7)
Socioeconomic status		
High	12.7 (8.8 ; 16.5)	12.6 (8.4 ; 16.8)
Medium	52.4 ()46.6 ; 58.1	53.8 (47.4 ; 60.1)
Low	34.9 (29.4 ; 40.2)	33.6 (30.5 ; 42.7)
MVPA		
< 60 min/d	**71.8 (68.2 ; 75.5)**	**41.6 (37.2 ; 45.9)**
≥ 60 min/d	**28.2 (24.5 ; 31.8)**	**58.4 (54.1 ; 62.8)**
Tanner Stage		
1 and 2 (pre-pubertal)	6.5 (5.2 ; 7.7)	6.6 (5.1 ; 7.6)
3 e 4 (pubertale)	66.4 (64.0 ; 68.8)	69.9 (67.5 ; 72.3)
5 (post-pubertal)	27.1 (24.9 ; 29.4)	23.7 (21.5 ; 26.0)
Waist circumference (cm)	**70.2 (69.7 ; 70.6)**	**74.2 (73.5 ; 74.8)**
Tobacco smoking		
Yes	13.7 (11.4 ; 16.0)	13.0 (106.1 ; 15.4)
No	86.3 (84.0 ; 88.6)	87.0 (84.6 ; 89.4)
Energy total intake (Kcal)	2,434 (2,392 ; 2,477)	2,471 (2,426 ; 2,516)
Sodium total intake (mg/d)	**2,027 (1,970 ; 2083)**	**2,558 (2,488 ; 2,628)**
Protein vegetal (g/d)	38.1 (37.3 ; 38.9)	38.4 (37.5 ; 39.3)
Protein animal (g/d)	56.2 (54.8 ; 57.5)	56.6 (55.0 ; 58.2)
Protein total (g/d)	94.5 (92.7 ; 96 ; 2)	95.3 (93.3 ; 97.4)

Arginine (g/d)	4.86 (4.76 ; 4.97)	4.93 (4.79 ; 5.05)
Asparaginic acid (g/d)	7.79 (7.62 ; 7.96)	7.89 (7.69 ; 8.09)
Cysteine (g/d)	1.19 (1.17 ; 1.22)	1.20 (1.17 ; 1.23)
Glutamic acid (g/d)	17.94 (17.57 ; 18.32)	18.11 (17.68 ; 18.54)
Glycine (g/d)	3.64 (3.56 ; 3.72)	3.69 (3.59 ; 3.78)
Histidine (g/d)	**2.23 (2.38 ; 2.48)**	**2.45 (2.39 ; 2.52)**
Iso ; leucine (g/d)	4.29 (4.19 ; 4.38)	4.33 (4.22 ; 4.44)
Leucine (g/d)	**6.71 (6.81 ; 7.12)**	**7.05 (6.87 ; 7.22)**
Lysine (g/d)	5.97 (5.83 ; 6.10)	6.03 (5.87 ; 7.22)
Methionine (g/d)	1.96 (1.91 ; 199)	1.97 (1.92 ; 2.02)
Phenylalanine (g/d)	3.96 (3.88 ; 4.05)	4.01 (3.92 ; 4.11)
Proline (g/d)	6.40 (6.26 ; 6.54)	6.47 (6.30 ; 6.61)
Serine (g/d)	4.30 (4.21 ; 4.39)	4.35 (4.25 ; 4.45)
Threonine (g/d)	3.17 (3.10 ; 3.23)	3.20 (3.12 ; 3.28)
Tryptophane (g/d)	3.54 (3.17 ; 3.62)	3.58 (3.49 ; 3.67)
Tyrosine (g/d)	**1.20 (0.99 ; 1.04)**	**1.03 (1.01 ; 1.06)**
Valine (g/d)	4.85 (4.75 ; 4.95)	4.91 (4.79 ; 5.03)
Diastolic Blood Pressure (mmHg)	**69.1 (68.4 ; 69.8)**	**68.0 (67.3 ; 68.8)**
Systolic Blood Pressure (mmHg)	**117.2 (116.3 ; 118.1)**	**126.7 (125.5 ; 127.8)**

CI95%: confidence interval of 95%; MVPA: Moderate to vigorous physical activity.
Significant associations between sexes ($p<0.05$) are in bold.

DISCUSSION

In this study we analyzed the association between protein and AA intake and BP levels in a large sample of European adolescents, and our main findings were: 1) protein intake (animal, vegetable and total) was negatively associated with DBP in boys; 2) in both sexes, histidine showed a positive association with SBP; 3) tyrosine intake was inversely associated with DBP among girls. From our knowledge, this is the first article analyzing these associations in adolescents; in addition, the observed results highlight the need to verify the role of protein/AA intake with BP, since available literature is scarce about this topic.

Table 2: Multiple linear regression analysis evaluating the association between amino acids and protein intake and blood pressure levels in girls. Beta coefficient (standardized) and their respective confidence intervals 95% (95% CI).

Aminoacids and proteins consumption (g/d)[a]	Diastolic Blood Pressure				Systolic Blood Pressure			
	Undajusted		Adjusted		Undajusted		Adjusted	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Protein vegetal	-0.94	(-6.43 ; 4.54)	-0.14	(-0.45 ; 0.17)	**9.03**	**(7.45 ; 17.33)**	-0.10	(-0.48 ; 0.27)
Protein animal	**-1.34**	**(-4.63 ; 1.95)**	-0.12	(-0.40 ; 0.15)	-1.36	(-6.02 ; 3.30)	-0.06	(-0.40 ; 0.27)
Protein total	-0.95	(-3.48 ; 1.58)	0.11	(-0.16 ; 0.39)	1.57	(-2.06 ; 5.20)	0.04	(-0.29 ; 0.39)
Aliphatic side chains								
Alanine	-0.01	(-0.06 ; 0.03)	**-1.41**	**(-2.78 ; -0.39)**	0.02	(-0.05 ; 0.08)	-0.70	(-2.67 ; 1.27)
Glycine	-0.01	(-0.07 ; 0.05)	0.07	(-0.67 ; 0.82)	0.02	(-0.06 ; 0.10)	-0.58	(-1.55 ; 0.41)
Isoleucine	-0.02	(-0.07 ; 0.03)	-0.39	(1.80 ; 1.02)	0.02	(-0.05 ; 0.09)	-0.67	(-2.53 ; 1.20)
Leucine	-0.01	(-0.04 ; 0.02)	0.72	(-0.50 ; 1.94)	0.01	(-0.03 ; 0.06)	0.15	(-1.54 ; 1.84)
Valine	-0.02	(-0.06 ; 0.03)	-0.40	(-1.84 ; 1.04)	0.02	(-0.04 ; 0.08)	-0.61	(-2.61 ; 1.39)
Aromatic side chains								
Phenilalanine	-0.02	(-0.07 ; 0.03)	0.27	(-1.44 ; 1.99)	0.03	(-0.05 ; 0.11)	-1.69	(-4.15 ; 0.78)
Tryptophane	-0.05	(-0.27 ; 0.16)	2.80	(-1.27 ; 6.86)	0.10	(-0.20 ; 0.40)	**4.41**	**(0.11 ; 9.96)**
Tyrosine	-0.03	(-0.09 ; 0.04)	**-2.68**	**(-4.46 ; -0.90)**	0.03	(-0.07 ; 0.12)	**-2.14**	**(-4.43 ; -0.01)**
Basic side chains								
Arginine	-0.01	(-0.05 ; 0.03)	0.45	(-0.08 ; 0.98)	0.02	(-0.04 ; 0.08)	0.51	(-0.18 ; 1.20)
Histidine	-0.02	(-0.10 ; 0.07)	0.59	(-0.39 ; 1.57)	0.03	(-0.09 ; 0.15)	**1.13**	**(0.18 ; 2.45)**
Lysine	-0.01	(-0.04 ; 0.03)	-0.05	(-0.73 ; 0.64)	0.08	(-0.04 ; 0.05)	0.44	(-1.40 ; 0.51)
Acidic side chains								
Asparaginic acid	-0.01	(-0.04 ; 0.02)	0.25	(-0.05 ; 0.56)	0.01	(-0.03 ; 0.05)	-0.20	(-0.61 ; 0.22)
Glutamic acid	-0.01	(-0.02 ; 0.01)	-0.02	(-0.20 ; 0.15)	0.09	(-0.09 ; 0.03)	-0.15	(-0.39 ; 0.09)
Hydroxyl side chains								
Serine	-0.02	(-0.07 ; 0.03)	0.48	(-0.51 ; 1.48)	0.03	(-0.04 ; 0.10)	1.11	(-0.17 ; 2.40)

Threonine	-0.02	(-0.08 ; 0.04)	-0.39	(-2.42 ; 1.63)	0.02	(-0.06 ; 0.11)	3.18	(-0.36 ; 5.99)
Sulfur-containing side chains								
Cysteine	-0.07	(-0.25 ; 0.10)	-1.29	(-3.61 ; 1.03)	0.08	(-0.17 ; 0.34)	0.60	(-2.60 ; 3.81)
Methionine	-0.02	(-0.13 ; 0.08)	**2.78**	**(0.98 ; 4.59)**	0.02	(-0.13 ; 0.17)	0.87	(-1.47 ; 3.21)
Cyclic side chain								
Proline	**-0.02**	**(-0.05 ; 0.01)**	-0.10	(-0.50 ; 0.29)	0.03	(-0.02 ; 0.07)	0.34	(-0.18 ; 0.86)
Akaike Information Criterion	6691.8476		6208.782		7068.4268		6514.8014	

*This analysis was adjusted for potential confounders: *City. Seasonality. Socioeconomic status. Parental education. Age (years). Age at menarche. Body mass index. Waist circumference. Physical activity. Tobacco smoking. Total energy and sodium intake.*
Significant associations are in bold.

Table 3: Multiple linear regression analysis evaluating the association between amino acids and protein intake and blood pressure levels in boys. Beta coefficient (standardized) and their respective confidence intervals 95% (95% CI).

Aminoacids and proteins consumption (g/d)[a]	**Diastolic Blood Pressure**				**Systolic Blood Pressure**			
	Undajusted		**Adjusted**		**Undajusted**		**Adjusted**	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Protein vegetal	**5.97**	**(0.15 ; 11.78)**	**-1.16**	**(-6.62 ; -0.20)**	9.04	(0.75 ; 17.33)	-0.38	(-1.06 ; 0.30)
Protein animal	-1.14	(-4.39 ; 2.11)	**-1.82**	**(-0.85 ; -0.08)**	-1.36	(-6.02 ; 3.30)	-0.51	(-1.09 ; 0.08)
Protein total	0.85	(-1.68 ; 3.38)	-0.55	(-3.68 ; 4.78)	1.57	(-2.06 ; 5.20)	0.51	(-0.07 ; 1.09)
Aliphatic side chains								
Alanine	0.01	(-0.04 ; 0.06)	0.72	(-1.46 ; 2.90)	0.02	(-0.05 ; 0.09)	1.89	(-0.37 ; 4.16)
Glycine	0.02	(-0.04 ; 0.07)	-0.35	(-1.85 ; 1.14)	0.02	(-0.06 ; 0.10)	-1.04	(-2.41 ; 0.35)
Isoleucine	0.02	(-0.03 ; 0.06)	-0.90	(-3.32 ; 1.53)	0.02	(-0.05 ; 0.09)	-0.07	(-2.45 ; 2.32)
Leucine	0.01	(-0.02 ; 0.04)	0.19	(-1.77 ; 2.15)	0.01	(-0.03 ; 0.06)	-0.35	(-2.37 ; 1.67)
Valine	0.02	(-0.03 ; 0.06)	-1.51	(-4.17 ; 1.14)	0.02	(-0.04 ; 0.08)	-1.56	(-3.79 ; 0.67)
Aromatic side chains								

Phenilalanine	0.02	(-0.03 ; 0.08)	2.09	(-0.73 ; 0.49)	0.03	(-0.05 ; 0.11)	0.02	(-2.33 ; 2.38)
Tryptophane	0.08	(-0.13 ; 0.29)	2.52	(-4.96 ; 10.00)	0.10	(-0.20 ; 0.40)	8.74	(-0.14 ; 16.06)
Tyrosine	0.03	(-0.04 ; 0.09)	-0.23	(-3.12 ; 2.65)	0.03	(-0.07 ; 0.12)	-0.51	(-3.35 ; 2.38)
Basic side chains								
Arginine	0.02	(-0.03 ; 0.06)	0.26	(-0.79 ; 1.30)	0.02	(-0.04 ; 0.08)	1.01	(-0.08 ; 1.94)
Histidine	0.02	(-0.06 ; 0.11)	0.27	(-1.44 ; 1.97)	0.03	(-0.09 ; 0.15)	**1.01**	**(0.08 ; 1.94)**
Lysine	0.01	(-0.03 ; 0.04)	-0.37	(-1.52 ; 0.77)	0.01	(-0.04 ; 0.05)	-0.47	(-1.55 ; 0.60)
Acidic side chains								
Asparaginic acid	0.01	(-0.02 ; 0.04)	0.18	(-0.35 ; 0.70)	0.01	(-0.03 ; 0.05)	-0.24	(-0.72 ; 0.24)
Glutamic acid	0.01	(-0.01 ; 0.04)	-0.05	(-0.31 ; 0.21)	0.01	(-0.01 ; 0.03)	-0.02	(-0.29 ; 0.25)
Hydroxyl side chains								
Serine	0.02	(-0.03 ; 0.07)	-1.26	(-2.79 ; 0.28)	0.03	(-0.04 ; 0.10)	0.48	(-0.99 ; 1.94)
Threonine	0.02	(-0.04 ; 0.08)	1.56	(-2.30 ; 5.41)	0.02	(-0.06 ; 0.11)	0.05	(-2.81 ; 2.91)
Sulfur-containing side chains								
Cysteine	0.05	(-0.13 ; 0.23)	-2.56	(-6.23 ; 1.12)	0.08	(-0.17 ; 0.34)	-3.51	(-7.13 ; 0.11)
Methionine	0.02	(-0.08 ; 0.13)	-0.785	(-3.39 ; 1.88)	0.02	(-0.13 ; 0.17)	-0.84	(-3.06 ; 1.36)
Cyclic side chain								
Proline	0.01	(-0.02 ; 0.05)	0.31	(-0.33 ; 0.95)	0.03	(-0.02 ; 0.07)	0.43	(-0.20 ; 1.05)
Akaike Information Criterion	5637.2316		1677.12462		6241.237		1949.21626	

*This analysis was adjusted for potential confounders: *City. Seasonality. Socioeconomic status. Parental education. Age (years). Body mass index. Waist circumference. Physical activity. Tobacco smoking. Total energy and sodium intake.*
Significant associations are in bold.

Existing differences in dietary patterns between boys and girls during adolescence [30] could explain the distinct associations observed between outcomes and exposures analyzed.

Protein intake (vegetal, animal and total) in boys was negatively associated with DBP. The potential role of diet protein content or the type of protein consumed on human BP is, however, unclear. Several observational studies [31, 32] and a recently published meta-analysis of randomized controlled trials [5] found that protein intake reduces BP levels in adults. This underlying mechanism could also occur in adolescents since proteins may provoke a slightly decrease in glucose and insulin; in fact, the hyperinsulinemic state has directly been associated with pathophysiology of hypertension. Another possible explanation is that protein has been associated with ion channels, which may indirectly influence the pathways of BP control [33]. Furthermore, results from the INTERMAP study showed an inverse association between vegetal protein intake and BP in elderly people [34]. We also found such relationship but only among boys. These findings are of great interest since vegetable-rich diets are recommended as part of a healthy lifestyle to prevent chronic diseases as hypertension.

Altorf-van der Kuil *et al.* [6] observed among adults that tyrosine intake was negatively associated with SBP, i.e. each gram of tyrosine decreased SBP in 2.4 mmHg. According to this, we also observed a negative association between tyrosine intake and DBP in girls. A plausible biological explanation is that tyrosine seems to increase expression of the dopamine D1 receptor, which may turn out in a decrease in cortisol blood concentrations and in a reduction of the sympathetic tone [35]. All these actions may contribute to reduce BP levels. These results were observed in animal models, for that reason, there is a need to verify if these mechanisms also occur in humans.

The intake of tryptophane seems to increase SBP levels in girls. This may be explained by the fact that tryptophane increases serotonin turnover in the brain, and, consequently, in turn increases the enzymatic activity 5-HIAA/5-HT+5-HIAA, which raises sympathetic activity and, consequently, SBP levels [36]. In agreement to other studies, [7], we found a positive association between methionine intake and DBP also in girls. This might be explained by the fact that methionine is an important precursor of homocysteine, which is associated with an increase in BP by inhibition of nitric oxide formation [37].

Some studies found that histidine can help to control BP since it acts as a precursor of nitric oxide in brain vasomotor centers [38]. However, our findings consistently showed an inverse association between histidine and SBP in both genders. Our results were different from those observed by Stamler et al.[39] who failed to find a significant association between histidine intake and BP levels in adults; however, they observed a positive association with glycine. This lack of agreement with other studies may be explained by differences in study methods and populations among others.

The multicenter design of our study and the diverse geographic origin of the sample, as well as the multilevel analysis conducted with a complete set of potential confounders are some of the main strengths of the present study. Nevertheless, the cross-sectional design of the study represents a limitation, as temporality cannot be established. In addition, diet was assessed by a self-reported method which is subject to certain degree of measurement error. Dietary data was obtained by two non-consecutive 24-hour dietary recalls. Although more recall days would have been desirable, dietary information was corrected for within- and between-person to mitigate in part this limitation [40]. In addition, the applied 24-hour dietary recall has previously been shown as an appropriate method to accurately assess diet in European adolescents [13, 14]. Dietary

recalls were not collected on Fridays and Saturdays due to logistical reasons. Previous findings suggest that energy intake tends to increase on weekends, and on Fridays [41, 42]; therefore, it would have been desirable to collect that information to take into consideration the apparent modification in dietary habits during weekends. Another limitation is that protein and AA serum concentrations were not assessed. Additionally, cultural differences in the preparation of foods as well as specific local conditions i.e. varying soils and water content might have influenced the analyses, however, these possible differences were mitigated by conducting multilevel analyses.

CONCLUSIONS

Protein intake might help male adolescents to keep DBP within normal values independently of other risk factors i.e. abdominal obesity/daily total calories intake/PA and sodium intake. Moreover, the association between AA and BP levels is controversial and depends on the AA intake *per se*. These findings are of great interest and should be taken into account when designing strategies aimed to prevent the onset of CVD and its related risk factors such as hypertension at this early and crucial period of life. More studies following a longitudinal design are needed to confirm these findings in adolescents.

COMPETING INTEREST

The remaining authors state no conflict of interest.

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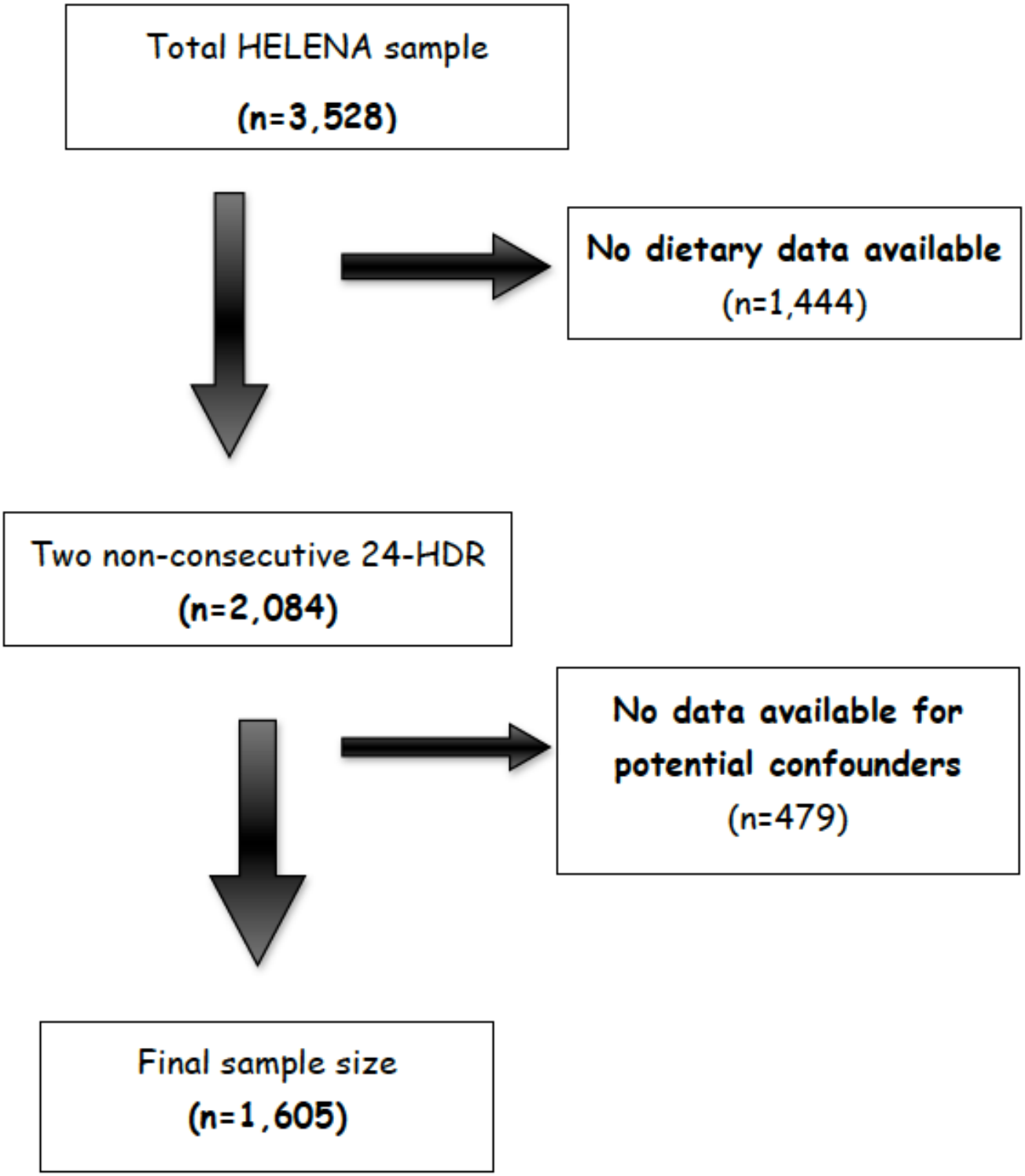
Supplemental file 1:

Final sample size flowchart. 24-HDR= 24-hour dietary recall.



3.8 PHYSICAL ACTIVITY MODIFIES THE ASSOCIATIONS BETWEEN GENETIC VARIANTS AND BLOOD PRESSURE IN EUROPEAN ADOLESCENTS – PLUBLISHED IN THE JOURNAL OF PEDIATRICS

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This manuscript has not been and will not be submitted to any other journal while it is under consideration by The Journal of Pediatrics.

The first draft of the manuscript was wrote by *Augusto César Ferreira de Moraes and Juan Miguel Fernández-Alvira*.

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: **AMe, JD, AK, AMa, DM, YM, KW, KW, MGG and LM;** Performed the experiments: **AMe, JD, AK, AMa, DM, JRR, YM, KW, KW, CB, MGG and LM;** Analyzed the data: **ACFM, JMFA, HBC** and **LAM;** Contributed reagents/materials/analysis tools: **ACFM, JMFA, HBC, AMe, JD, JRR, IL** and **LAM;** Wrote the manuscript: **ACFM, JMFA, HBC** and **LAM;** Others - critical and important scientific contribution: **AMa, JD, AK, AMe, DM, YM, JR, IL, KW, KW, CB, MGG. ACFM** and **JMFA** has primary responsibility for the final content. All authors read and approved the final manuscript.

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ABSTRACT

We hypothesized that physical activity (PA) and sedentary behavior levels could modify the associations between known genetic variants blood pressure (BP)-associated genes in European adolescents. Meeting current PA recommendations (≥60 min/d of PA) was able attenuate the deleterious effect of the *NOS3* rs3918227 polymorphism on systolic BP in European adolescents.

Keywords: lifestyle behaviors; polymorphism; cardiovascular risk; adolescents.

INTRODUCTION

Hypertension is a major public health problem in the developed world and a major risk factor for cardiovascular disease (CVD)[1]. Recent studies have suggested an association between BP levels and certain genetic loci, with special potential gene regulatory mechanisms at the *MTHFR* and *NOS3* loci [2, 3]. Additionally, BP is known to be affected by several environmental factors, including among physical activity (PA) and sedentary behaviors (SB) as predictors of higher BP [4].

As genetic influence on BP has been observed already during adolescence [5], determining to what extent BP-related genes are modulated by environmental factors such PA and SB during adolescence could help to further understand and prevent high BP at early stages.

Thus, we hypothesized that PA and SB levels could modify the associations between genetic variants known BP-associated variants in European adolescents (**Figures 1; online only**).

METHODS

Sample size

A total of 3,528 adolescents (1,845 girls) from nine European countries, participated of the HELENA study. A detailed description of the HELENA methodology has been published elsewhere [6, 7].

A total of 1009 adolescents (12.5–17.5 years old; 532 girls); with valid data on BP levels, physical activity measured by accelerometry, sedentary behavior assessed by questionnaire and a complete set of confounders were included in the analyses of this study.

Outcome

Blood Pressure

Systolic and diastolic BP were measured by an arm blood pressure oscillometric monitor device (OMRON® M6 (HEM 70001)) and following the recommendations for adolescent populations [8], complete data collection procedures have been described in earlier [9].

Independent variables

Genotyping

Fasting blood samples were collected by venipuncture at school after a 10-h overnight fast, this specific methods has been previously described [10].. For the purposes of the present study, six SNPs in three genes previously associated with hypertension[3, 11] were selected (rs1205 and rs1130864 in *CRP*, rs1801131 and rs1537516 in *MTHFR* and rs1800779 and rsc3918227 in *NOS3*. Genotyping success rates were greater than 99.7%. The genotype distributions for all polymorphisms respected the Hardy-Weinberg equilibrium ($p>0.25$).

Lifestyle variables

Physical activity (PA) was measured with accelerometers (Actigraph GT1M,) during seven consecutive days, with a minimum of 8 recorded hours/day for at least 3

days [12]. Following current PA recommendations [13], subjects were categorized as "meeting the current PA recommendations" (≥ 60 min/d of moderate to vigorous PA) or "not meeting the PA recommendations" (< 60min/d of moderate to vigorous PA).

Sedentary behavior (SB) was assessed by questionnaire about the usual time spent in front of the television, computer and/or playing video games. Total sedentary time was calculated by summing hours in week and week-end days. Then, a categorical variable was derived with the following categories: 0-2 h/d; >2 – 4 h/d; ≥4 h/d according to Dunstan et al. [14].

Statistical analyses

Descriptive analyses are presented as means and percentages, 95% confidence intervals (95% CI). The impact of the six polymorphisms (using the additive model) on diastolic and systolic BP levels were estimated by multilevel linear regression models using mixed effects intercept adjusted for potential confounders. Center was used as context variable.

To test for the existence of an interaction between each SNP and lifestyle variables the same models were applied by adding the SNPs x lifestyle cross-product (e.g. rs1205 x PA) in the model.

Significance was set at *p*-values <0.05, after controlling for multiple testing using the Bonferroni correction ($p<0.05$/number of tests; i.e. for physical activity for $p\leq 0.025$; for sedentary behavior $p\leq 0.017$ for 2 sided).

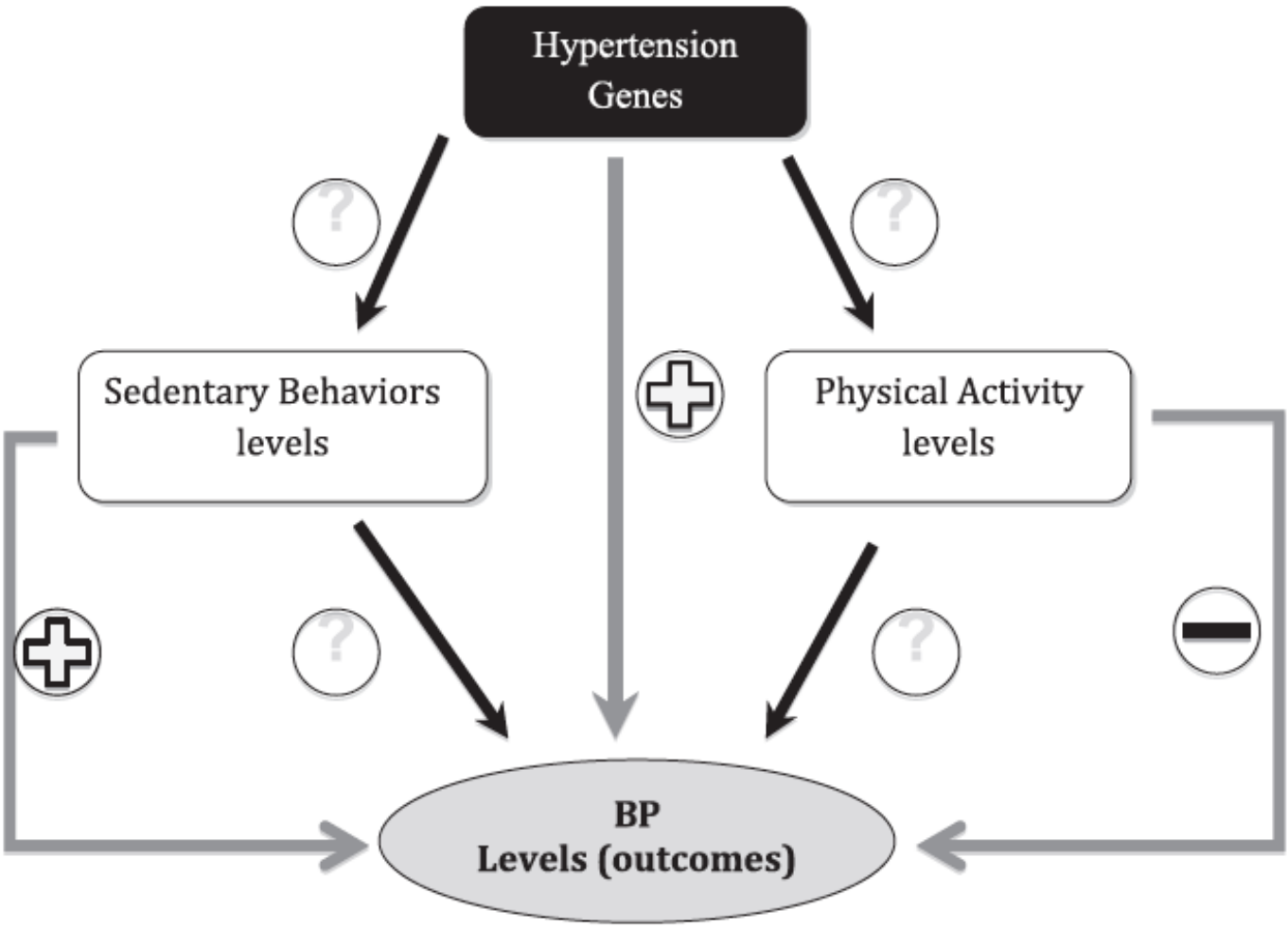

Figure 1. Conceptual framework of the associations between genes, lifestyle behaviors, and BP levels. Gray arrows represent direct association described in the literature; black arrows represent attenuate associations analyzed in this article.

RESULTS

Boys presented significant higher prevalence of high MVPA (≥ 60 min/d) and high SB (> 4 h/d) than girls. Boys had also higher WC and Systolic BP than girls, whereas girls had higher diastolic BP levels than boys (**Table 1)**.

The *CRP* rs1130864 and *NOS3* rs3918227 SNPs were associated with both BP levels. Indeed, adolescents carrying the minor allele of rs1130864 had higher diastolic BP (p=0.003) and systolic BP (p=0.041) levels than carriers of the major allele. Similarly, adolescents carrying the minor allele of rs3918227 had higher systolic BP (p=0.026) and diastolic BP (p=0.026) levels than carriers of the major allele. Then,

adolescents bearing the minor allele of the *MTHFR* rs1537516 SNP had higher values of diastolic BP than the other adolescents (p=0.017; **Figures 2; online only**)

Table 1: Characteristics of the subjects in the HELENA study.

Variables	Girls mean or % (95%CI)	Boys mean or % (95% CI)
Age (years)	14.7 (14.6 – 14.8)	14.8 (14.7 – 14.9)
Education mother		
Lower education	9.7 (7.1 – 12.2)	6.2 (4.0 – 8.4)
Lower secondary education	24.7 (21.0 – 28.4)	28.8 (24.7 – 32.9)
Higher secondary education	31.5 (27.5 – 35.5)	29.6 (25.5 – 33.8)
University degree	34.2 (30.1 – 38.2)	35.4 (31.1 – 39.7)
Tanner Stage		
1 and 2 (pre-pubertal)	7.3 (5.0 – 9.6)	7.1 (4.7 – 9.5)
3 and 4 (pubertale)	65.6 (61.6 – 70.1)	64.4 (59.9 – 68.9)
5 (post-pubertal)	26.8 (59.9 – 68.9)	28.5 (24.3 – 32.8)
MVPA		
< 60 min/d	**73.5 (69.0 – 77.9)**	**40.4 (35.2 – 45.7)**
≥ 60 min/d	**26.5 (22.1 – 30.9)**	**59.6 (54.3 – 64.8)**
Sedentary behavior by questionnaire		
< 2 h/d	**44.5 (40.2 – 48.8)**	**21.9 (18.1 – 25.7)**
2 - 4 h/d	**36.9 (32.7 – 41.1)**	**37.3 (32.8 – 41.7)**
> 4 h/d	**18.6 (15.2 – 21.9)**	**40.8 (36.3 – 45.3)**
Waist circumference (cm)	**70.6 (69.9 – 71.3)**	**73.7 (72.5 – 74.5)**
Systolic Blood Pressure (mmHg)	**118.1 (117.0 – 119.2)**	**126.2 (12.48 – 127.6)**
Diastolic Blood Pressure (mmHg)	**69.8 (68.8 – 70.7)**	**68.1 (67.1 – 69.1)**
MTHFR gene (% polymorphisms minor allele**)**		
rs1801131	11.3 (8.6 – 14.0)	8.8 (6.2 – 11.3)
rs1537516	0.8 (0.2 – 1.0)	0.4 (0.2 – 1.0)
CRP gene		
rs1205	9.6 (7.1 – 12.1)	7.5 (5.2 – 9.9)
rs1130864	9.2 (6.7 – 11.7)	9.8 (7.2 – 12.5)
NOS3 gene		
rs1800779	15.2 (12.2 – 18.3)	18.7 (15.2 – 22.2)
rs3918227	0.4 (0.1 – 0.9)	0.4 (0.2 – 1.0)

CI95%: confidence interval of 95%;; MVPA: Moderate to vigorous physical activity.
Significant difference (p< 0.05) between sexes are in bold.

We did not detect any significant interaction between the levels of SB, the polymorphisms and BP levels (data not shown). In contrast, a significant interaction was only observed for the *NOS3* rs3918227 polymorphism (*p*=0.034) and systolic BP

levels. The minor allele of rs3918227 was associated with higher systolic BP levels if the adolescents did not meet the PA recommendations (+ 5.6 mmHg per allele, p=0.014) than if they met the PA recommendations (+ 3.8 mm Hg per allele, p=0.048; **Figure 3A and 3B**).

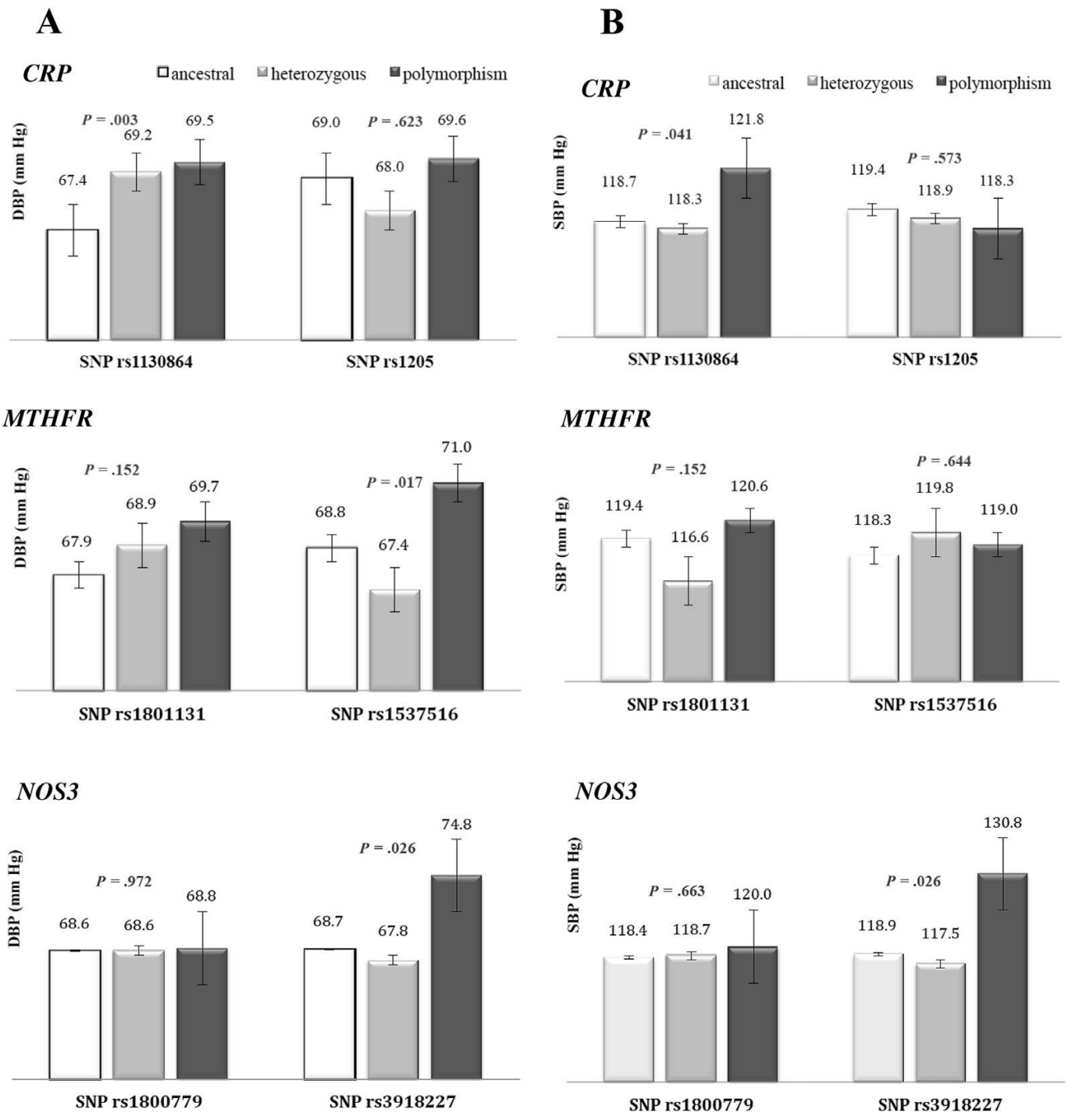

Figure 2. Means and 95% CIs of BP levels, A, DBP and B, SBP used to evaluate the direct association between BP levels and each SNPs polymorphisms in European adolescents.

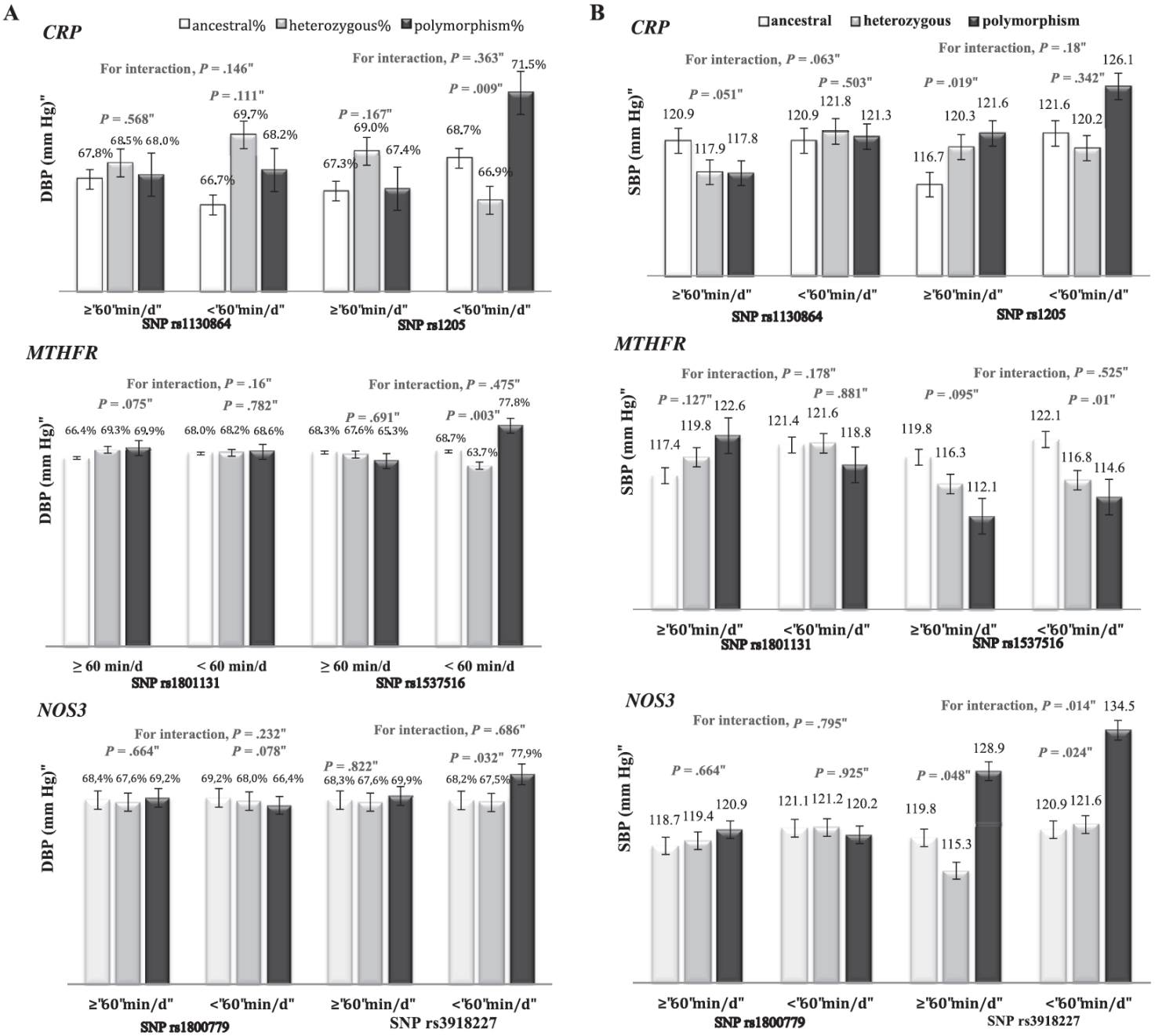

Figure 3. Means (adjusted for potential confounders: city, sex, maternal education, waist circumference, and tobacco smoking) and 95% CIs (error bars) of BP levels each categories of the physical activity ($60 min/d and <60 min/d), A, DBP and B, SBP used to evaluate the interaction effect and association between BP levels and each SNPs polymorphisms in European adolescents (n = 1009).

DISCUSSION

Our results suggest that performing ≥ 60 min/d of PA modifies the effect of the *NOS3* rs3918227 polymorphism on systolic BP levels in adolescents.

Genome-wide association studies identified several genes (notably *CRP*, *MTHFR* and *NOS3*) associated with higher BP and development of arterial hypertension

in adults [2, 11]. These results are important as it seems that these genes have already some effects at an early age of life (adolescence).

We detected an interaction between physical activity, the *NOS3* rs3918227 polymorphism and systolic BP levels. Similar results were observed in another European study [5]. Therefore, our study reinforces, with a better accurate method of physical activity assessment, the finding that PA may strengthen the production and effect of nitric oxide in the regulation of BP through endothelial vasodilatation.

The results are consistent in different adolescent populations[5], which strengthens the conclusions and adds consistency to our report (Hill's principles). The results show that increasing PA levels could be an effective way of controlling BP in individuals with a genetic predisposition towards hypertension.

We concluded: *i*) the *CRP*, *MTHFR* and *NOS3* genes are positively associated with BP levels; *ii*) there was no significant interaction with SB; *iii*) meeting current PA recommendations (≥60 min/d of PA) can attenuate the deleterious effect of the *NOS3* rs3918227 polymorphism on systolic BP in European adolescents.

COMPETING INTEREST

The remaining authors state no conflict of interest.

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3.10 INCIDENCE OF HIGH BLOOD PRESSURE IN CHILDREN – EFFECTS OF PHYSICAL ACTIVITY AND SEDENTARY SEHAVIOR: THE IDEFICS STUDY STUDY – SUBMITTED IN BMJ

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ABSTRACT

Objective: High blood pressure (HBP) is one of the most important risk factors for cardiovascular diseases and it has a high prevalence in pediatric populations. However, the determinants of the incidence of Pre-HBP and HBP in children, such as physical activity (PA) and sedentary behaviors (SB) is not well known .

Study Design: A total of 16,224 children were recruited by complex sampling population-based survey in eight European countries. At baseline (T0), 5,221 children were selected for accelerometer measurements; 5,061 children were re-examined 2 years later (T1). All measures were collected by standardized protocols at T0 and T1. We estimated the incidence of Pre-HBP and HBP and evaluate the effect of PA and SB in the development of both outcomes, by computing relative risks and the corresponding 95% confidence intervals (RR, 95% CI), adjusted for potential confounders.

Results: Incidences of Pre-HBP and HBP per year were: 121 new cases/1000 children and 110 new cases/1000 children, respectively. We found that children maintaining SB > 2 h/d during the two year follow-up showed a RR of having HBP of 1.28 (1.03 - 1.60). Children in T1 not performing the recommended amount of PA (<60 min/d) have a RR of HBP of 1.53 (1.12 to 2.09). We found no association between pre-HBP and the behaviors.

Conclusion: The incidence of pre-HBP and HBP is high in European children. Maintaining sedentary behaviors during childhood increases the risk of developing HBP after two years of follow-up.

Keywords: hypertension; lifestyle behaviours; cohort study; multicenter study; children.

INTRODUCTION

Chronic non-communicable diseases are the main source of disease burden worldwide and are thus a major public health problem [1]. Among non-communicable diseases, hypertension has been shown to have the highest prevalence in adults [2], and studies have shown that blood pressure (BP) levels in childhood and adolescence greatly impact the development of hypertension in adulthood [3].

Among the factors that may influence blood pressure levels (e.g. genetics, intrauterine development, socioeconomic status, tobacco use, total and abdominal obesity), physical activity (PA) and sedentary behaviors (SB) have been shown inverse [4] and direct associations[5,6], respectively, with blood pressure in children.

Although the effects of PA and SB on BP have mainly been examined in isolation, there are studies suggesting that these behaviors have an aggregate effect in children[7]; however, few studies have quantified the association between combined PA/SB levels and cardiovascular risk in children, like blood pressure. On the other hand, PA/SB levels are associated with sociodemographic and economic variables. The influence of sociodemographic factors on PA/SB has been described in a review [8]. There is no consensus in the literature regarding socioeconomic variables as determinants of these behaviours since such differences may be attributed to the demographic context and characteristics of the populations studied rather than the individual [9,10].

Reproducing the same results in different population groups with different characteristics would increase their biological plausibility and provide a higher level of scientific evidence. For this reason, we have included results from a multi-national European study in this report. We tested our hypothesis, in cohort studies conducted

with children within the IDEFICS study (***I****dentification and prevention of* ***D****ietary- and lifestyle-induced health* ***EF****fects* ***I****n* ***C****hildren and infant****S***).

Thus, we hypothesized that low levels of PA and high levels of SB may contribute to the development of high blood pressure (HBP).

METHODS

Study Population

The IDEFICS study (***I****dentification and prevention of* ***D****ietary- and lifestyle-induced health* ***EF****fects* ***I****n* ***C****hildren and infant****S***) is an epidemiological multicenter European study, aiming to identify nutritional and lifestyle-associated aetiological factors of childhood obesity and related morbidities. A cohort of 16,224 children aged 2–9 years (51% of eligible sample), recruited from eight European countries (Germany, Hungary, Italy, Cyprus, Spain, Estonia, Sweden and Belgium), was examined in the baseline survey (T0) and it was the starting point of the prospective study with the largest European children's cohort established to date. They were assessed between September 2007 and May 2008 according to a standardized protocol. Details of the procedures of the IDEFICS project have been previously published[11,12]. These children were followed up longitudinally to assess their development and to determine the aetiological associations between baseline predictors and selected follow-up end points by a follow-up survey 2 years later at T1 (September 2009 to May 2010).

As accelerometry was measured only in a random subset of children from every center (due to availability of accelerometers), when the objective measurement of physical activity (PA) was included in the analyses, the sample size was reduced. The present analysis was performed in 5,221 children (32.2% of the sample; Boys=51%; age=6.1±1.8 years; mean±s.d.), with a complete set of data including: systolic blood

pressure (SBP), diastolic blood pressure (DBP), height, exposures [PA intensities, sedentary behavior (SB)] and confounding variables (**Figure 1)**. Parents or legal guardians, provided written informed consent to participate in the full programme or in a selected set of examination modules. For each survey center, the approval of the local Ethical Committee was obtained.

Outcomes

Data collection procedures were described previously[13]. An arm BP oscilometric monitor device WelchAllyn 42008™, previously validated in this age group was used.[14] It was previously tested for reliability and reproducibility in the IDEFICS project[13]. Two BP readings were taken after 10-min rest, with a 5-min interval between them, and the lowest reading was recorded. The inter-observer coefficients' of variation were below 5% for both BP levels.

The outcomes for this study are: Pre-HBP and HBP[15]. Pre-HBP was defined as SBP or DBP between 90th to 95th percentil for age and height; and HBP defined as SBP or DBP above the 95th percentile for age and height too.

Principal Exposures

PA and SB levels were considered independent variables.

Physical Activity: was measured using a uniaxial accelerometer (Actigraph model GT1M). Recordings were for at least 6h/d for at leat 3 days (2 weekdays and 1 day of weekend/holiday). The sampling interval (epoch) was set at 15 seconds. A measure of average total volume activity (hereafter called total PA) was expressed as the sum of recorded counts divided by total daily registered time expressed in minutes (counts/min; cpm). The cut-offs to define the PA intensity categories were derived from previously-validated cut-offs[16], with time spent in light PA (minutes) defined as the sum of time-per-day in which counts per epoch were 26 to 573 cpm.

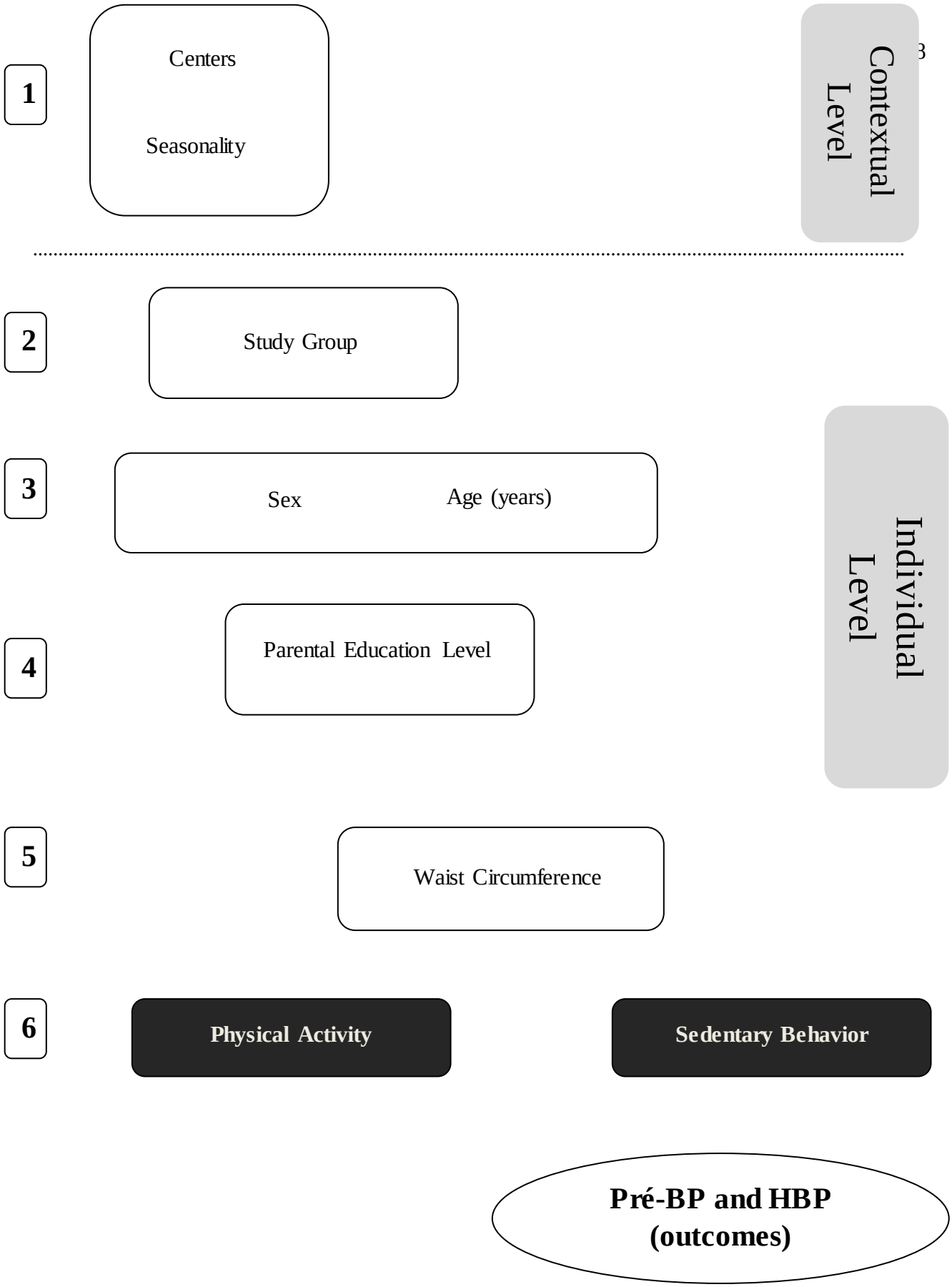

Figure 1: Theoretical conceptual model of the association between contextual and individual variables on children's' blood pressure categories. The effect of each variable on the outcome was adjusted for other variables in the same model or above in the hierarchical model. Variables with $p > 0.2$ were not included in the subsequent adjustment models. HBP= High Blood Pressure.
The principals independents variables are described in level 6.

The time engaged in moderate PA was calculated based upon a cut-off of 574 to 1,002 cpm per epoch. The time engaged in vigorous PA was calculated based upon a cut-off of ≥1,003 cpm per epoch. In addition, the time spent at the 'effective' intensity level was calculated as the sum of time spent in moderate + vigorous PA (MVPA).

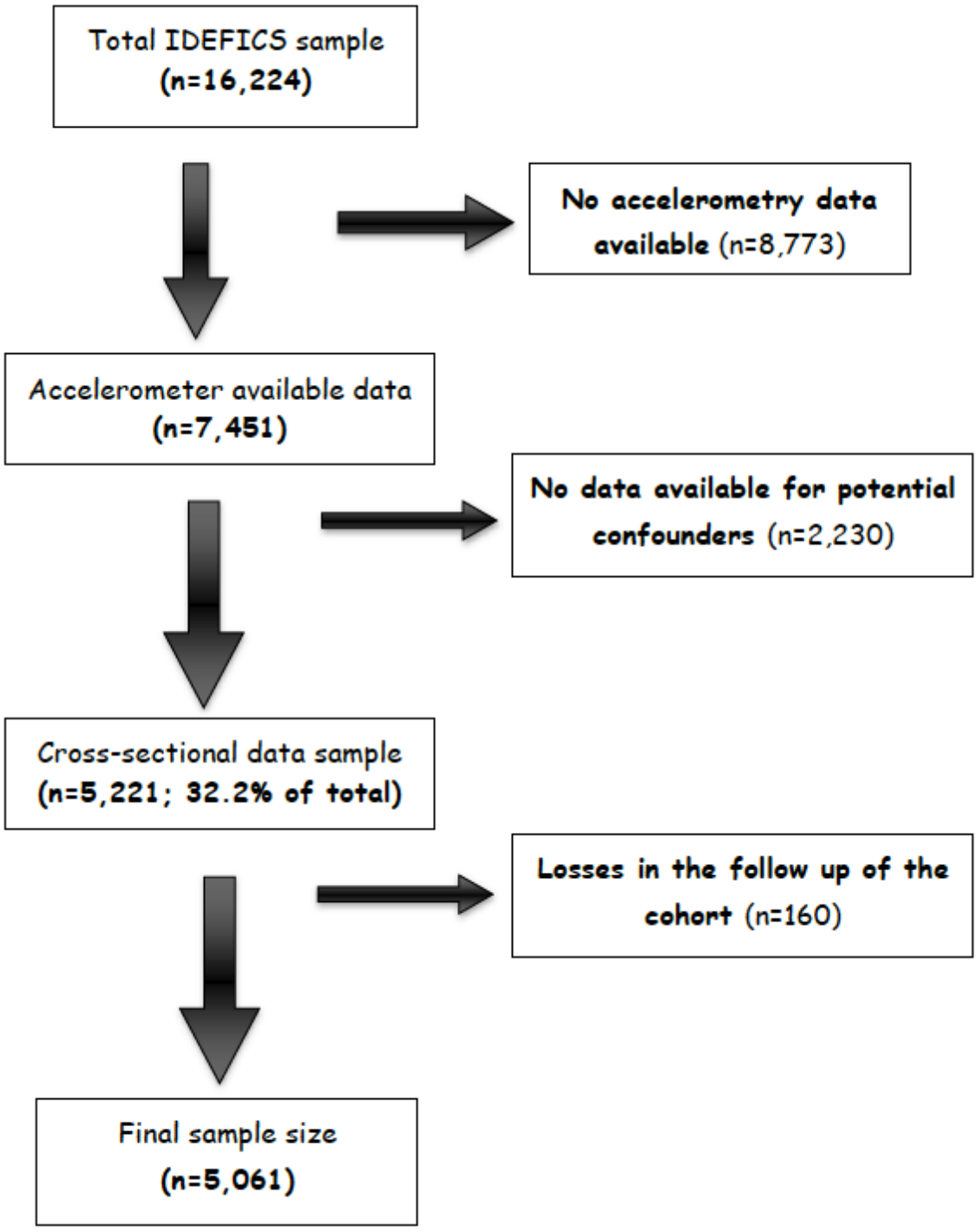

Figure 2: Final sample size flowchart

Following current PA guidelines[17], subjects were classified in T0 and T1 as: meeting current PA recommendations when they accumulated at least 60 min/d of MVPA and not meeting current PA recommendations when MVPA was <60 min/d. We also established the variable change in PA based on the distribution in PA categories in T0 and T1; subjects were classified into the following categories: always ≥ 60 min/d (meeting current PA recommendations in both T0 and T1); ≥60 min/d → <60 min/d (meeting current PA recommendations in T0 to not meeting current PA recommendations in T1); <60 min/d → ≥60 min/d (not meeting current PA recommendations in T0 to meeting current PA recommendations T1); and always <60 min/d (not meeting current PA recommendations in T0 and T1).

Sedentary Behaviors: The parental questionnaire was used to obtain information on children's sedentary behaviors'. Parents reported hours of TV/DVD/video viewing and computer/games-console use both for a typical weekday and weekend day. For the purpose of the current analysis, children's daily TV/DVD/ video and computer/games-console use were summed to obtain the total screen time per day (the whole week). The used questionnaire, had previously been tested for its reliability and validity in this population[18].

Thereafter, participants were classified into two groups according to the American Academy of Pediatrics (AAP's) guidelines on total screen time: ≤2 h/d and >2h/d [19]. We also established the variable change in SB based on the distribution in SB categories in T0 and T1; subjects were classified into the following categories: always < 2h/d (meeting current SB recommendations in both T0 and T1); < 2h/d → ≤ 2h/d (meeting current SB recommendations in T0 to not meeting current SB recommendations in T1); ≤ 2h/d → < 2h/d (not meeting current SB recommendations in

T0 to meeting current SB recommendations T1); and always ≤ 2h/d (not meeting current SB recommendations in T0 and T1).

Potential confounders

The potential confounders for this study were divided in two groups: Contextual Factors and Individual Factors, and described bellow:

Contextual Factors

Centres in 8 European countries: Belgium, Cyprus, Estonia, Greece, Germany, Hungary, Italy, Spain and Sweden.

- Seasonality: A variable was computed by recoding the original variable "blood drawing date" into "seasonality", as follows: winter (from 21st December to 20th March, coded as 1), autumn (from 21st September to 20th December, coded as 2), spring (from 21st March to 20th June, coded as 3), and summer (from 21st June to 20th September, coded as 4), as performed in previous studies. As the IDEFICS study was performed during the academic year, only a few children (n=2%) were assessed in the first days of summer They were included along with those assessed during spring. Therefore, the final variable was composed of three groups: winter (coded as 1), autumn (coded as 2), and spring (coded as 3).

Individual Factors

- Study Group: The children were divided in two groups: Intervention and Control. The intervention group received a community-based intervention program with multicomponent education topics to promote healthy lifestyle. Children received nutrition education at school and community activities were performed to prevent obesity/overweight and metabolic syndrome components. The control group did not received any intervention.

- Age group: 2 - 5 years; 6 - 9 years and 10 - 12 years (only T1 analyses);
- Parental education of the family provider: determined with a self-reported questionnaire and categorized according to the *International Standard Classification of Education* (ISCED)[20] in five levels: ISCED 1= illiterate; ISCED 2= up to 4th year of primary school; ISCED 3= completed primary school; ISCED 4= completed high school; and ISCED 5= completed higher education.
- Waist circumference: It was measured at the midpoint between the lowest rib cage and the top of the iliac crest with a non-elastic tape to the nearest 0.1 cm. The intraobserver technical errors of measurement were between 0.53 and 1.75 cm and interobserver reliability were greater than 94.9% [21], for this circumference.

Statistical analysis

The descriptive analyses were performed by mean (continuous variables) and percentage (categorical variables) and respective 95% confidence intervals (95% CI).

We calculated the cumulative incidence and 95%CI of both outcomes: Pre-HBP and HBP for total and principal exposures. The magnitude of these associations was subsequently expressed in, unadjusted and adjusted, relative risk (RR) and 95%CI. Multinomial multilevel regression models using mixed effects intercept were applied to estimate the effect of PA and SB on Pre-HBP and HBP incidence [22,23]. The context variable used was the center.

For the adjusted analysis we developed a conceptual framework (**Figure 2**) previously separated into five levels (the association of these levels is not shown): 1) center, seasonality; 2) sex; age (years); 3) parental education; 4) waist circumference; 5) PA and SB. In this model, variables were controlled for those in the same level but also

for those in the higher one [24]. P-values $\leq$ 0.20 were adopted in the univariate analysis [24] (as necessary to include a variable in the multivariate analysis and, then, it was entered through the hierarchical model method following the levels above) or when there was more than 10% modification in RR of any variable already in the model.

Multilevel analyses were performed with two objectives: 1st) to test the associations between BP categories and two separate measures of individual behaviors; 2nd) to test the extent to which country-specific characteristics and contextual variables mediate the associations between BP categories and PA and SB.

Significance was set at *p*-values <0.05 and the Stata 12 (Stata Corp., College Station, TX, USA) was used for all statistical calculations. All analyses were adjusted for the clustered nature of the sample using the "svy" set of commands.

RESULTS

We performed sensitivity analyzes in the sample by comparing the prevalence's of the outcomes among children who had complete data (T0 and T1), and those who did not have complete data (only T0). We found no significant differences (p>0.05) in prevalence's in this analysis. **Figure 1** presents a description of the study sample, which represents 32.2% of the total IDEFICS Study sample. A strong point of our study is that we only had 3.1% loss in the two years of follow-up (n=5,061).

Table 1 shows the characteristics of the sample at both times of data collection. The only variable found showing significant differences between boys and girls was the increased prevalence of high sedentary behaviors (> 2h/d) both at T0 and T1, being higher in males than in females.

Cumulative incidences of both outcomes (Pre-HBP and HBP) according to the main exhibits examined are presented in **Table 2**. Significant effects were only found in

the incidence of HBP for PA and SB at follow-up in cohort and in those maintaining high SB (> 2h/d) at follow-up. Children who perform <60 min/d of PA at the time of the second data collection or maintained higher SB (> 2h/d) during the period of follow-up are at risk for HBP (**Table 3)**.

Table 1: Descriptive analysis of characteristics of the sample on cross–sectional and cohort, and their respective confidence intervals 95% (95% CI), according to independent variables.

	Cross–sectional (n= 5,221)				**Cohort (n= 5,061)**			
Independents Variables	**Males (n= 2,638)**		**Female (n= 2,583)**		**Males (n= 2,485)**		**Female (n= 2,576)**	
	%	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Age (years)								
2 – 5 years	42,8	(40,9–44,6)	39,6	(37,8–41,6)	16,5	(9,1–11,5)	15,5	(14,1–16,9)
6 – 9 years	57,2	(55,4–59,1)	60,4	(58,4–62,2)	69,3	(66,2–69,8)	70,3	(68,5–72,2)
10 – 12 years	---	--------	---	--------	14,2	(13,4–16,2)	14,3	(12,9–15,6)
Countries								
Belgium	6,3	(5,4–7,2)	7,5	(6,5–8,5)	14,2	(12,7–15,4)	13,8	(12,4–15,1)
Cyprus	7,6	(6,6–8,6)	7	(6,0–8,0)	5,1	(4,3–6,0)	6,7	(5,7–7,7)
Estonia	16,1	(14,7–17,5)	16,9	(15,5–18,4)	20,1	(18,5–21,6)	21,5	(19,9–23,1)
Germany	11,5	(10,3–12,7)	12,9	(11,6–14,2)	14,5	(13,2–15,9)	13,8	(12,4–15,1)
Hungary	16,3	(14,9–17,7)	16,4	(14,9–17,8)	5,7	(4,8–6,6)	5,5	(4,6–6,4)
Italy	12,2	(10,9–13,4)	11,4	(10,2–12,6)	12,6	((11,3–13,9)	10,4	(9,2–11,6)
Spain	20,1	(18,6–21,6)	19,2	(17,6–20,7)	20,1	(18,5–21,7)	19,4	(17,9–20,9)
Sweden	9,9	(8,8–11,1)	8,7	(7,6–9,8)	7,8	(6,8–8,9)	9	(7,9–10,1)
Parental education level (ISCED)								
ISCED 1	2,1	(1,5–2,6)	2,3	(1,7–2,9)	2,2	(1,6–2,8)	2,4	(1,8–3,0)
ISCED 2	6,4	(5,4–7,3)	6,2	(5,3–7,1)	7,5	(6,4–8,6)	6,4	(5,5–7,4)
ISCED 3	34,5	(32,7–36,3)	33,2	(31,3–35,0)	32,5	(30,6–34,4)	32,1	(30,3–34,0)
ISCED 4	17	(15,6–18,5)	18,8	(17,3–20,4)	21,2	(19,5–22,8)	20,1	(19,3–22,5)
ISCED 5	40	(38,1–41,9)	39,6	(37,6–41,4)	36,6	(34,7–38,6)	38,2	(36,3–40,1)
Physical activity								
< 60 min/d	78,4	(76,8–79,9)	91,4	(90,3–92,5)	74,3	(7,26–76,0)	90,4	(89,2–91,5)
≥ 60 min/d	21,6	(20,1–23,2)	8,6	(7,5–9,7)	25,7	(24,0–27,4)	9,6	(8,5–10,8)
Sedentary behavior								
≤ 2 h/d	63,7	(61,9–65,6)	70,5	(68,8–72,3)	51,1	(49,1–53,2)	60,1	(58,2–62,0)
> 2 h/d	**36,3**	**(34,4–38,1)**	**29,5**	**(27,7–31,2)**	**48,9**	**(46,9–50,9)**	**39,9**	**(38,0–41,8)**
Nutritional status by BMI								
Undernutrition	10,2	(9,0–11,3)	10,6	(9,4–11,8)	10,3	(9,1–11,5)	10,1	(8,9–11,3)
Euthrofic	72,5	(70,8–74,2)	69,5	(67,8–71,3)	68	(66,2–69,8)	67,8	(66,0–69,6)

Overweight	10,8	(9,7–12,0)	13,6	(12,3–15,0)	14,8	(13,4–16,2)	16,2	(14,8–17,6)
Obese	6,5	(5,6–7,5)	6,3	(5,3–7,2)	6,8	(5,8–7,8)	5,9	(5,0–6,8)
Blood Pressure								
Normal	78,8	(77,2–80,4)	75,5	(73,8–77,2)	84,5	(83,1–86,0)	83,7	(82,3–85,2)
Pre–HBP	12,1	(10,9–13,4)	13,2	(11,8–14,5)	8,8	(7,7–9,9)	8,4	(7,3–9,4)
HBP	9,1	(7,9–10,2)	11,3	(10,0–12,5)	6,7	(5,7–7,6)	7,9	(6,8–8,9)

Significant associations are in bold.
HBP= High Blood Pressure

Table 2: Cumulative incidence and respectives confidence interval 95% (95% CI) of the outcomes per 1,000 individual lifestyle behaviours.

Independents Variables	Incidence	
	Pre–HBP	**HBP**
Physical activity (PA) in Cross–sectional		
≥ 60 min/d	84 (56 – 112)	66 (41 – 91)
< 60 min/d	81 (69 – 92)	75 (64 – 86)
Physical activity (PA) in Cohort		
≥ 60 min/d	83 (61 – 104)	40 (25 – 55)
< 60 min/d	63 (54 – 72)	65 (56 – 74)
PA changes (Cross–sectional ==> Cohort)		
Always ≥ 60 min/d	68 (43 – 93)	43 (23 – 63)
≥ 60 min/d ==> < 60 min/d	52 (40 – 63)	42 (31 – 52)
< 60 min/d ==> ≥ 60 min/d	105 (68 – 143)	35 (13 – 58)
Always < 60 min/d	74 (61 – 87)	86 (72 – 99)
Sedentary behavior (SB) in Cross–sectional		
≤ 2 h/d	67 (56 – 78)	57 (47 – 67)
> 2 h/d	66 (54 – 79)	65 (53 – 77)
Sedentary behavior (SB) in Cohort		
≤ 2 h/d	66 (56 – 76)	55 (46 – 63)
> 2 h/d	69 (54 – 83)	73 (58 – 88)
SB changes (Cross–sectional ==> Cohort)		
Always ≤ 2 h/d	67 (56 – 79)	56 (45 – 67)
≤ 2 h/d ==> > 2 h/d	62 (45 – 80)	51 (35 – 67)
> 2 h/d ==> ≤ 2 h/d	64 (37 – 91)	61 (35 – 87)
Always > 2 h/d	70 (53 – 88)	78 (59 – 96)
Total	121 (53 – 188)	110 (93 – 162)

HBP= High Blood Pressure.

Table 3: Relative risk and respectives confidence interval 95% (RR, 95%CI) by multilevel Poisson according lifestyle behaviours changes

Independents Variables	Pre-High Blood Pressure		High Blood Pressure	
	RR (CI 95%) Unadjusted	**RR (CI 95%) Adjusted***	**RR (CI 95%) Unadjusted**	**RR (CI 95%) Adjusted***
Random Effects Intercept	0,06 (0,03 - 0,10)	0,08 (0,04 - 0,15)	0,06 (0,03 - 0,10)	0,08 (0,04 - 0,15)
Physical activity (PA) in cross-sectional				
≥ 60 min/d	1,00	1,00	1,00	1,00
< 60 min/d	0,93 (0,64 - 1,37)	0,89 (0,61 - 1,31)	1,10 (0,82 - 1,49)	0,93 (0,68 - 1,27)
Physical activity (PA) in cohort				
≥ 60 min/d	1,00	1,00	1,00	1,00
< 60 min/d	0,85 (0,63 - 1,16)	0,83 (0,61 - 1,13)	**1,67 (1,25 - 2,24)**	**1,53 (1,12 - 2,09)**
PA changes (cross-sectional ==> cohort)				
Always ≥ 60 min/d	1,00	1,00	1,00	1,00
≥ 60 min/d ==> < 60 min/d	0,84 (0,54 - 1,32)	0,80 (0,51 - 1,27)	1,05 (0,71 - 1,54)	0,97 (0,64 - 1,47)
< 60 min/d ==> ≥ 60 min/d	1,26 (0,73 - 2,16)	1,23 (0,72 - 2,12)	0,67 (0,38 - 1,19)	0,65 (0,36 - 1,18)
Always < 60 min/d	1,03 (0,67 - 1,57)	0,98 (0,64 - 1,51)	**1,66 (1,16 - 2,37)**	1,43 (0,97 - 2,12)
Sedentary behavior (SB) in cross-sectional				
≤ 2 h/d	1,00	1,00	1,00	1,00
> 2 h/d	1,05 (0,82 - 1,34)	1,04 (0,81 - 1,33)	**1,24 (1,04 - 1,48)**	1,20 (0,99 - 1,44)
Sedentary behavior (SB) in cohort				
≤ 2 h/d	1,00	1,00	1,00	1,00
> 2 h/d	1,03 (0,82 - 1,31)	1,01 (0,80 - 1,28)	1,17 (0,98 - 1,39)	1,16 (0,97 - 1,40)
SB changes (cross-sectional ==> cohort)				
Always ≤ 2 h/d	1,00	1,00	1,00	1,00
≤ 2 h/d ==> > 2 h/d	0,96 (0,70 - 1,35)	0,95 (0,69 - 1,29)	1,07 (0,84 - 1,35)	1,06 (0,82 - 1,35)
> 2 h/d ==> ≤ 2 h/d	0,93 (0,61 - 1,43)	0,94 (0,61 - 1,44)	1,15 (0,85 - 1,56)	1,07 (0,78 - 1,46)
Always > 2 h/d	1,08 (0,81 - 1,44)	1,05 (0,78 - 1,44)	**1,32 (1,07 - 1,63)**	**1,28 (1,03 - 1,60)**
Random Effects - Countries	0,73 (0,42 - 1,29)	0,72 (0,41 - 1,27)	1,21 (0,71 - 2,06)	1,18 (0,70 - 2,02)
Akaike Information Criterion	2076,51	2065,9	3334,77	3098,58

*This analysis was adjusted for potential confounders: country, seasonaliy, sex, age, parental education and waist circumference. Significant associations are in bold.

DISCUSSION

To the best of our knowledge, this is the first study examining the incidence of HBP in children, and exploring the effect of PA and SB on this incidence. We analyzed the incidence of pre-HBP and HBP in a large sample of European children from eight different countries, and also the effect of PA and SB in the incidences of these outcomes. We found a high incidence of both outcomes during the follow-up of two years. Children fitting with the PA recommendation (≥ 60 min/d) in T1 or maintaining high SB (> 2h/d) at follow-up are at high risk of developing HBP. Our findings are relevant, because HBP is considered the risk factor with the highest attributable fractions for cardiovascular diseases (CVD) mortality (40.6%) [25] and some studies have shown that if HBP is developed in childhood and adolescence. Therefore, this could be crucial for developing CVD such as stroke and myocardial infarction in adulthood [3]. Including data from a cohort study adds consistency and temporality to our report as some of the results were similar in different populations and the risk factor came first than the outcome (Hill's principles) [26].

Our results corroborate studies that have evaluated, in cross-sectional studies, the association of PA on BP levels [27,28]. Several mechanisms can explain the positive effects PA induces on BP levels. There is strong evidence that the sheer stress caused by regular PA has a powerful effect on the release of vasodilator factors produced by the vascular endothelium [29], such as nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) [30], and the children that perform physical activity less than <60/min/d have lower vasodilation capacity of the endothelium and this could be the biological mechanism by which they develop HBP.

An important result we found was that children who maintained SB> 2 h / d during the two-year follow-up showed a high incidence of HBP. There are several

possible physiological mechanisms by which SB may contribute to increased BP, and more research is needed to analyze the pathophysiological processes of increased BP due to high SB. One possible biological explanation is that SB change the myokine response in the skeletal muscle and these alterations promote the endothelial dysfunction in the cardiovascular system by increase of the pro-inflamatory adipokines. Consequently, this increased could be the start of the pathological processes of atherosclerosis, and progressively develop into hypertension [31].

The results of this study are in agreement with some cross-sectional studies suggesting that lower PA level is associated with higher levels of BP [32]. Additionally, present results corroborate with previous survey observations[33] and a recent review[5] suggesting that SB is an independent risk factor for detrimental cardiovascular outcomes independent of PA level. Our results are of importance and highlight youth should be encouraged to engage in recommended levels of MVPA and reduce excessive time spent in screen-based sedentary behavior. In adolescents our group found that recommended levels of MVPA could attenuates the harmful effects of SB in increased blood pressure [6].

The behavioral patterns under consideration during childhood tend to continue into adulthood [34] and high levels of sedentary behaviors in adults increase the risk of mortality from cardiovascular diseases [35,36].

A limitation of this study is that it was not possible to adjust the analysis for other potentially BP-associated factors in either of the large children sample, such as genetics or intrauterine development, but we developed an adjusted analysis for large potential set of confounders. On the other hand, the diverse geographic origin of the samples, the cohort design consequently, temporal sequence between risk factor and

outcome can be established, the use of objective measures to assess PA and SB and multilevel adjusted analysis are some of the main strengths of our study.

CONCLUSIONS

According to our results, the incidence of pre-HBP and HBP is high in European children, low levels of PA are a risk factor for developing HBP and to maintain sedentary behaviors increases the risk of developing HBP after two years of follow-up. These results suggest that regular PA should be promoted and SB discouraged in children to prevent high blood pressure and its consequences in adulthood.

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4. CRITICAL ANALYSIS OF OUR RESULTS

This thesis is a compilation of 10 papers (systematic reviews and originals), six of which have been published or have been accepted for publication.

Our systematic review found that the worldwide prevalence of high blood pressure (HBP) is high in adolescents, and higher prevalences have been noted in developing countries. This result is alarming, as PAE is the primary risk factor for the development of coronary heart disease and stroke. In this review, we found that the instrumentation used to measure blood pressure is directly associated with the prevalence of HBP. Hence, research in this area should involve the use of previously validated methods and equipment tested specifically for adolescents.

One of the scientific concerns that arose (and remains) during my PhD research is the overvaluation of the p-value in scientific and biomedical research, a concern that persists due to a scientific paper by RA Fernandes et al. The authors noted an accuracy of 0.61 for resting heart rate as a predictor of cardiometabolic disorders: the p-value was significant (<0.05); therefore, the authors concluded that resting heat rate is a good predictor of subsequent diseases and ignored the 60% accuracy value. Our article regarding the same issue noted similar values (58.4% accuracy).

Experts believe that a good predictor should have ≥ 90% accuracy. There are other points from that article worth discussing (the use of categorized and continuous outcomes), but what stands out most is the overvaluation of the p-value. Epidemiologists must utilize statistical indicators to allow our findings to be interpreted, as conclusions must be drawn based on these statistics. The primary journals in the field (International Journal of Epidemiology, American Journal of Epidemiology;

Epidemiology Reviews) request that p-values are not included in tables but agree that the 95% confidence interval is an appropriate indicator.

Epidemiologists utilize indicators of population health, and interpretations of study results must be appropriate to avoid erroneous conclusions by experts and the general population. As a scientist recently told Regina Nuzzo* in an article published in Nature, as follows:

"P-values, the 'gold standard' of statistical validity, are not as reliable the many scientists assume."

Another interesting result of our study was that no socioeconomic factors specific to families demonstrated a relationship with blood pressure in either Brazilian or European adolescents, aside from a relationship between the two parameters that was observed only in boys. This may indicate that boys are more dependent on family than girls, as girls receive health care separate from boys.

In an article published in Plos One, we observed that the relationships between physical activity and sedentary behavior and blood pressure are different for boys and girls: they also differ between studies. We also found that physical activity mitigates the possible deleterious effects of sedentary behavior in adolescents. Another positive factor of physical activity is that this behavior also minimizes the effects of genes that predispose European adolescents to high blood pressure. Therefore, physical activity is an important behavior that should be promoted by public policy and by schools because it helps control blood pressure and attenuates the effects of risk factors.

Protein intake (plant and animal) was negatively associated with blood pressure levels in European boys, whereas the relationship between amino acid intake and blood pressure was inconsistent in this population. These results are consistent with those of randomized clinical trials involving adults and represent examples of other behaviors

* Nuzzo R. Scientific method: statistical errors. Nature. 2014 Feb 13;506(7487):150-2.

and dietary adjustments that may be promoted to control and reduce blood pressure in adolescents.

We had the opportunity to test our hypothesis regarding the effects of physical activity and sedentary behavior on blood pressure in a European cohort study (IDEFICS). The results of this study indicate that the incidence of HBP is high in children and adolescents. European physical activity exerted protective effects against the development of PAE. Sedentary behavior increases this risk. This result is troubling because research has shown that sedentary behavior increases during adolescence, which increases the risk of developing HBP.

* Nuzzo R. Scientific method: statistical errors. Nature. 2014 Feb 13;506(7487):150-2.

5. CONCLUSIONS

The data presented allow us to conclude the following:

i. The worldwide prevalence of HBP is high in adolescents and is higher in developing countries; methodology influences its prevalence;

ii. The performance of ≥ 60 min of physical activity per day helps to control blood pressure and reduces the effects of sedentary behavior and genes associated with elevated blood pressure;

iii. Socioeconomic factors in families have an inverse relationship with blood pressure in European boys;

iv. Protein consumption is associated with lower blood pressures in European children, and the relationship between amino acid intake and blood pressure is inconsistent in this population;

v. The incidence of high blood pressure in children and adolescents is high among Europeans, and sedentary behavior is the primary risk factor for this outcome;

vi. Scientists should avoid making interpretations and drawing conclusions based only on p-values.

APPENDIX

1. Statement of Project Approval - Ethics Committee for Research - CEP

MEDICINA
USP
COMITÊ DE ÉTICA EM PESQUISA

APROVAÇÃO

O Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade de São Paulo, em sessão de **27/02/2013, APROVOU** o Protocolo de Pesquisa nº **066/12** intitulado: **"PRESSÃO ARTERIAL ELEVADA E AGREGAÇÃO DE FATORES DE RISCO EM ADOLESCENTES: UM ESTUDO MULTICÊNTRICO"** apresentado pelo **Departamento de MEDICINA PREVENTIVA**

Cabe ao pesquisador elaborar e apresentar ao CEP-FMUSP, os relatórios parciais e final sobre a pesquisa (Resolução do Conselho Nacional de Saúde nº 196, de 10/10/1996, inciso IX.2, letra "c").

Pesquisador (a) Responsável: Heráclito Barbosa de Carvalho
Pesquisador (a) Executante: Augusto César Ferreira de Moraes

CEP-FMUSP, 01 de Março de 2013.

Prof. Dr. Roger Chammas
Coordenador
Comitê de Ética em Pesquisa

APPENDIX 2. First page of the Published Articles and Confirmation emails of the Accepted Articles of this PhD Thesis

Assunto:	Medicine® MD-D-14-00444: Editor Decision
De:	"Medicine" <em@editorialmanager.com>
Para:	augustocesar.demoraes@usp.br;
Data:	08 de setembro de 2014 13:18:33 BRT

ACCEPTED

Aug 28, 2014

RE: MD-D-14-00444, entitled "Prevalence of high blood pressure in 122,053 adolescents: a systematic review and meta-regression"

Dear Prof. de Moraes:

Thank you for revising and resubmitting your manuscript. The Editors appreciate your efforts. We are pleased to accept your revised manuscript for publication in The Journal of Pediatrics. Please note the slight title change, as written above. We have made other editorial changes, which you will see in the proofs.

We will forward your manuscript to Elsevier, Inc. Before the final publication date, the publisher will send you galley proofs and other relevant material. Please read the proofs carefully and contact the publisher if anything is unclear or incorrect; the authors have final responsibility for the accuracy of the publication.

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de Moraes et al. BMC Pediatrics 2014, 14:117
http://www.biomedcentral.com/1471-2431/14/117

BMC Pediatrics

COMMENTARY Open Access

Potential biases in the classification, analysis and interpretations in cross-sectional study: commentaries – surrounding the article "resting heart rate: its correlations and potential for screening metabolic dysfunctions in adolescents"

Augusto César Ferreira de Moraes[1,2*†], Alex Jones Flores Cassenote[3†], Luis A Moreno[2,4] and Heráclito Barbosa Carvalho[1]

Abstract

Background: Resting heart rate reflects sympathetic nerve activity. A significant association between resting heart rate (HR) and all causes of cardiovascular mortality has been reported by some epidemiologic studies. Despite suggestive evidence, resting heart rate (RHR) has not been formally explored as a prognostic factor and potential therapeutic outcome and, therefore, is not generally accepted in adolescents.

Discussion: The core of the debate is the methodological aspects used in "Resting heart rate: its correlations and potential for screening metabolic dysfunctions in adolescents"; the points are: cutoff used for cluster RHR, two different statistical models used to analyze the same set of variables, one for continuous data, and another for categorical data; interpretation of p-value < 0.05, sampling process involving two random stages, analysis of design effect and the parameters of screening tests.

Summary: Aspects that must be taken into account for evaluation of a screening test to measure the potential for discrimination for a common variable (population with outcome vs. no outcome population), the main indicators are: sensitivity, specificity, accuracy, positive predictive value and negative predictive value. The measures of argumentation equality (CI) or difference (p-valor) are important to validate these indicators but do not indicate quality of screening.

Keywords: Resting heart rate, Screening test, P-value, High glucose, High triglycerides

Background

Recently, Fernandes et al. published an article aimed at analyzing the potential effects of screening and resting heart rate (RHR) on cardiometabolic risk in adolescents [1] in this respected journal. We read the manuscript with great interest, since RHR reflects sympathetic nerve activity [2,3], and it is an easily accessible clinical measurement. A significant association between resting HR and all-causes of cardiovascular mortality has been reported in some epidemiological studies [2,4-6].

After studying the article, we decided to take the opportunity to propose a healthy debate on the methodological aspects used by Fernandes et al. [1]. With this debate, we hope to contribute to the enrichment of the reader, especially with regard to statistical analysis and interpretation of results.

The aim of this article is to present a critical appraisal of methodological aspects of the article "Resting heart rate: its correlations and potential for screening metabolic dysfunctions in adolescents" presented by BMC *Pediatrics*.

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ORIGINAL ARTICLE

Prevalence of cardiovascular risk factors among Latin American adolescents: a multilevel analysis

ACF de Moraes[1,2,3,4,5], C Musso[6], MN Graffigna[6], J Soutelo[6], M Migliano[6], HB Carvalho[2,8] and G Berg[6,7,8]

High blood pressure (HBP) and obesity is a well-established major risk factor for stroke and coronary heart disease. However, the literatures are scarce about these informations in adolescents from low-and-middle income countries. This school-based survey was carried out among students from Maringá (Brazil) and Buenos Aires (Argentina) selected random sampling. We studied 991 Brazilian adolescents (54.5% girls) in the age range of 14–18 years. In Argentina, we studied 933 adolescents (45.9% female) in the age range of 11–17 years. The outcomes of this study are general obesity, abdominal obesity and HBP. The associated factors analysed were gender, age and health behaviours. The prevalence of obesity was 5.8% in Brazil and 2.8% in Argentina, the prevalence of abdominal obesity was 32.7% in Brazil and 11.1% in Argentina, the prevalence of HBP was 14.9% in Brazil and 13.5% in Argentina. The multilevel analysis showed that older adolescents (>14 years old) have a little likelihood of being overweight, whereas male adolescents are more likely to be obese and have HBP. The abdominal obesity in both indicators were not associated with the independent variables. The prevalence of cardiovascular risk factors is high in Latin American adolescents independent of each country, and was associated with male gender.

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Keywords: cardiovascular health; pediatrics; population; risk factors; low-and-middle countries; multicentre study

INTRODUCTION

Cardiovascular diseases are the main sources of disease burden worldwide, and constitute a major public health problem in many countries.[1] Several studies have demonstrated that general obesity (measured by body mass index (BMI)), abdominal obesity and high blood pressure (HBP) in adolescence are a good predictor for cardiovascular disease in adulthood.

In epidemiological studies, anthropometry has been considered as an efficient method for diagnosis of obesity.[2,3] The measurement of waist circumference (WC) represents a good marker of abdominal obesity. The other anthropometric indicator of abdominal fatness is waist-to-height ratio; this is an easily measurable anthropometric index and predicts cardiovascular disease, diabetes and other metabolic risk factors.[4] The advantage of measuring waist-to-height ratio is that a single value could be useful in different ethnic, age and sex groups,[5] while WC requires population-specific boundary values.[6] Abdominal obesity, evaluated through the WC, has a central role in the metabolic syndrome, an entity that predisposes affected people to the development of diabetes and cardiovascular disease, and is associated with insulin resistance.[7,8]

On the other hand, HBP is an established major risk factor for stroke and coronary heart disease.[9] the literature highlights, particularly in developing countries,[1] increasing prevalence of childhood obesity in the past decades, which may influence the prevalence of HBP in adolescents.

Abdominal obesity and HBP are components of metabolic syndrome according to the criterion of the NCEP-ATP III (National Cholesterol Education Program's Adult Treatment Panel III).[10] Nevertheless, the high prevalence of abdominal obesity and HBP, and the analysis of the association of these outcomes with modifiable risk are scarce in adolescents from developing countries. It is therefore essential to try to identify and analyse the most significant variables involved in the development of metabolic syndrome in this population. The objective of this study was to evaluate the prevalence of cardiovascular risk factors among Latin American adolescents from two countries.

SUBJECTS AND METHODS

The study was carried out in the city of Maringá, located in the northwest of Paraná State (PR), Southern Brazil, which has a population of ~330 000 (51 428 adolescents, 50.1% female) and in Buenos Aires, capital city of Argentina, which has a population of 2 891 000 (53% female). In Brazil, a formal request to conduct this survey was sent to and subsequently accepted by the school boards of several schools in the city, and those adolescents who met the inclusion criteria were admitted in the study. In Argentina, from a sample of 1023 high school adolescents who underwent a medical mandatory examination to be admitted in the High School of Buenos Aires University, those who met the inclusion criteria were included. All the participants were from the city of Buenos Aires or its suburbs, belonged to middle class and 96.2% of them were white Caucasians. Recruitment of students took place from 5 to 30 May 2008. The study included 991 adolescents (540 females and 451 males, 14.0–17.5 years) from Maringá and 943 (429 females and 514 males; 11–14 years) from Buenos Aires. The complete methodology of this study has been described earlier.[11,12]

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PLOS ONE

Independent and Combined Effects of Physical Activity and Sedentary Behavior on Blood Pressure in Adolescents: Gender Differences in Two Cross-Sectional Studies

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Abstract

Objectives: To examine the independent and combined association of physical activity (PA) and sedentary behavior (SB) on both systolic (SBP) and diastolic blood pressure (DBP) in adolescents from two observational studies.

Methods: Participants from two cross-sectional studies, one conducted in Europe (n = 3,308; HELENA study) and the other in Brazil (n = 991; BRACAH study), were selected by complex sampling. Systolic and diastolic blood pressure (outcomes), PA and SB, both independently and combined, and potential confounders were analyzed. Associations were examined by multilevel linear regression.

Results: Performing the recommended amount of PA (≥60 min/d) attenuated the effect of SB on DBP in BRACAH study girls and in boys from both studies. In contrast, PA did not attenuate the effects of SB on the SBP of girls in the HELENA study. The combination of less than recommended levels of PA with 2–4 h/d of sedentary behavior was found to be associated with increased SBP in boys from both studies.

Conclusions: Meeting current PA recommendations could mediate the association between SB and DBP in both sexes. In boys, the joint effect of low levels of PA and excessive sedentary activity increases SBP levels. Longitudinal studies are required to confirm these findings.

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Applied nutritional investigation

Vitamins and iron blood biomarkers are associated with blood pressure levels in European adolescents. The HELENA study

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ABSTRACT

Objectives: Previous research showed that low concentration of biomarkers in the blood during adolescence (i.e., iron status; retinol; and vitamins B_6, B_{12}, C, and D) may be involved in the early stages of development of many chronic diseases, such as hypertension. The aim was to evaluate if iron biomarkers and vitamins in the blood are associated with blood pressure in European adolescents.

Methods: Participants from the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study (N = 1089; 12.5–17.5 y; 580 girls) were selected by complex sampling. Multilevel linear regression models examined the associations between iron biomarkers and vitamins in the blood and blood pressure; the analyses were stratified by sex and adjusted for contextual and individual potential confounders.

Results: A positive association was found in girls between RBC folate concentration and systolic blood pressure (SBP) (β = 3.19; 95% confidence interval [CI], 0.61–5.77), although no association between the vitamin serum biomarkers concentrations and diastolic blood pressure (DBP) was

The HELENA Study was financially support by the European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034). The writing group takes sole responsibility for the content of this article. This study was also supported by a grant from the Spanish Ministry of Health: Maternal, Child Health and Development Network (number RD08/0072), grant from the Spanish Ministry of Education (EX-2008-0641; AP-2008-03806) and the Swedish Heart-Lung Foundation (20090635). ACdeM received scholarship from São Paulo Research Foundation— FAPESP (proc. 2011/11137-1 and 2011/20662-2). LAM received scholarship of visiting professor from the Brazilian government by Science without Borders Program by CNPq (National Counsel of Technological and Scientific Development) and CAPES (Coordination of Improvement of Higher Education Personnel) (proc. 007/2012). The GENUD Research Group co-financed by the European Regional Development Fund (MICINN-FEDER). The authors declare that there are no conflicts of interest.

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Physical Activity Modifies the Associations between Genetic Variants and Blood Pressure in European Adolescents

Augusto César Ferreira de Moraes, PhD[1,2,3], Juan Miguel Fernández-Alvira, PhD[2,3], Heráclito Barbosa Carvalho, PhD[1], Aline Meirhaeghe, PhD[4], Jean Dallongeville, PhD[4], Anthony Kafatos, PhD[5], Ascensión Marcos, PhD[6], Dénes Molnar, PhD[7], Yannis Manios, PhD[8], Jonatan R. Ruiz, PhD[9], Idoia Labayen, PhD[10], Kurt Widhalm, PhD[11], Christina Breidenassel, PhD[12], Marcela Gonzalez-Gróss, PhD[12,13], and Luis A. Moreno, PhD[2,3,*]

We hypothesized that physical activity and sedentary behavior could modify the associations between known genetic variants blood pressure-associated genes in European adolescents. Meeting current physical activity recommendations (≥60 minutes/day) was able attenuate the deleterious effect of the *NOS3* rs3918227 polymorphism on systolic blood pressure in European adolescents. *(J Pediatr 2014; ■: ■-■).*

Hypertension is a major public health problem and risk factor for cardiovascular disease.[1] Recent studies have suggested an association between blood pressure (BP) levels and certain genetic loci, with special potential gene regulatory mechanisms at the *MTHFR* and *NOS3* loci.[2,3] In addition, BP is known to be affected by several environmental factors, among which are physical activity and sedentary behaviors, as predictors of higher BP.[4]

As genetic influence on BP would have been already observed during adolescence,[5] determining to what extent BP-related genes are modulated by environmental factors such as physical activity and sedentary behaviors during adolescence could help to further understand and prevent high BP at early stages.

Thus, we hypothesized that physical activity and sedentary behavior levels could modify the associations between genetic variants known BP-associated variants in European adolescents (**Figure 1**; available at www.jpeds.com).

Methods

A total of 3528 adolescents (1845 girls) from 9 European countries participated in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study. A detailed description of the HELENA methodology has been published elsewhere.[6,7] The Human Research Review Committees of the centers involved approved the protocol following the Helsinki Declaration rules.

A total of 1009 adolescents (12.5-17.5 years old; 532 girls), with valid data on BP levels, physical activity measured by accelerometry, sedentary behavior assessed by questionnaire, and a complete set of confounders were included in the analyses of this study.

BP	Blood pressure
DBP	Diastolic blood pressure
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
SBP	Systolic blood pressure

Systolic BP (SBP) and diastolic BP (DBP) were measured by an arm BP oscillometric monitor device (OMRON M6, HEM 70001; Omron, Kyoto, Japan) and following the recommendations for adolescent populations,[8] complete data collection procedures have been described in earlier.[9]

Independent Variables

Genotyping. Fasting blood samples were collected by venipuncture after a 10-hour overnight fast, as previously described.[10] For the purpose of the present study, 6 single nucleotide polymorphisms in 3 genes previously associated with hypertension[3,11] were selected (rs1205 and rs1130864 in *CRP*, rs1801131 and rs1537516 in *MTHFR*, and rs1800779 and rsc3918227 in *NOS3*). Genotyping success rates were greater than 99.7%. The genotype distributions,

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APPENDIX 3. First page of the Published Articles like a Co-Author

Hypertension Research (2011) 34, 913–914

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COMMENTARY

Evaluating risk factors in hypertension screening in children and adolescent

Augusto César Ferreira de Moraes and Heráclito Barbosa de Carvalho

Hypertension Research (2011) **34,** 913–914; doi:10.1038/hr.2011.73; published online 9 June 2011

The pediatric obesity epidemic has increased significantly over the last three decades.[1] This increase represents a problem for the healthcare system, as being overweight is directly associated with an increased probability of developing metabolic syndrome and high blood pressure.[2,3]

The screening performed by Fernandes *et al.*[4] used body mass index (BMI) and dual-energy X-ray absorptiometry (DXA), which is a more accurate method for assessing body composition, as DXA assesses three aspects of body composition: bone mineral, lipids (triglycerides, phospholipid membranes and so on) and lipid-free soft tissue. From these data, we can estimate fat mass, lean soft tissue mass, fat-free mass, soft tissue mass, total body mass and percent fat mass.[5] Moreover, this method is difficult logistically because the evaluation requires an appropriate location (laboratory or clinic) and in epidemiological studies, this is not always possible, as the individuals would have to travel to the laboratory/clinic to perform the evaluation.

However, we should highlight the benefits and drawbacks of this type of study, as the number of individuals who may benefit is small.[6] Moreover, the method used by Fernandes *et al.*[4] is simple and rarely causes complications in subjects.[5] Regarding the measurement of blood pressure, the authors used an electronic device validated for this population. Thus, our initial conclusion is that the methods used to assess obesity and body fat were appropriate and that high blood pressure posed no risk to the health of the individuals, which is an important point in screening studies.

Faced with this difficulty, anthropometry has been considered an efficient and feasible method for diagnosing obesity in epidemiological studies because of its simplicity and cost effectiveness. The principal anthropometric indicators used in studies have been BMI as an indicator of general obesity and waist circumference as an indicator of abdominal obesity.[7] The international reference has been used for the classification of obesity based on BMI, which allows the possibility of comparison between studies from different countries. However, among adolescents, these cutoffs have shown low positive predictive value for excess body fat.[8] Therefore, it is more appropriate to use diagnostic criteria for obesity developed from samples of Brazilian adolescents, as they better represent the miscegenation of this population.

Another important point in the article by Fernandes *et al.*[4] was to compare the predictor performance of blood pressure at different cutoff points for obesity from the body fat. The differences in results may be partly explained by such methodological aspects. Another factor that may have influenced the recorded prevalence is the question of measurement accuracy. Differential or non-differential misclassification effects (error due to disease status or exposure) of obesity prevalence are unpredictable and may have caused the underestimation or overestimation of the true prevalence. In the context of this study, it is likely that the validity of the diagnostic criteria and tools used varied for each characteristic of the adolescents studied.[9]

Wald and Morris[10] have been investigating risk factors as potential screening tests, as some risk factors are directly associated with a disease and would suggest the need for screening. These analyses are important, because risk factors are usually poor predictors of disease, and this is reflected in the low positive predictive values presented by Fernandes *et al.*,[4] which were $<60\%$ (Figure 1).

BMI and body fat are optimal risk indices for the development of hypertension.[4] Thus, the most important contribution of this study was to demonstrate the high prevalence of

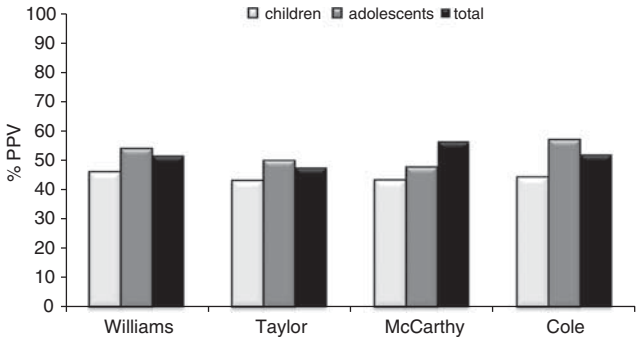

Figure 1 Perceptual (%) values of positive predictive value (PPV) for diagnosis of the elevated blood pressure from Fernandes *et al.*[5] according to obesity cutoff points proposed by the authors described.

Professor ACF de Moraes is at the Post-Graduate Program in Science, Children Institute, School of Medicine, University of São Paulo, São Paulo, São Paulo, Brazil and GEPECIN, Nutritional Science Group Research, Maringá, Paraná, Brazil and GEPEMENE, Nutrition, Exercise and Metabolism Research Group, Londrina, Paraná, Brazil and OLFIS, Latin American Epidemiological Research Group, Bucaramanga, Santander, Colombia, and HB de Carvalho is at the Department of Preventive Medicine, School of Medicine, University of São Paulo, São Paulo, São Paulo, Brazil.
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obesity and high blood pressure in children and adolescents in a developing country. This demonstrates the need for obesity reduction intervention in this population, as multicomponent intervention can have beneficial effects on several cardiovascular risk factors.

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Nutrition, Metabolism & Cardiovascular Diseases

Sedentary behaviour and clustered metabolic risk in adolescents: The HELENA study☆

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KEYWORDS
Sedentary behaviour;
Physical activity;
Clustered metabolic risk;
Videogames

Abstract *Background and aims:* Although sedentary behaviours are linked with mortality for cardiovascular reasons, it is not clear whether they are negatively related with cardio-metabolic risk factors. The aim was to examine the association between time engaged in television (TV) viewing or playing with videogames and a clustered cardio-metabolic risk in adolescents.
Methods and results: Sedentary behaviours and physical activity were assessed in 769 adolescents (376 boys, aged 12.5–17.5 years) from the HELENA-CSS study. We measured systolic blood pressure, HOMA index, triglycerides, TC/HDL-c, VO_2max and the sum of four skinfolds, and a clustered metabolic risk index was computed. A multilevel regression model (by Poisson) was performed to calculate the prevalence ratio of having a clustered metabolic risk. In boys,

☆ A list with all the HELENA members is shown as Supplementary data.

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OBESITY PREVENTION (A MUST, SECTION EDITOR)

Obesity Prevention in Latin America

Juliana Kain · Sonia Hernández Cordero · Diana Pineda · Augusto Ferreira de Moraes · Daniel Antiporta · Tatiana Collese · Elsie Costa de Oliveira Forkert · Laura González · Juan Jaime Miranda · Juan Rivera

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Abstract In Latin American countries, obesity prevalence has increased significantly as a result of rapid urbanization and an improvement in socioeconomic conditions. We report the prevalence of overweight and/or obesity and prevention efforts in five countries: Mexico, Colombia, Brazil, Peru, and Chile. In children, the highest and lowest rates of obesity are found in Chile (23 % in 6-year-olds) and Peru (1.8 % in those <5 years), respectively. In adults, Mexico and Chile present similar high rates of obesity (around 35 %), whereas in Brazil and Colombia, the rates are around 20 % and 16.5 %, respectively. In general, the highest prevalence occurs in low-income women. Every country has developed initiatives to target obesity, from the government to the private sector and academia, mainly at the health sector and school settings. Food labeling is being addressed, but has not been implemented yet. Two interventions are described, a community-based in Mexico and a school-based in Chile. Because the increase in chronic diseases, especially diabetes, has paralleled that of obesity, effective prevention efforts are urgently needed.

Keywords Obesity · Prevention · Latin America

Introduction

During the past three decades, people living in Latin American countries have experienced extensive demographic, epidemiologic, and socioeconomic changes, showing improvements in overall health and educational indicators [1]. These processes, known as epidemiologic transition, have occurred at a different pace from country to country and are closely related to increasing urbanization and a reduction in poverty levels, producing changes in dietary and physical activity patterns in all age groups. These changes have been directly

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Original Article

Sleep time and cardiovascular risk factors in adolescents: The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study

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ABSTRACT

Objective: We aimed to examine the association between adolescents' sleep time and a cardiometabolic risk score. A second aim was to examine associations between sleep time and individual cardiometabolic risk factors.
Methods: Adolescents ($N = 699$; ages, 12.5–17.5 years) participating in the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study were examined. Sleep time was reported by a questionnaire. Physical activity (PA) was assessed by accelerometry ($n = 497$). Cardiometabolic risk factors measurements included insulin resistance, blood pressure, adiposity markers, cardiorespiratory fitness, and blood lipids. A cardiovascular disease risk score was computed. Associations were examined by a multilevel regression analysis (linear for individual risk factors and Poisson for the clustered risk score).
Results: For school days no association was found between sleep time and cardiometabolic risk factors. At weekend days, the prevalence ratio (PR) of having a clustered risk score increased by 15% for each additional hour of sleep controlling for age, sex, and socioeconomic status (SES); however, the prevalence disappeared when adjusting for PA.
Conclusions: In European adolescents sleep time is not associated with cardiometabolic risk factors when important confounders are considered. Future research about sleep cardiovascular risk factors should register other sleep dimensions (sleep patterns or disturbances) to provide a better insight in this scientific field.

1. Introduction

Coronary heart disease is a leading cause of death globally. Although genetic influences have been reported [1], nine risk factors, including smoking, history of hypertension or diabetes mellitus, waist/hip ratio, dietary patterns, physical activity (PA), consumption of alcohol, blood apolipoproteins, and psychosocial factors, are significantly associated with acute myocardial infarction and explain more than 90% of the population-attributed risk [2]. A cause of concern is that cardiometabolic risk factors appear in the first decade of life. For example, a recent study found that half of adolescents in North America present one cardiometabolic risk factor (e.g., dyslipidemia, glucose intolerance, hypertension, obesity) [3]. This finding is relevant for public health, as cardiometabolic risk factors in adolescents predict adult carotid artery intima

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BMC Pediatrics

RESEARCH ARTICLE Open Access

The relationship between visceral fat thickness and bone mineral density in sedentary obese children and adolescents

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Abstract

Background: Among adults, obesity has been positively related to bone mineral density. However, recent findings have pointed out that abdominal obesity could be negatively related to bone density. The above mentioned relationship is not clear among pediatric populations. Therefore, this cross-sectional study analyzed the relationship between thickness of abdominal adipose tissue and bone mineral variables in sedentary obese children and adolescents.

Methods: One hundred and seventy five obese children and adolescents (83 male and 92 female) with ages ranging from 6 to 16 years-old were analyzed. Bone mineral content and density were estimated by dual-energy X-ray absorptiometry and ultrasound equipment which estimated the thickness of the abdominal adipose tissue. Pubertal stage was self-reported by the participants.

Results: The mean age was 11.1 (SD = 2.6). Thickness of the abdominal adipose tissue was negatively related to bone mineral density ($r = -0.17$ [$r_{95\%CI}$: -0.03;-0.32]), independent of gender, pubertal stage and other confounders ($\beta = -0.134 \pm 0.042$ [$\beta_{95\%CI}$: -0.217; -0.050]).

Conclusions: In sedentary obese children and adolescents abdominal obesity is negatively related to bone mineral density, suggesting a potential link between abdominal obesity and osteoporosis.

Keywords: Child, Adolescents, Obesity, Bone size, Bone density, Ultrasonography

Background

In modern society osteoporosis is a highly occurring disease and constitutes a public health concern due to its impact on public costs [1]. Early life has been pointed out as a crucial period in the development of osteoporosis. Childhood and adolescence are phases of the human development during which the adult bone mass density is determined and, therefore, problems during this period of life could compromise bone health in adulthood [2].

Worldwide, children and adolescents are widely affected by obesity and its comorbidities [3-6]. Despite these related comorbidities, overweight/obesity has been associated with a lower occurrence of osteoporosis in adulthood. However, body weight is composed of lean and fat mass and the actual effect of the adipose tissue on bone mineral density (BMD) is not clear.

Moreover, the distribution of the adipose tissue could be a relevant confounder in this complex process that links obesity to osteoporosis. Recently, Bhupathiraju et al. [7] analyzing Porto Rican adults (47–79 years) observed that a higher abdominal fat mass (in kg) is related to a lower BMD, but the amount of visceral and subcutaneous abdominal adipose tissue were not assessed. Furthermore, there is an absence of data about this issue in pediatric populations. Understanding the relationship between pediatric obesity and bone health is relevant for health professionals [8-10], because childhood and adolescence are two critical periods in the prevention and development

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ORIGINAL ARTICLE

Inflammation profile in overweight/obese adolescents in Europe: an analysis in relation to iron status

M Ferrari[1], M Cuenca-García[2], J Valtueña[1,3], LA Moreno[4,5], L Censi[1], M González-Gross[3,6], O Androutsos[7], CC Gilbert[8], I Huybrechts[9], J Dallongeville[10], M Sjöström[11], D Molnar[12], S De Henauw[13,14], S Gómez-Martínez[15], ACF de Moraes[4,16], A Kafatos[17], K Widhalm[18] and C Leclercq[1] on behalf of the HELENA Study Group[19]

BACKGROUND/OBJECTIVES: The objectives of this study were to investigate the relationship between inflammatory parameters (CRP, c-reactive protein; AGP, α1-acid glycoprotein), iron status indicators (SF, serum ferritin; sTfR, soluble transferrin receptor) and body mass index (BMI) z-score, fat-free mass (FFM) and fat mass (FM) in European adolescents. Differences in intake for some nutrients (total iron, haem and non-haem iron, vitamin C, calcium, proteins) were assessed according to BMI categories, and the association of nutrient intakes with BMI z-score, FM and FFM was evaluated.

METHODS: A total of 876 adolescents participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence-Cross Sectional Study were included in the study sample.

RESULTS: Mean CRP values (standard error; s.e.) were significantly higher in overweight/obese adolescents (1.7 ± 0.3 and 1.4 ± 0.3 mg/l in boys and girls, respectively) than in thin/normal-weight adolescents (1.1 ± 0.2 and 1.0 ± 0.1 mg/l in boys and girls, respectively) ($P < 0.05$). For boys, mean SF values (s.e.) were significantly higher in overweight/obese adolescents (46.9 ± 2.7 μg/l) than in thin/normal-weight adolescents (35.7 ± 1.7 μg/l) ($P < 0.001$), whereas median sTfR values did not differ among BMI categories for both boys and girls. Multilevel regression analyses showed that BMI z-score and FM were significantly related to CRP and AGP ($P < 0.05$). Dietary variables did not differ significantly among BMI categories, except for the intake of vegetable proteins, which, for boys, was higher in thin/normal-weight adolescents than in overweight/obese adolescents ($P < 0.05$).

CONCLUSIONS: The adiposity of the European adolescents was sufficient to cause chronic inflammation but not sufficient to impair iron status and cause iron deficiency.

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INTRODUCTION

There is growing evidence that obesity is characterised by a chronic, low-grade, systemic inflammation[1,2] that may have a causal role in the development of several diseases such as cardiovascular diseases, insulin resistance and type 2 diabetes, as well as metabolic disturbances such as metabolic syndrome,[3,4] but the underlying mechanisms are unclear. The concept of obesity-related inflammation is supported by previous studies that showed a correlation between levels of inflammatory mediators, acute-phase proteins and body weight.[5] The relationship between c-reactive protein (CRP), an acute-phase reactant, and obesity has undergone intense investigation because its elevation was shown to be correlated to the increase of body weight and adiposity in both adults and adolescents.[6,7] The regulation of cytokines by adipose tissue for the synthesis of acute-phase proteins could be an explanation for the association between CRP and obesity.[8] However, it is not known whether adipocytokines are related specifically to CRP or to other acute-phase proteins produced by the liver in obesity. Other inflammatory markers such as alpha 1-acid glycoprotein (AGP) were found to be increased in obese adult subjects.[5,9]

In some studies, overweight and obese children appear to be at a higher risk of iron deficiency than those having normal body weight,[10–12] and similar findings have been reported in adults as well.[11,13,14] A full understanding of the mechanisms that could explain the observed low iron status in obese subjects has not yet been reached. One proposed explanation is that obese children and adolescents are at a higher risk of iron deficiency because

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[19]See Appendix.
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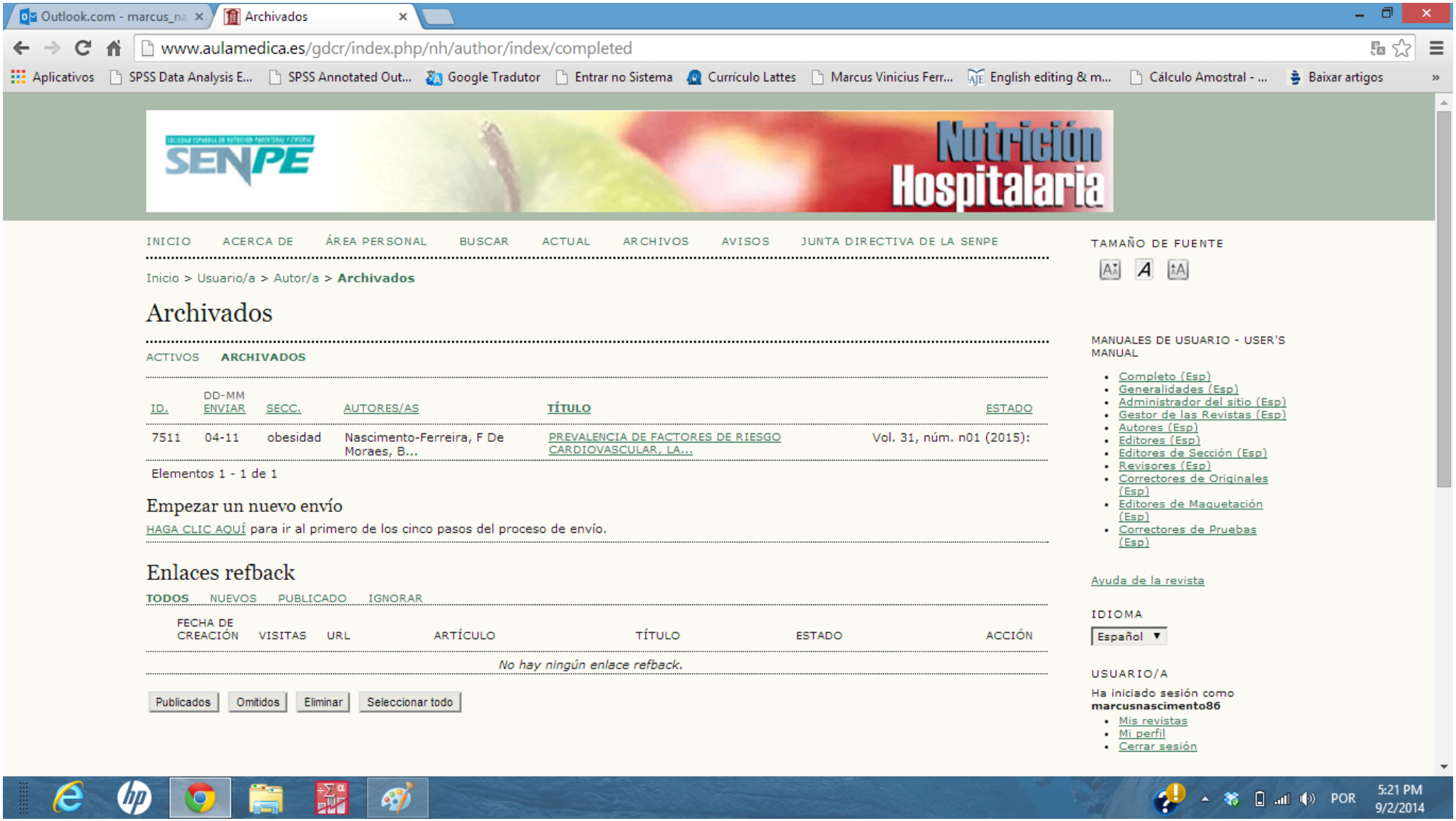
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