#### **ORIGINAL CONTRIBUTION**



## Relative validity of the Planetary Health Diet Index by comparison with usual nutrient intakes, plasma food consumption biomarkers, and adherence to the Mediterranean diet among European adolescents: the HELENA study

Leandro Teixeira Cacau<sup>1,2,3</sup> · Giles T. Hanley-Cook<sup>4</sup> · Inge Huybrechts<sup>5</sup> · Stefaan De Henauw<sup>3</sup> · Mathilde Kersting<sup>6</sup> · Marcela Gonzalez-Gross<sup>7,8</sup> · Frederic Gottrand<sup>9</sup> · Marika Ferrari<sup>10</sup> · Esther Nova<sup>11</sup> · Manuel J. Castillo<sup>12</sup> · Dénes Molnár<sup>13</sup> · Kurt Widhalm<sup>14</sup> · Odysseas Androutsos<sup>15,16</sup> · Yannis Manios<sup>15,17</sup> · Peter Stehle<sup>18</sup> · Carl Lachat<sup>4</sup> · Dirce Maria Marchioni<sup>2</sup> · Luis A. Moreno<sup>1,7,19,20</sup>

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#### Abstract

**Purpose** The EAT-*Lancet* Commission proposed an evidence-based global reference diet to improve human health within planetary boundaries. Recently, the Planetary Health Diet Index (PHDI) was developed based on the EAT-*Lancet* recommendations and validated among Brazilian adults. However, the relative validity of the PHDI in adolescents has yet to be assessed. Thus, we aimed to evaluate the relative validity of the PHDI in European adolescents.

**Methods** We used cross-sectional data from 1804 adolescents (12.5-17.5 years) enrolled in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study. The PHDI (0-150 points) was calculated based on dietary intake data from two non-consecutive 24-h dietary recalls. Associations between the PHDI and usual nutrient intakes, plasma food consumption biomarkers, and adherence to the Mediterranean diet were evaluated using multivariable-adjusted mixed-effects linear regression models.

**Results** Higher PHDI score was associated with greater intakes of nutrients predominantly from plant-source foods, such as vegetable protein, vitamin E, and folate and with lower intake of nutrients predominately from animal-source foods, such as total and saturated fat, cholesterol, and animal protein. Furthermore, a higher PHDI score was also positively associated with plasma  $\beta$ -carotene, vitamin C, vitamin D, folate, and ferritin concentrations, while negatively associated with transfatty acids concentration. Moreover, higher PHDI was related to a greater adherence to the Mediterranean dietary pattern. **Conclusions** The PHDI showed good relative validity among adolescents in the HELENA study. Hence, future research should assess adherence to the PHDI and long-term health outcomes.

Keywords EAT-Lancet diet · Sustainable diets · Diet quality · Relative validity · Biomarkers · Usual intake

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Leandro Teixeira Cacau lcacau@usp.br

Extended author information available on the last page of the article

#### Introduction

Global food systems are major contributors to adverse environmental impacts [1, 2] and poor-quality diets are the primary driver of morbidity and mortality worldwide [3]. Transitioning from contemporary diets to energy balanced, health supporting, and sustainable diets has been increasingly recognized as an important pathway to reduce food-system related mortality rates [4, 5], greenhouse gas emissions (GHGE), water use, and land use [5–7]. According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) [8], sustainable healthy diets have to fulfill four criteria: (i) promote all dimensions of individuals' health; (ii) have low environmental pressure and impact; (iii) are accessible, affordable, safe, and equitable; and iv) are culturally acceptable worldwide.

In 2019, the EAT–*Lancet* Commission on "Healthy Diets from Sustainable Food Systems" released a scientific report that proposed a balanced sustainable reference diet to promote human health within planetary boundaries. This global reference diet predominantly consists of vegetables, fruits, whole grains, legumes, nuts, and unsaturated oils, includes only a low to moderate amount of seafood and poultry, and no or a low quantity of red meat, animal fats, added sugar, refined grains, and starchy vegetables [2]. Willett and colleagues stated that the EAT–*Lancet* diet is nutritionally adequate and thus suitable for healthy adults and children older than 2 years [2].

At present, the EAT-*Lancet* diet is widely debated in the scientific community [9, 10], but it has nonetheless been used as a reference diet to build specific dietary recommendations in the context of local food cultures [11–13] and been compared against the recommendations for a healthy diet from the Dietary Guidelines for Americans [14]. Moreover, the global adoption of this reference diet was estimated to reduce global deaths by 11.6 million annually (i.e., 23.6% deaths per year) and decrease GHGE by 42%, freshwater use by 10%, and nitrogen application by 15% [2, 7]. However, the EAT-*Lancet* diet is affordable only in predominantly high-income countries, which represents a key constraint to adherence in low- and middle-income countries [15, 16].

Monitoring and evaluating adherence to the EAT-Lancet dietary recommendations might allow more accurate and robust evidence on the relationships with human health and environmental sustainability outcomes. Dietary quality indices are useful for this purpose, as they are tools to assess nutrient adequacy, appraise geographic differences and temporal changes in diets, and evaluate adherence to dietary guidelines [17–19]. Recently, the Planetary Health Diet Index (PHDI) was proposed to assess adherence to the reference diet proposed by the EAT-Lancet Commission for healthy and sustainable diets and uses the dietary targets in its cutoff points to ensure diet quality and environmental sustainability. Cutoff points are based on energy-adjusted values and are given a continuous scoring system, which allows greater discrimination between different levels of adherence to recommendations. The PHDI has five components to encourage intake (i.e. nuts and peanuts, legumes, whole grains, fruits and vegetables), five optimal components to account for foods that should be consumed within a specific range (i.e. eggs, fish, and fruits seafood, tubers and potatoes, dairy and vegetable oils), two components to promote plant variety intake (i.e. proportion of dark green vegetables and proportion of red and orange vegetables)

and four components to moderate (i.e. red meats, chicken and substitutes, animal fats, and added sugars), totaling 16 components and a score that can vary from 0 to 150 points [20, 21]. Previous studies have shown that higher PHDI was related to lower GHGE [20], greater overall diet quality [20], lower cardiometabolic risk profile [22], and lower odds for overweight and obesity among Brazilian adults [21, 23].

Although adherence to the PHDI was evaluated in a subgroup of adolescents participating in a national, populationbased study in Brazil [24], the index's relative validity has yet to be assessed among adolescents. Therefore, this study aimed to investigate the relative validity of the PHDI by comparison with usual nutrient intakes, plasma food consumption biomarkers, and adherence to the Mediterranean diet among European adolescents enrolled in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study.

#### **Methods**

#### Study design

The present study analyzed data from the HELENA study. In brief, HELENA was a multicenter cross-sectional study conducted among adolescents from ten European cities: (i) Athens, Greece, (ii) Dortmund, Germany, (iii) Ghent, Belgium, (iv) Heraklion, Crete, (v) Lille, France, (vi) Pécs, Hungary, vii) Rome, Italy, viii) Stockholm, Sweden, (ix) Vienna, Austria, (x) Zaragoza, Spain. The sample size was calculated using the mean body mass index (kg/m<sup>2</sup>) and variance values for each sex and age-specific strata, a 5% significance level ( $\alpha$ ), and an error of ± 0.3. Consequently, at least 300 adolescents from each country were required (300 for each country\*10 countries = 3000 participants). Data collection was carried out between 2006 and 2007. Further details on the sample, objectives, and data collection methods used in the HELENA study were previously published [25, 26].

#### Ethics

All participants and their parents gave written informed consent, and the study protocol was approved by the ethics committee of each city involved according to the Declaration of Helsinki 1964 (revision of Edinburgh 2000) and the International Conference of Harmonization for Good Clinical Practice [27].

#### **Study population**

Exclusion criteria in the HELENA study were as follows: age < 12.5 or > 17.5 years, no measurement of weight and/or height, completion of less than 75% of the tests, participating

simultaneously in a clinical trial, or an acute infection during the week prior to the examination [26]. In total, 3528 adolescents were recruited for participation in the HELENA study. Participants from Heraklion (Crete) and Pécs (Hungary) were excluded for the current analyses, as no nutrient intake data were obtained from these two cities due to logistical problems.

Thus, in the present study, only adolescents who provided data from two non-consecutive 24 h dietary recalls (24H-DRs) were included, resulting in 2330 participants. However, adolescents who were under-reporters were excluded for our analyses, as previous research using HELENA data reported that the validity of food and nutrient intakes as compared to the "true" intake (i.e., using plasma food consumption biomarkers and the Triad method) were superior when excluding under-reporters from the analyses [28]. Under-reporters were identified using the Goldberg method [29] and were considered when the ratio of energy intake over the estimated basal metabolic rate was lower than 0.96 [30]. After excluding under-reporters, the final sample size, composed by the plausible reporters, was 1804 adolescents (52.6% females). The group of under-reporters had higher PHDI compared to plausible reporters (46.1 vs 44.3; P = 0.008), consisted of a significantly higher percentage of females (57.8% vs 52.6%; P = 0.036), but with no differences in age (P=0.100), as reported in previous HELENA studies [31, 32].

Blood samples were collected in a randomly selected subset of the HELENA study population (*n* 1089), of whom 697 provided two 24H-DRs. However, the exclusion of under-reporters led to a final sample size of 552 adolescents (52.3% females) with biomarker concentration data. Characteristics of adolescents for whom no biochemical parameters were collected were compared to those included in the sub-sample analyses. No significant differences in PHDI score (P = 0.662), age (P = 0.310), and sex (P = 0.144) were observed between these two groups.

#### **Dietary assessment**

Dietary intake data were obtained using the HELENA Dietary Assessment Tool (HELENA-DIAT), a selfadministered computer-assisted 24H-DR [33]. In brief, the HELENA-DIAT was based on the Young Adolescents Nutrition Assessment (YANA-C) software, previously validated among Flemish adolescents [34]. The HELENA-DIAT was improved and culturally adapted for use among European adolescents, by including national dishes to capture country-specific dietary patterns. The HELENA-DIAT has been used and is recommended to assess dietary intake in European children and adolescents [35]. Participants were invited to complete two 24H-DR using the HELENA-DIAT on non-consecutive days in a period of two weeks, during school hours. To support the adolescents in case they required any clarifications to complete the HELENA-DIAT, a trained dietitian was present.

To obtain energy and nutrient intakes, the German Food Code and Nutrient Database (Bundeslebensmittelschlüssel, vII.3.1, Karlsruhe, Germany) was used. This food composition database contained the largest number of nutrients and food items. To illustrate, approximately 12,000 coded foods, menus, and menu components with up to 158 nutrients are available for each product [36]. The Multiple Source Method (MSM) was used to estimate the usual nutrient intakes by removing intra-person variance. MSM is a statistical program available open access online: https://msm.dife.de. First an individual's nutrient intakes were computed and based on these data, a population consumption distribution was modeled [37, 38].

#### Planetary health diet index

To assess adherence to the EAT-*Lancet* dietary recommendations, we used the PHDI. In brief, the PHDI considers all EAT-*Lancet* food groups and has a gradual scoring system, i.e., the components are scored according to the quantity of consumption [20, 21]. The scores are computed as a caloric intake ratio, i.e., for any given PHDI component, the caloric intake ratio was defined as the sum of calories from all foods classified in that component divided by the total calories from all PHDI foods. The total daily energy intake for the calculation of the PHDI components considered only the food groups recommended by the EAT-Lancet (i.e., it does not include alcohol consumption). In this case, we name it "PHDI total daily energy", to differentiate it from the total daily energy intake, which considers all foods and beverages consumed.

Briefly, the PHDI has 16 components divided into four categories: (i) adequacy components (nuts and peanuts, fruits, legumes, vegetables, and whole grain cereals), (ii) optimum components (eggs, dairy products, fish and seafood, tubers and potatoes, and vegetable oils), (iii) ratio components (dark green vegetables/total vegetables and red–orange vegetables/total vegetables), and (iv) moderation components (red meat, chickens and substitutes, animal fats, and added sugars). The adequacy, optimum, and moderation components are scored from 0 to 10 points, while the ratio components are scored from 0 to 5 points. The total score ranges from 0 to 150 points, and higher scores indicate greater adherence to the EAT-*Lancet* reference diet.

Further detail on the PHDI development, scoring criteria, cutoff points, and validity and reliability can be found elsewhere [20].

#### Mediterranean diet score

Adherence to the Mediterranean diet was used as a proxy of diet quality, since this dietary pattern has been extensively researched with regard to its positive effects on human health [39, 40], besides also being widely considered as a sustainable diet [41, 42]. The Mediterranean Diet Score (MDS), which has been described elsewhere, was computed as the sum of nine components, including both foods and nutrients [43]. To summarize, vegetables, fruits and nuts, cereals and tubers, legumes, fish, dairy products, and unsaturated to saturated fat ratio were considered healthy food subgroups and were scored 1 point when the intakes were above the sexspecific median and 0 when the intakes were below the cutoff, whereas meat products (including processed meat) and alcohol consumption were classified as unhealthy foods and scored inversely. Therefore, the range of the MDS was from 0 to 9 points. Thereafter, the adherence was categorized as low adherence ( $\leq 4$  points) and high adherence ( $\geq 5$ ) points.

#### Socioeconomic status

Socioeconomic status (SES) was examined by parental education and the Family Affluence Scale (FAS) [44], using a questionnaire filled in by the adolescents. Parental education level of mother and father were categorized into three groups using the International Standard Classification of Education (ISCED): lower education (primary education or lower secondary education); higher secondary education; and higher education or university degree [45]. A modified version of the FAS [44] was used as a proxy indicator of SES. The FAS considers parameters such as car ownership, having an own bedroom, internet availability, and computer ownership. Adolescents were scored from 0 (lowest) to 8 (highest) and further re-categorized into low FAS score (0–4) and high FAS score (5–8) [45].

# Blood samples and plasma food consumption biomarkers

Blood samples were collected in the morning after an overnight fasting period by venipuncture at school, following a standardized blood collection protocol. Details on the sample processing and transportation have been previously published [46]. For the present study, nutritional biomarkers and biomarkers with important roles in nutrient metabolism were selected as objective indicators of dietary intake [32, 47].

Plasma 25-hydroxyvitamin D was analyzed by enzymelinked immuno-sorbent assay (ELISA) using a kit (OCTEIA 25-Hydroxy Vitamin D) from Immunodiagnostic Systems (Frankfurt, Germany) and measured with a Sunrise<sup>™</sup> Photometer by TECAN (Hamburg, Germany). The sensitivity of this method is 5 nmol/L for 25-hydroxyvitamin D and the variation is below 6%. Plasma cobalamin and folate were measured in heparin plasma by means of a competitive immunoassay using the Immunolite 2000 analyser (DPC BiermannGmbH, Bad Nauheim, Germany). Plasma retinol,  $\alpha$ -tocopherol, vitamin C, and  $\beta$ -carotene were analyzed using reversed-phase HPLC (Sykam Gilching) using UV detection (UV-Vis 205, Merck). Serum phospholipid fatty acid composition was measured by capillary GC (model 3900; Varian GmbH, Darmstadt, Germany) after extraction performed by thin-layer chromatography. Serum triacylglycerols were determined enzymatically on the Dimension RxLclinical chemistry system (Dade Behring, Schwalbach, Germany) using the manufacturer's reagents and instructions. All these parameters were analyzed at the University of Bonn (Germany). Holo-transcobalamin was measured at the Biochemistry Lab at UPM (Madrid, Spain) by microparticle enzyme immunoassay (Active B12Axis-Shield Limited) with the use of AxSym (Abbott Diagnostics, S.A.). Serum ferritin was measured using an in-house sandwich ELISA at the Human Nutrition Laboratory of the National Research Institute on Food and Nutrition (INRAN, Rome, Italy).

#### Data analyses

Data management and analyses were performed using Stata® version 14.2 (Statistical Software for Professionals, College Station, Texas, USA). All statistical tests were two-sided, and P values < 0.05 were considered statistically significant.

The nutrient residual (energy-adjusted) method was used to adjust the usual nutrient intake for total energy intake, since nutrient consumption is associated with total energy intake either because they contribute directly to energy intake or because individuals who consume more total energy also eat, on average, more of all specific nutrients [48]. After this procedure, the normality was evaluated visually (histograms) and based on the skewness of the data distributions. Vitamin A and cobalamin intake and plasma holo-transcobalamin were log-transformed to achieve a more normal distribution.

Descriptive statistics were expressed as means and 95% confidence intervals (95% CI) for continuous variables, whereas categorical variables were expressed as frequencies and percentages (%). PHDI scores were compared across sex (boys and girls), age groups (12.5–13.9, 14–14.9, 15–15.9, and 16–17.5 years), FAS score (low: 0–4 points; high: 5–8 points), and parental education level (both low and high). Student's *t*-test and ANOVA were used to test differences in the PHDI across subgroups.

To assess the associations between the PHDI and usual energy (kcal) and energy-adjusted nutrient intakes, we fitted mixed-effects linear regression models with a random intercept for study center, adjusted for sex (male *vs* female) and age (years) (i.e., potential confounders).

The same model was fitted to assess the associations between the PHDI and plasma food consumption biomarkers, with adjustments for sex, age and total daily energy intake. In addition, we used an identical model to evaluate the relationship between the PHDI and the MDS. For this analysis, the PHDI and the MDS were standardized (i.e., z-scores), as crude scores have distinct scoring ranges (i.e., 0-150 vs 0-9 points, respectively).

#### Results

The PHDI score was normally distributed in the HELENA study (Fig. 1) and the average score was 44.3 (95% CI 43.7–44.9) points, which represents 29.5% of the maximum possible total score (Table 1). At the components level, the highest average scores were observed for fruits and animal fats, followed by whole cereals, and vegetables. In contrast, the lowest scores were observed for red meat, eggs, fish and seafood, nuts and peanuts, and tubers (Fig. 2).

On average, younger adolescents (12.5–13.9 years old) had higher PHDI scores than older adolescents (16–17.5 years old). Furthermore, adolescents whose parents had a higher education level had higher PHDI scores as compared to those whose parents had lower education level. Adolescents from Mediterranean countries and those with higher adherence to the Mediterranean diet score had higher PHDI scores when compared to those from non-Mediterranean countries and with lower adherence to the Mediterranean diet. No differences were observed over sex and FAS categories (Table 1).

Table S1 presents the usual dietary intake of key micronutrients among adolescents in HELENA. On average,



Fig. 1 Distribution of the Planetary Health Diet Index (PHDI) among adolescents (n 1804) in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study

 Table 1
 Planetary Health Diet Index (PHDI) among subgroups of adolescents in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study (n 1804)

	n	%	PHDI		
			Mean	95%	CI
Total	1804	100	44.8	44.2	45.4
Age (years)					
12.5–13.9 years	587	32.5	45.1	44.0	46.2
14-14.9 years	445	24.7	44.9	43.7	46.2
15–15.9 years	421	23.3	44.0	42.6	45.4
16-17.5 years	351	19.5	42.5	41.0	43.9
Sex					
Male	855	47.4	43.7	42.8	44.7
Female	949	52.6	44.8	43.9	45.7
FAS					
Low FAS score (0-4 points)	782	43.5	44.5	43.6	45.5
High FAS score (5–8 points)	1015	56.5	44.2	43.3	45.0
Maternal education level					
Lower (secondary) education	551	32.0	42.6	41.5	43.8
Higher secondary education	540	31.3	43.0	41.9	44.1
Higher education or university degree	633	36.7	46.7	45.6	47.8
Paternal education level					
Lower (secondary) education	576	34.4	42.5	41.4	43.5
Higher secondary education	468	28.0	43.7	42.4	45.0
Higher education or university degree	630	37.6	46.3	45.2	47.4
European region					
Mediterranean countries	607	33.6	46.4	45.3	47.7
Non-Mediterranean countries	1197	66.4	43.2	42.4	44.0
Mediterranean Diet Score					
Low $(\leq 4)$	936	51.9	41.2	40.3	42.0
High $(\geq 5)$	868	48.1	47.7	46.7	48.6

FAS Family Affluence Scale

micronutrient intakes were higher than the Institute of Medicine's age and sex-specific Estimated Average Requirements (EARs) [49–51]. Only mean calcium, vitamin D, and folate intakes were lower than their respective EARs.

A one-point increment in the PHDI score was associated with a lower total energy intake (kcal/d) ( $\beta = -2.834$ ; 95% CI - 4.701, - 0.967; P = 0.003). For macronutrients, a onepoint increase in the PHDI score was associated with a small but significantly decrease in total protein (g/d) ( $\beta = -0.067$ ; 95% CI - 0.124, - 0.011; P = 0.018), animal protein (g/d) ( $\beta = -0.167$ ; 95% CI - 0.225, - 0.109; P < 0.001), total fat (g/d) ( $\beta = -0.097$ ; 95% CI - 0.145, - 0.048; P < 0.001), saturated fat (mg/d) ( $\beta = -61.557$ ; 95% CI - 86.428, - 36.747; P < 0.001), polyunsaturated fat (mg/d) ( $\beta = -14.376$ ; 95% CI - 26.852, - 1.899; P = 0.024), and cholesterol intakes (mg/d) ( $\beta = -1.101$ ; 95% CI - 1.432, - 0.770; P < 0.001). In contrast, a one-point increase in the PHDI score was



**Fig. 2** Average scores of the 16 Planetary Health Diet Index (PHDI) components among adolescents (n 1804) in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study. Note that ratio

components are scored from 0 to 5 points. DGV, dark green vegetables; ReV, red-orange vegetables

positively associated with carbohydrates (g/d) ( $\beta$ =0.301; 95% CI 0.168, 0.435; *P*<0.001), fiber (g/d) ( $\beta$ =0.106; 95% CI 0.09, 0.122; *P*<0.001), and vegetable protein intakes (g/d) ( $\beta$ =0.095; 95% CI 0.067, 0.123; *P*<0.001) (Table 2).

A unit increase in the PHDI score was significantly associated with greater usual intakes of most vitamins evaluated (P < 0.05), except for niacin ( $\mu$ g/d) which decreased substantially ( $\beta = -20.091$ ; 95% CI - 34.491, - 5.690; P = 0.006). In addition, no statistical associations were observed for usual cobalamin, retinol, and vitamin D intakes. Furthermore, higher PHDI scores were also positively associated with the usual intakes of all minerals assessed, except for heme iron ( $\mu$ g/d), which slightly decreased ( $\beta$  = - 6.121; 95% CI: - 8.618, - 3.624; *P* < 0.001) (Table 2).

Table 3 indicates the positive associations between higher PHDI scores and the following plasma food consumption biomarkers (all P < 0.05):  $\beta$ -carotene (ng/mL), vitamin C (mg/L), vitamin D (nmol/L), plasma folate (nmol/L), ferritin (mcg/L). In contrast, a negative association was found with trans-fatty acids (mol/L) ( $\beta = -2.613$ ; **Table 2** Associations between the Planetary Health Diet Index(PHDI) and the residual energy-adjusted usual intakes of macro- andmicronutrients among adolescents in the Healthy Lifestyle in Europeby Nutrition in Adolescence (HELENA) study (n 1804)

	PHDI (n 1804)			
	β	95% CI		P value
Energy (kcal/d)	- 2.834	- 4.701	- 0.967	0.003
Macronutrients				
Carbohydrates (g/d)	0.301	0.168	0.435	< 0.001
Fiber (g/d)	0.106	0.09	0.122	< 0.001
Protein (g/d)	-0.067	-0.124	-0.011	0.018
Animal protein (g/d)	- 0.167	-0.225	- 0.109	< 0.001
Vegetable protein (g/d)	0.095	0.067	0.123	< 0.001
Total fat (g/d)	- 0.097	- 0.145	-0.048	< 0.001
Saturated fat (mg/d)	- 61.557	- 86.428	- 36.747	< 0.001
Monounsaturated fat (mg/d)	- 15.643	- 35.979	4.693	0.132
Polyunsaturated fat (mg/d)	- 14.376	-26.852	- 1.899	0.024
Cholesterol (mg/d)	- 1.101	- 1.432	-0.770	< 0.001
Vitamins				
Thiamine (µg/d)	2.062	1.066	3.058	< 0.001
Riboflavin (µg/d)	3.443	2.107	4.778	< 0.001
Niacin (µg/d)	- 20.091	- 34.491	- 5.690	0.006
Folate (µg/d)	1.058	0.896	1.221	< 0.001
Cobalamin (µg/d)	0.001	-0.008	0.007	0.839
Vitamin C (mg/d)	0.759	0.568	0.951	< 0.001
Beta-carotene ( $\mu$ g/d)	24.061	20.138	27.983	< 0.001
Retinol ( $\mu$ g/d)	- 0.002	- 0.003	- 0.001	0.010
Vitamin E ( $\mu$ g/d)	24.868	17.251	32.485	< 0.001
Vitamin D (µg/d)	-0.001	- 0.003	0.003	0.835
Minerals				
Potassium (mg/d)	9.112	7.169	11.054	< 0.001
Calcium (mg/d)	2.827	1.838	3.815	< 0.001
Magnesium (mg/d)	0.957	0.756	1.158	< 0.001
Heme iron $(\mu g/d)$	- 6.121	- 8.618	- 3.624	< 0.001
Non-heme iron ( $\mu$ g/d)	33.233	25.168	41.298	< 0.001
Copper ( $\mu$ g/d)	2.920	1.479	4.362	< 0.001
Manganese ( $\mu$ g/d)	13.963	9.873	18.054	< 0.001
Zinc ( $\mu$ g/d)	10.879	3.890	17.868	0.002
Phosphorus (mg/d)	1.610	0.773	2.448	< 0.001
Iodine ( $\mu$ g/d)	0.224	0.121	0.327	< 0.001

Mixed effects linear regression models with a random intercept for the study center, and adjusted for sex and age (years)

95% CI – 3.257, – 1.970; P = 0.009), while non-significant association were observed for retinol (ng/mL), tocopherol (pmol/L), holo-transcobalamin (pmol/L), and n-3 fatty acids (mol/L).

Lastly, a standard deviation increment in the PHDI score was associated with an increase in the standardized Mediterranean diet score (*z*-score) ( $\beta$ =0.298; 95% CI 0.244: 0.332; *P*<0.001) (Table 4).

**Table 3**Associations between the Planetary Health Diet Index(PHDI) and plasma concentrations of food consumption biomarkersamong adolescents in the Healthy Lifestyle in Europe by Nutrition inAdolescence (HELENA) study (n 543)

	PHDI (n 543)			
	β	95% CI		P value
Retinol (ng/mL)	- 0.611	- 1.258	0.036	0.064
$\beta$ -carotene (ng/mL)	2.094	0.954	3.234	< 0.001
Vitamin C (mg/L)	0.021	0.001	0.041	0.036
Vitamin D (nmol/L)	0.175	0.024	0.325	0.023
Tocopherol (µg/mL)	- 0.004	- 0.017	0.009	0.565
Plasma folate (nmol/L)	0.076	0.013	0.139	0.018
Holo-transcobalamin (pmol/L)	0.002	- 0.000	0.005	0.086
Ferritin (µg/L)	0.049	0.003	0.095	0.036
n-3 fatty acids (mol/L)	0.132	- 0.165	0.430	0.383
Trans-fatty acids (mol/L)	- 2.613	- 3.257	- 1.970	0.009

Mixed effects linear regression models with a random intercept for the study center, and adjusted for sex, age (years), and total energy intake (kcal/d)

**Table 4**Associations between the Planetary Health Diet Index(PHDI) and the Mediterranean Diet Score (MDS) among adolescents in the Healthy Lifestyle in Europe by Nutrition in Adolescence(HELENA) study (n 1804)

	PHDI (z-score) (n 1804)					
MDS (z-score)	β	95% CI		P value		
Model crude	0.254	0.215	0.293	< 0.001		
Model adjusted	0.298	0.244	0.332	< 0.001		

Mixed effects linear regression models with a random intercept for the study center, and adjusted for sex, age (years), and total energy intake (kcal/d)

#### Discussion

In this observational study among European adolescents, we evaluated the relative validity of the PHDI, an index constructed to measure the adherence to the healthy and sustainable reference diet proposed by the EAT-*Lancet* Commission. In accordance with a priori hypotheses, our findings indicate that the PHDI was positively associated with usual nutrient intake and plasma food consumption biomarkers from predominately plant-source foods and adherence to the Mediterranean diet, while negatively associated with nutrients predominately from animalsource foods.

The average score achieved among participants was only 29.5% of the maximum possible total score, indicating that study participants were far from achieving the EAT-*Lancet* recommendations. Similarly, in a national population-based study in Brazil carried out between 2017 and 2018, the mean PHDI among participants < 19 years was only 29.1% of the maximum possible total score. In a cohort study in Brazil with adults and older adults, the average PHDI score was 60.4 points, representing about 40% of the total score [20].

Our study showed that a one-point increase in the PHDI was associated with a decrease in the usual intake of total energy and the energy-adjusted usual intake of total and animal source protein, total fat, saturated and polyunsaturated fats, and cholesterol, and with the vitamin niacin. The directions of the associations with usual nutrient intakes were expected, and analogous to a cross-sectional study conducted in Brazil [20], as adolescents consuming excessive quantities of animal source foods receive zero point for the respective PHDI components [2, 20].

In contrast with our results, Vyncke et al. [32] evaluated diet quality in HELENA using the Diet Quality Index for Adolescents (DQI-A) and reported positive associations for almost all nutrients evaluated (including those from predominantly animal foods), with the exception of total energy and carbohydrates, which were both negatively associated with the DOI-A. In another HELENA study evaluation, Aparicio-Ugarriza et al. [47] evaluated adherence to the Mediterranean diet using an adapted Mediterranean Diet Score for Adolescents (MDS-A) and the Mediterranean Diet Quality Index for children and adolescents (KIDMED) and also found positive associations for both diet indices with all nutrients evaluated. These findings may be due to the fact that these studies used diet quality indices based on dietary recommendations for adolescents who fail to consider planetary health [32, 47], while the PHDI was based on the EAT-*Lancet* reference diet for individuals  $\geq 2$  years, which considered human health within planetary boundaries [2]. In alignment with our results, Monge-Rojas et al. [52] proposed a diet quality index for adolescents in Costa Rica and observed negative relationships with total fat, monounsaturated, polyunsaturated, and saturated fats, while fiber and certain micronutrients were positively associated with the score. Comparably, Wong et al. [53] found that adolescents with higher diet quality evaluated using the New Zealand Diet Quality Index for Adolescents (NZDQI-A) had lower intakes of total, saturated, monounsaturated, and polyunsaturated fats, and higher intakes of vitamin C and iron.

When comparing, the PHDI score in our study with plasma food consumption biomarkers, it was whose to be positively associated with  $\beta$ -carotene, vitamin C, vitamin D, plasma folate, and ferritin, while negatively associated with trans-fatty acids. These results also corroborate the relative validity of the indicator, since these biomarkers were already described in the literature as biomarkers of food/nutrient intake and diet quality [54].

Food consumption biomarkers have also been recognized as useful tools to help describe dietary patterns, since they provide data that is complementary to self-reported dietary assessment methods [54]. Indeed, plasma carotenoids are correlated with fruit and vegetables intakes [54–56], while serum ascorbic acid is positively associated with dietary intake of vitamin C [54, 57]. Vitamin D concentrations was also related with dietary intake [58]. Plasma folate concentrations were previously described as biomarkers of folate intake, although with weak correlations, since many factors can influence their bioavailability [58]. Moreover, correlations between the trans-fatty acid intake and the serum trans-fatty acid levels were previously described [59, 60]. Likewise, to our findings of positive associations between the PHDI and  $\beta$ -carotene, vitamin C, vitamin D, folate, and ferritin, and negative associations with trans-fatty acids, Vyncke et al. [32] reported positive relationships between the DQI-A with vitamin D and holo-transcobalamin. Furthermore, Aparicio-Ugarriza et al. [47] indicated positive associations between the MDS-A and vitamin D, vitamin C, folate,  $\beta$ -carotene, and n-3 fatty acids, and between the KIDMED score and vitamin D, vitamin C, folate, holotranscobalamin, total homocysteine, and  $\beta$ -carotene. Moreover, both studies also found negative, but non-significant associations between their diet quality indices and trans-fatty acids. In addition, other studies have identified relationships between either the Healthy eating index (HEI) and the Diet Quality Index-Revised and serum  $\beta$ -carotene concentrations [61-63]. Weinsteinet al. [63] also reported a correlation between the HEI and serum vitamin C, folate, vitamin B12, and retinol, whereas Bach-Faig et al. [64] observed associations between higher adherence to the Mediterranean diet and greater serum  $\beta$ -carotene, vitamin C, and folate concentrations.

The PHDI score was also positively associated with better adherence to the MDS, which is a widely accepted model of a healthy sustainable dietary pattern [41, 42, 65]. The Mediterranean diet has certain similarities to the EAT-*Lancet* diet, such as the recommendations of a high consumption of plant-based foods and a low consumption of animal-source foods. However, the Mediterranean diet was advocated to help prevent cardiovascular disease and promote cardiovascular health, while the EAT-*Lancet* diet was developed to promote human and planetary health, with dietary recommendations also based on the environmental impacts of food production and consumption [2, 66].

Besides these aforementioned results, it is worth highlighting that this is the first study to evaluate the validity of a diet quality index built to evaluate the adherence to a healthy and sustainable diet proposed by the EAT-*Lancet* Commission. Since the EAT-*Lancet* report stated that this reference diet can be followed by children over 2 years, studies that assess the adherence to this diet may be important to provide specific dietary recommendations to children and adolescents, focusing in the sustainability of food consumption and production, with alignment of the FAO/WHO recommendations [8]. In our case, we observed the highest scores, but still only half of the possible points, for fruits, whole cereals, and vegetables, indicating a sub-optimal consumption of these food groups. On the other hand, eggs, fish, vegetable oils and red meat had the lowest scores, indicating excessive consumption of these food groups, according to the targets of the EAT-*Lancet* diet.

The PHDI was positively associated with non-heme iron and serum ferritin, while negatively associated with heme iron. This result was expected, since animal foods are a source of heme iron and the EAT-*Lancet* diet recommends no or low intake of red meat. Previous studies with the HELENA sample reported low prevalence of iron deficiency and great agreement with the EAR cutoff values, indicating that this population has an adequate consumption and status for iron, although one of these studies reported a prevalence of iron depletion of around 14% for boys and 21% for girls, indicating that attention should be paid to the prevalence of iron depletion found [67, 68].

PHDI does not correlate with B12 blood levels, hence care should be given to mitigate this. According to the EAT-Lancet Commission report, shifting to the reference diet improves intake of most micronutrients, including iron, zinc, calcium, folate, and vitamin A. However, a recent study reported that the EAT-Lancet diet may need some adaptations to improve the intake of iron, calcium, zinc and B12, due to the low amount of animal sources foods [69]. Another point raised was the presence of foods rich in phytates, which can reduce the absorption of micronutrients, including iron. The authors suggest changing the recommended values for some food groups (for example, increasing consumption of animal source foods) and including seeds, while slightly decreasing foods rich in phytates. They advocate a reference diet rich in minimally processed, nutrient-dense foods. However, that study considered only adults aged  $\geq 25$  years old [69]. Therefore, an assessment of the adaptation for adolescents may be necessary and further studies may adequately address this question, to ensure the nutritional adequacy of the EAT-Lancet diet and, subsequently, the adaptation of the PHDI for adolescents. For now, the EAT-Lancet Commission suggests that iron supplementation could be an alternative to increase iron intake without the consequences of high red meat intake, while B12 supplementation may be necessary, since B12 is low in plant-based diets [2], but this point must be deeply discussed and based on nutritional and scientific guidelines, to ensure the best alternative: whether to increase the intake of nutrient-rich foods (include minimally processed animal source foods) or use fortification and supplementation.

It is important to highlight that the PHDI is an index to measure adherence to the targets of the EAT-*Lancet* diet. Therefore, for instance, we have not made any adaptations to the original dietary recommendations. In our view, it is important first to see how the population (in our case, the adolescents in the HELENA study) adheres to this reference diet, to propose adaptations afterwards. Our results indicate that the PHDI had relative validity and reliability as a tool to measure adherence to the EAT-*Lancet* diet in this population. Further studies should evaluate the relationship between PHDI and health outcomes, environmental impacts and nutrient adequacy in adolescents, to ensure that the purpose of healthy and adequately sustainable diet is appropriate for all.

Moreover, as far as we know, to date, only one study compared the EAT-Lancet reference diet targets with the food consumption of children [70]. Bäck et al. [70] evaluated Finnish preschoolers aged between 3 and 6years-old and observed that the consumption of plant foods such as legumes, nuts, whole cereals, and vegetables were very low, while the consumption of red meat and dairy was about fivefold the recommended, tubers and starchy vegetables over two and half times higher and added sugar as well. In addition, Vallejo et al. [71] proposed a binary 18-component diet score and evaluated the adherence to the EAT-Lancet reference diet in adolescents ( $\geq 15$  years of age) from Dortmund. They observed that those with the highest scores (>11 points) have lower consumption of total protein, animal protein, added sugar, and cholesterol, while higher vegetables protein and total fiber intake.

Our study has several key strengths. We used a welldeveloped large and culturally diverse sample of European adolescents. Data collection followed standardized procedures and strict protocols in each country included in the HELENA study. Food consumption was assessed using the recommended HELENA-DIAT, an automated tool to collect 24H-DRs. In addition, we modeled usual intakes using MSM software, which removed the intra-person variance (i.e., day-to-day variability) of dietary intakes. Furthermore, we computed a dietary index that was previously associated with overall dietary quality and lower GHGE in a Brazilian population. Lastly, we evaluated the PHDI's relative validity by also comparing to plasma food consumption biomarkers, which are free of a social desirability bias, independent of memory lapse, and not based on a participant's ability to describe the type and quantity of food consumed, as more "objective" outcomes of dietary intake.

However, our study is also subject to limitations. As is well-known, diet is a complex variable and, in particular, dietary recalls are prone to measurement errors. Of note is that, at the time of data collection, sustainable diets and planetary health were little discussed topics, which might explain the poor adherence to the EAT-*Lancet* recommendations. One possible limitation of PHDI is that the cutoff points were constructed based on the original values recommended by the EAT-*Lancet* Commission without considering local contextual differences. However, this can also be viewed as a positive aspect since PHDI can be applied in diverse settings and studies to evaluate adherence to the original EAT-*Lancet* recommendations. Future studies may investigate nutrient adequacy and the PHDI score to better assess whether we need to adapt cutoffs for some food groups.

In conclusion, the PHDI is a valid index of overall diet quality among European adolescents, given the observed associations with usual nutrient intakes, plasma food consumption biomarkers, and the Mediterranean diet in the HELENA study. In conjunction with previous research on the PHDI, our findings warrant (prospective) analyses of the PHDI and health-related outcomes among adolescents.

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Author contributions The authors' contributions were as follows. LTC, DMM, and LAM designed the research; IH advised on the data curation; LTC carried out the data analyses; LTC developed the first draft and revised the manuscript; GTH-C supported with written the draft. DMM and LAM advised on statistical analyses. LAM supervised LTC. GTH-C, IH, SDH, MK, MG-G, FG, MF, EN, MJC, DM, KW, OA, YM, PS, CL, DMM, and LAM critically reviewed and revised the manuscript.

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**Data availability** The analytic code of the PHDI computation will be made available upon request pending to the corresponding author.

#### Declarations

**Conflict of interest** The authors declares that they have no conflict of interest.

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### **Authors and Affiliations**

Leandro Teixeira Cacau<sup>1,2,3</sup> · Giles T. Hanley-Cook<sup>4</sup> · Inge Huybrechts<sup>5</sup> · Stefaan De Henauw<sup>3</sup> · Mathilde Kersting<sup>6</sup> · Marcela Gonzalez-Gross<sup>7,8</sup> · Frederic Gottrand<sup>9</sup> · Marika Ferrari<sup>10</sup> · Esther Nova<sup>11</sup> · Manuel J. Castillo<sup>12</sup> · Dénes Molnár<sup>13</sup> · Kurt Widhalm<sup>14</sup> · Odysseas Androutsos<sup>15,16</sup> · Yannis Manios<sup>15,17</sup> · Peter Stehle<sup>18</sup> · Carl Lachat<sup>4</sup> · Dirce Maria Marchioni<sup>2</sup> · Luis A. Moreno<sup>1,7,19,20</sup>

- <sup>1</sup> Growth, Exercise, Nutrition and Development (GENUD) Research Group, Facultad de Ciencias de La Salud, Universidad de Zaragoza, Zaragoza, Spain
- <sup>2</sup> Department of Nutrition, School of Public Health, University of São Paulo, Av. Dr. Arnaldo, 715, Cerqueira César, São Paulo, SP 01246-904, Brazil
- <sup>3</sup> Department of Public Health and Primary Care, Ghent University, Ghent, Belgium
- <sup>4</sup> Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
- <sup>5</sup> Nutritional Epidemiology Group, Nutrition and Metabolism Section, International Agency for Research on Cancer, World Health Organization (IARC/WHO), Lyon, France

- <sup>6</sup> Research Department of Child Nutrition, University Hospital of Pediatrics and Adolescent Medicine, Ruhr-University Bochum, Bochum, Germany
- <sup>7</sup> Centro de Investigación Biomédica en Red de Fisiopatología de La Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
- <sup>8</sup> ImFINE Research Group, Department of Health and Human Performance, Universidad Politécnica de Madrid, Madrid, Spain
- <sup>9</sup> Institute for Translational Research in Inflammation (INFINITE), University Lille, Lille, France
- <sup>10</sup> Council for Agricultural Research and Economics, Research Center for Food and Nutrition, Rome, Italy

- <sup>11</sup> Department of Metabolism and Nutrition, Institute of Food Science and Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain
- <sup>12</sup> Department of Physiology, Faculty of Medicine, University of Granada, Granada, Spain
- <sup>13</sup> Department of Pediatrics, Medical School, University of Pécs, Pécs, Hungary
- <sup>14</sup> Department of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria
- <sup>15</sup> Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Athens, Greece

- <sup>16</sup> Department of Nutrition and Dietetics, University of Thessaly, Trikala, Greece
- <sup>17</sup> Institute of Agri-Food and Life Sciences, Hellenic Mediterranean University Research Centre, Heraklion, Greece
- <sup>18</sup> Department of Nutrition and Food Sciences, University of Bonn, Bonn, Germany
- <sup>19</sup> Instituto Agroalimentario de Aragon (IA2), Zaragoza, Spain
- <sup>20</sup> Instituto de Investigacion Sanitaria Aragon (IIS Aragon), Zaragoza, Spain