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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

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found. In boys, retinol was positively associated with DBP (β = 3.84; 95% CI, 0.51–7.17) and vitamin B_6 was positively associated with SBP (β = 3.82; 95% CI, 1.46–6.18). In contrast, holotranscobalamin was inversely associated with SBP (β = −3.74; 95% CI, −7.28 to −0.21).
Conclusions: Levels of RBC folate and vitamin B_6 in blood may affect BP in adolescents. In this context, programs aimed at avoiding high BP levels should promote healthy eating behavior by focusing on the promotion of vegetable proteins and foods rich in vitamin B_{12} (i.e., white meat and eggs), which may help to achieve BP blood control in adolescents.

Introduction

High blood pressure (BP) 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 (CVD) in adolescents [2] and studies have shown that BP levels in childhood and adolescence are mediators of developing hypertension in adulthood [3].

A recent study [4] reported that dietary phosphorus, magnesium, iron, thiamin, folacin, and riboflavin were inversely associated with systolic BP (SBP) in adults. Additionally, dietary folic acid and riboflavin were negatively associated with diastolic BP (DBP). Also in adolescents, nutritional biomarkers are associated with health, and studies have demonstrated low serum biomarkers concentrations during adolescence (i.e., iron status, retinol, and vitamins B_6, B_{12}, C, and D). These biomarkers present anti-inflammatory and antioxidant effects, and their low serum concentrations maybe an important factor in the early stages of the pathogenesis of many chronic diseases [5].

Most studies analyzing the association of serum biomarkers 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 past decades [8].

We hypothesized that low levels of vitamins and low levels of iron biomarkers were both related to higher levels of BP 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 obtained data from a sample of European adolescents on a broad battery of nutritional and health-related parameters. Data collection took place in 2006 and 2007 in 10 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 or 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 to 17.5 y. All of the analyses conducted on the HELENA data are adjusted for a weighting factor to balance the sample according to the theoretical age distribution foreseen. In all, 3528 adolescents (1845 girls), were considered eligible for the HELENA analyses. The HELENA protocol established that blood samples were obtained randomly from one-third of the population sample. To make maximum use of the data, all valid data on BP levels were included in here. Consequently, sample sizes vary for the different BP levels and biomarkers. In all, 1089 adolescents (ages 12.5–17.5 y old; 580 girls) from the HELENA study met the inclusion criteria and were included in the analyses. Distribution per city was: Stockholm, 94; Dortmund, 117; Ghent, 119; Lille, 82; Vienna, 128; Pecs, 136; Rome, 115; Zaragoza, 110; Athens, 92; and Heraklion, 96. We performed sensitivity analyzes in the sample by comparing levels of BP and all confounder variables among the 1089 adolescents who had blood sample data, and the 2439 who did not.

Sensitivity analyses were performed to compare BP 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, therefore, avoiding selection bias.

Outcome

Blood pressure

Blood pressure measurements were performed following the recommendations for adolescent populations. SBP and DBP were measured with the use of the arm blood pressure oscillometric monitor device OMRON® model HEM 7001, which has been approved by the European Hypertension Society [10]. These data collection procedures were described previously [11]. Briefly, participants were seated in a separate, quiet room for 10 min with their backs supported and feet on the ground. Interobserver coefficients of variation were 2.1% and 3.6% for SBP and DBP, respectively. Two BP readings were taken with a 10-min interval between; the lowest reading was recorded, according European Hypertension Society [10].

Independent variables

The blood sampling procedure and sample logistics were described in detail elsewhere [12]. Briefly, fasting blood samples were collected by venipuncture at school between 0800 and 1000 after a 10-h overnight fast. Samples for the different analyses were manipulated in situ, as described here, 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 as previously described [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 enzyme-linked immunosorbent assay (ELISA) [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 (hemoglobin, red blood cell [RBC], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC] 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 (RP-HPLC) using ultraviolet detection (Sykam Gilching Germany) in serum. The vacutainer was centrifuged for 15 min at 3500g at 4°C. Standards (β-carotene, retinol, α-tocopherol), hexane, and isopropanol were obtained from Sigma Aldrich (Germany) and all were 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, heparin tubes were put immediately on ice and centrifuged within 30 min (3500g 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°C to 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 ultraviolet detection (Sykam, Gilching, Germany). The CV of the method was 1.7%.s.

Vitamin B_6, B_{12} (cobalamin and holotranscobalamin), homocysteine, and folate plasma and 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′-