Flavonoid Intake From Cocoa-Based Products and Adiposity Parameters in Adolescents in Spain

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Background: Cocoa-based products are a good source of flavonoids, which may have beneficial effects on metabolic health.

Objective: The aim of this study is to assess the relationship between flavonoids from cocoa-based products and adiposity parameters in adolescents.

Methods: A cross-sectional study was conducted involving 944 adolescents aged 11–14 years enrolled in the SI! Program for Secondary Schools trial in Spain with available baseline data from food frequency questionnaires and anthropometric measurements [weight, height, waist circumference (WC), and fat mass percentage (%FM) by bioimpedance analysis]. Fat mass index (FMI) and waist-to-height ratio (WHtR) were obtained by dividing fat mass by height and WC by height, respectively. Body mass index (BMI), WC, and FMI for age and gender z-score were calculated. Overweight/obesity was defined as BMI ≥ 85th percentile and excess adiposity as %FM or FMI ≥ 75th percentile. WC ≥ 90th percentile and WHtR with a 0.5 threshold were considered as criteria of abdominal obesity. Multilevel mixed-effect regressions were used to evaluate the association between flavonoids from cocoa-based products and adiposity parameters. Municipalities and schools were considered random effects.

Results: Participants with a higher flavonoid intake from cocoa-based products had lower WC z-score [B = −0.04, 95% CI (−0.07; −0.01), P-for trend = 0.045] and WHtR [B = −0.01, 95% CI (−0.02; −0.01), P-for trend < 0.001]. They also had lower probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), P-for trend = 0.001]. Inverse associations were observed between flavonoids from cocoa powder and BMI z-score [B = −0.08, 95% CI (−0.12; −0.05), P < 0.001], WC z-score [B = −0.06, 95% CI (−0.11; −0.02), P = 0.003], WHtR [B = −0.01, 95% CI (−0.01; −0.00), P < 0.001],
Obesity, which is characterized by abnormal or excessive body fat accumulation, is a serious public health problem worldwide (1, 2). Excessive adiposity in children and adolescents leads to metabolic disorders such as vascular dysfunction and subclinical indicators of atherosclerosis, increasing the risk of cardiovascular disease and mortality in adulthood (2, 3).

Marked by physiological and emotional changes, adolescence is a critical period for managing obesity. Behavioral modifications, particularly fomenting physical activity and healthy dietary patterns are one of the best strategies used in primary health care settings to reduce obesity among adolescents (2, 4). A diet based on polyphenol-rich foods is of interest because of the antioxidant and anti-inflammatory effect and their influence on physiological and molecular pathways related to body weight maintenance (5–8). The positive impact of cocoa-based products on obesity has been attributed to their content of flavonoids (a large class of phenolic compounds), specifically flavanols (catechins and procyanidins) (9, 10). Systematic reviews and meta-analyses support the beneficial effect of cocoa flavonoids on cardiovascular risk factors since they are reported to favorably improve blood pressure, lipid profile, inflammation, and adiposity parameters (11–13). However, as most of the research in this field has been performed in adults, there is a need for studies on adolescents to establish dietary recommendations for the consumption of cocoa-based products in this target population, always within the framework of a healthy lifestyle. Therefore, this study aimed to investigate the association between flavonoid intake from cocoa-based products and adiposity parameters in a large sample of adolescents in Spain.

INTRODUCTION

Obesity, which is characterized by abnormal or excessive body fat accumulation, is a serious public health problem worldwide (1, 2). Excessive adiposity in children and adolescents leads to metabolic disorders such as vascular dysfunction and subclinical indicators of atherosclerosis, increasing the risk of cardiovascular disease and mortality in adulthood (2, 3).

Conclusions: A higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and a lower probability of presenting abdominal obesity.

Keywords: (poly)phenols, catechin, epicatechin, proanthocyanidins, cardiometabolic, obesity

MATERIALS AND METHODS

Study Population

The SI! (Salud Integral-Comprehensive Health) Program for Secondary Schools trial (NCT03504059) is a cluster-randomized controlled intervention trial conducted in adolescents from 24 secondary schools in Spain and conducted from 2017 to 2021. The main objective of this trial was to evaluate the effectiveness of an educational intervention to promote cardiovascular health at schools. A detailed description of the original study design and recruitment procedures has been previously published (14). Parents or caregivers provided assent and written informed consent before entering the study.

The present cross-sectional study derived from the SI! Program for Secondary Schools trial was carried out using baseline data (2017) collected from 944 participants with available information on food consumption frequency, and whose total energy intake ranged from 803 to 4,013 kcal/day in boys and 502 to 3,511 kcal/day in girls (15) (Figure 1).

Assessment of Flavonoids From Cocoa-Based Products

Dietary intake was assessed by a validated semi-quantitative food frequency questionnaire (15, 16). Cocoa-based product intake was expressed in grams and included cocoa powder (25% of pure cocoa), dark (more than 70% pure cocoa), and milk chocolate (about 30% pure cocoa). Cookies, pastries, and beverages made of cocoa-based products were not considered due to the lack of information on their content of flavonoids. Flavonoids from cocoa-based products were estimated using the Phenol-Explorer database (http://www.phenol-explorer.eu), which included flavanols, such as catechin, epicatechin, cinnamattins, and proanthocyanidins (dimers, trimers, 4–6 mers, 7–10 mers, polymers, and monomers), and flavonol-like quercetin (17, 18). In brief, flavonoids from cocoa-based products were estimated (mg/100 g fresh food weight) for each food and then multiplied by intake of the respective foods (g/day). Total flavonoids from cocoa-based products were estimated as the sum of intakes of the individual flavonoids (catechin, epicatechin, cinnamattins, proanthocyanidins, and quercetin). Energy-adjusted flavonoid intake was calculated by the residual method established by Willet et al. (19).

Assessment of Adiposity Parameters

All participants were evaluated by trained staff, who performed the anthropometric measurements of weight, height, and waist circumference (WC) according to standard procedures (14). Weight was obtained to the nearest 0.1 kg using a digital scale (OMRON BF511) and height to the nearest 0.1 cm with a portable SECA 213 stadiometer. WC was measured to the nearest 0.1 cm. To minimize measurement errors, WC was measured three times and a mean value was calculated. The percentage of fat...
mass (\%FM) was estimated by bioelectrical impedance using a
tetrapolar OMRON BF511 and fat mass weight was calculated as
the product of fat percentage and body weight.

Body mass index (BMI) was calculated as body weight in
kilograms divided by height squared in meters (kg/m²). The
fat mass index (FMI) and waist-to-height ratio (WHtR) were
obtained by dividing fat weight (kg) by height squared (m²)
and WC (cm) by height (cm), respectively. Age- and gender-
specific BMI, WC z-scores, and FMI z-scores (standard deviation
score) were calculated according to the Center for Disease
Control growth references and the National Health and Nutrition
Examination Survey data (20–23).

Overweight was defined as BMI at or above the 85th percentile
to less than the 95th percentile, and obesity as equal to or greater
than the BMI 95th percentile (21, 22). Participants with a BMI
percentile equal to or above the 85th percentile were classified
as overweight/obese (21, 22). Abdominal obesity was defined
by WC at or above the 90th percentile and/or WHtR equal
to or above the 0.5 threshold (20, 24, 25). Finally, participants
with a \%FM and/or FMI greater than or equal to the age- and
gender-specific 75th percentile were classified as excess adiposity,
according to published reference data for \%FM and FMI (23, 26).

Assessment of Covariates

Intake of energy, foods, and nutrients was determined from
the semi-quantitative food frequency questionnaire that was
previously described, with the use of values from Spanish food
composition tables (27, 28).

Information on physical activity was obtained with the use
of accelerometers and a standardized questionnaire. Physical
activity was measured using accelerometers (Actigraph wGT3X-
BT, ActiGraph, Pensacola, USA) worn on the non-dominant
wrist for 7 days, except during water-based activities (14).
Moderate-to-vigorous physical activity was estimated according
to the cut points of Chandler et al. and is presented as the
average minutes of moderate-to-vigorous physical activity per
day (29). In participants with missing accelerometer data (n =
48), the information from the QAPACE survey (Quantification

\[ \text{BMI} = \frac{\text{Body weight (kg)}}{\text{Height (m)}^2} \]
\[ \text{FMI} = \frac{\text{Fat weight (kg)}}{\text{Height (m)}^2} \]
\[ \text{WHtR} = \frac{\text{WC (cm)}}{\text{Height (cm)}} \]
Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant. Significant differences are bolded.

### Statistical Analysis

A descriptive analysis of the population was carried out using mean (SD) and frequency distribution. Participants were categorized into quintiles of energy-adjusted flavonoids from cocoa-based products (Q1: <12.1, Q2: 12.1–32.0, Q3: 32.1–53.2, Q4: 53.3–83.8, and Q5 >83.8 mg/day). One-way analysis of variance, including Bonferroni post-hoc test, and chi-square analysis were performed to assess the differences in means and frequencies across quintiles of flavonoids from cocoa-based products, respectively.

Multilevel mixed-effects linear regression models with robust error variance were used to evaluate the association between quintiles of flavonoids from cocoa-based products with the anthropometric measurements (BMI z-score, WC z-score, WHR, %FM, and FMI z-score) as continuous variables. Multilevel generalized logistic regression was performed to study the association between quintiles of flavonoids from cocoa-based products and adiposity parameters (BMI ≥ 80th to <95th percentile, BMI ≥ 95th percentile, WC ≥ 80th to 95th percentile, and WHtR ≥ 0.5). The fixed effects were gender (girls/boys), age (continuous, year), Tanner maturation stage (from I to V), physical activity (≥60 min/≤60 min moderate-to-vigorous physical activity), parental education (university studies/lower than university studies), intake of energy (continuous, Kcal/day), sweetened products like breakfast cereals (continuous, g/day), pastries (continuous, g/day), sugar-sweetened beverages (continuous, g/day), meat, and processed meat (continuous,
TABLE 2 | Dietary food intake of participants according to quintiles of flavonoids from cocoa-based products (mg/day).

|                        | Overall (n = 944) | Q1 (< 12.1) | Q2 (12.1–32.0) | Q3 (32.1–53.2) | Q4 (53.3–83.8) | Q5 (> 83.8) | P-for trend |
|------------------------|-------------------|-------------|----------------|----------------|----------------|-------------|-------------|
| Fish products, g/day   | 86.6 (55)         | 103.3 (67.2)<sup>a</sup> | 89.2 (52.6)<sup>b</sup> | 81.6 (47.0)<sup>b</sup> | 76.1 (49.6)<sup>b</sup> | 82.9 (53.1)<sup>b</sup> | <0.001      |
| Meat, g/day            | 172.6 (80.4)      | 207.4 (107.9)<sup>a</sup> | 161.2 (82.8)<sup>a</sup> | 172.5 (87.5)<sup>b</sup> | 155.8 (75.2)<sup>b</sup> | 166.0 (86.6)<sup>b</sup> | <0.001      |
| Processed meat, g/day  | 7.0 (8.9)         | 8.7 (8.5)<sup>a</sup> | 7.1 (7.1)<sup>a,b</sup> | 6.6 (6.3)<sup>c</sup> | 6.7 (6.3)<sup>a,b,c</sup> | 6.1 (5.4)<sup>d</sup> | 0.001       |
| Dairy products, g/day  | 406.6 (253.9)     | 448.4 (275.8)<sup>a</sup> | 404.9 (254.3)<sup>b</sup> | 382.4 (231.5)<sup>a,b</sup> | 357.7 (193.6)<sup>b</sup> | 439.9 (293.3)<sup>a</sup> | 0.269       |
| Refined grains, g/day  | 113.2 (69.4)      | 139.8 (85.8)<sup>a</sup> | 115.6 (70.1)<sup>a</sup> | 107.2 (59.1)<sup>b</sup> | 105.7 (62.9)<sup>b</sup> | 97.6 (58.7)<sup>b</sup> | <0.001      |
| Wholegrains, g/day     | 18.5 (32.2)       | 19.2 (33.6)<sup>a</sup> | 18.9 (32.0)<sup>a</sup> | 15.1 (25.9)<sup>a</sup> | 19.7 (34.1)<sup>a</sup> | 19.7 (34.8)<sup>a</sup> | 0.814       |
| Breakfast cereals, g/day | 14.6 (19.7)      | 17.8 (29.1)<sup>a</sup> | 15.3 (18.8)<sup>a</sup> | 13.4 (15.2)<sup>a</sup> | 14.6 (16.9)<sup>a</sup> | 12.1 (14.7)<sup>a</sup> | 0.008       |
| Legumes, g/day         | 60.6 (44.2)       | 72.0 (64.7)<sup>a</sup> | 59.6 (37.8)<sup>a,b</sup> | 58.4 (36.2)<sup>d</sup> | 57.2 (37.3)<sup>d</sup> | 55.6 (36.4)<sup>d</sup> | 0.001       |
| Vegetables, g/day      | 205.3 (150.6)     | 246.4 (199.9)<sup>a</sup> | 200.9 (137.9)<sup>d</sup> | 194.9 (154.7)<sup>d</sup> | 190.4 (129.7)<sup>d</sup> | 193.5 (131.3)<sup>d</sup> | 0.001       |
| Fruits, g/day          | 334.7 (250.7)     | 430.6 (290.0)<sup>a</sup> | 338.6 (216.2)<sup>a</sup> | 310.3 (243.1)<sup>d</sup> | 292.4 (208.5)<sup>d</sup> | 301.1 (263.3)<sup>d</sup> | <0.001      |
| Nuts, g/day            | 11.3 (14.5)       | 16.1 (19.7)<sup>a</sup> | 10.8 (13.7)<sup>a</sup> | 9.4 (10.8)<sup>a</sup> | 9.7 (11.7)<sup>a</sup> | 10.6 (14.1)<sup>a</sup> | 0.141       |
| Olive oil, g/day       | 16.6 (14.5)       | 17.8 (13.6)<sup>a</sup> | 17.2 (17.2)<sup>a</sup> | 16.9 (13.6)<sup>a</sup> | 15.3 (12.8)<sup>a</sup> | 16.1 (15.0)<sup>a</sup> | 0.014       |
| Cocoa-based products, g/day | 7.4 (7.6)   | 2.6 (2.5)<sup>a</sup> | 3.9 (3.5)<sup>a</sup> | 5.7 (4.1)<sup>a</sup> | 8.0 (5.3)<sup>a</sup> | 16.6 (10.3)<sup>d</sup> | <0.001      |
| Sugar-sweetened beverages, g/day | 54.8 (95.1) | 73.2 (123.2)<sup>a</sup> | 52.4 (96.5)<sup>a,b</sup> | 50.6 (94.5)<sup>b</sup> | 55.3 (81.3)<sup>a,b</sup> | 42.3 (69.4)<sup>a</sup> | 0.007       |
| Pastry products, g/day | 69.1 (52.5)       | 74.2 (54.6)<sup>a</sup> | 67.2 (53.9)<sup>a</sup> | 68.4 (53.5)<sup>a</sup> | 66.5 (46.6)<sup>a</sup> | 73.0 (53.2)<sup>a</sup> | 0.561       |

Data are expressed as mean (SD).

Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation.

Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables.

<sup>a,b,c</sup>Data sharing the different letters are statistically different after Bonferroni post-hoc test. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant. Significant differences are bolded.

Results

General Characteristics of the Study Participants

The characteristics of the cohort stratified by quintiles of flavonoid intake from cocoa-based products are shown in Table 1. Based on BMI z-score, 18% of adolescents presented overweight and 9% obesity. Regarding the abdominal obesity parameters, 16% of participants had a WC greater than the 90th percentile, and 23% had a high WHtR (>0.5 threshold). Finally, regarding the excess of adiposity, 8 and 16% of adolescents had %FM and FMI equal to or greater than the 75th percentile, respectively. Compared to the lowest quintile, participants in the highest quintile tended to have slightly lower BMI, WC, and WC z-score, although the differences were not significant in the univariate analysis.

The mean cocoa-based product intake was 7.4 (7.6) g/d, equivalent to one tablespoon of cocoa powder or one square piece of a chocolate bar. More than 90% of the participants reported daily intake of at least one cocoa-based product, from them, 75% (N = 700) reported intake of cocoa powder, 31% (N = 294) dark chocolate, and 68% (N = 644) milk chocolate. The mean flavonoid intake from cocoa-based products was 57.4 (74.5) mg/day, where 26.6 (35.3) mg/day were from cocoa powder, 24.0 (62.9 mg/day) from dark chocolate, and 6.7 (11.3) mg/day from milk chocolate (Data not shown). Participants with a
higher intake of flavonoids from cocoa-based products tended to consume lower fish, meat, processed meat, refined grains, (poly)phenol-rich foods (legumes, vegetables, fruits, and nuts), and sugar-sweetened beverages (Table 2). In addition, compared to the lowest quintile, participants in the highest quintile of flavonoids from cocoa-based products had a lower energy intake and macro and micronutrients (Table 3), except for vitamin A. A higher intake of total dietary flavonoids was observed in the highest quintile, but lower values of phenolic acids, lignans, and other (poly)phenol intake were observed in the same group (Table 3).

**TABLE 3 | Nutrients and (poly)phenols intake of participants according to quintiles of flavonoids from cocoa-based products (mg/day).**

| Nutrients | Overall (n = 944) | Q1 (<12.1) (n = 189) | Q2 (12.1–32.0) (n = 189) | Q3 (32.1–53.2) (n = 189) | Q4 (53.3–83.8) (n = 189) | Q5 (>83.8) (n = 188) | P-for trend |
|-----------|-------------------|-----------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------|
| Energy, Kcal/day | 2,539.2 (601.8) | 2,013.4 (435.9)a | 2,510.9 (490.9)b | 2,402.6 (662.7)c | 2,307.3 (614.7)d | 2,461.3 (622.8)e | <0.001 |
| Carbohydrates, g/day | 303.2 (65.8)a | 255.8 (65.7)b | 240.6 (64.3)c | 236.3 (71.3)d | 246.7 (73.3)e | <0.001 |
| Fiber, g/day | 29.4 (10.7) | 29.1 (8.9)b | 27.6 (9.9)b | 27.1 (10.3)b | 27.6 (10.7)b | <0.001 |
| Proteins, g/day | 120.9 (33.3) | 118.9 (27.8)b | 115.9 (30.8)c | 108.3 (30.3)d | 117.5 (35.5)e | <0.001 |
| SFA, g/day | 36.8 (11.5) | 35.8 (9.5)b | 34.6 (10.6)c | 32.7 (10.8)c | 36.8 (11.7)b | <0.001 |
| MUFA, g/day | 48.5 (16.2) | 48.1 (15.9)b | 46.4 (15.3)b | 43.8 (15.6)b | 47.2 (16.5)b | <0.001 |
| PUFA, g/day | 19.7 (6.8) | 19.4 (5.7)b | 18.7 (6.2)b | 18.2 (7.3)b | 18.5 (6.9)b | <0.001 |
| Calcium, mg/day | 1,012.9 (391.0) | 1,198.1 (403.5)b | 1,001.8 (354.7)c | 959.7 (362.2)c | 896.9 (321.2)b | <0.001 |
| Vitamin A, µg/day | 1,476.0 (1,465.2) | 1,849.9 (1,719.5)b | 1,542.8 (1,795.6)b | 1,342.6 (1,107.7)c | 1,177.2 (754.9)b | 1,487.6 (1,837.2)b | 0.001 |
| Vitamin D, µg/day | 5.1 (2.6) | 6.1 (3.0)b | 5.2 (2.4)b | 4.8 (2.4)b | 4.6 (2.5)b | 4.7 (2.4)b | <0.001 |

**TABLE 4 | Association between flavonoid intake from cocoa-based products (mg/day) and anthropometric measurements.**

| Anthropometric variables | Q1 (<12.1) (8, 95% CI) | Q2 (12.1–32.0) (8, 95% CI) | Q3 (32.1–53.2) (8, 95% CI) | Q4 (53.3–83.8) (8, 95% CI) | Q5 (>83.8) (8, 95% CI) | P-for trend |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------|
| BMI z-score | Reference | 0.25 (0.10; 0.40) | 0.29 (0.17; 0.41) | 0.06 (−0.04; 0.15) | −0.07 (−0.25; 0.10) | <0.001 |
| WC z-score | Reference | 0.09 (−0.01; 0.19) | 0.23 (0.17; 0.29) | 0.06 (−0.13; 0.24) | −0.04 (−0.07; −0.01) | 0.045 |
| WHtR | Reference | −0.01 (−0.02; 0.11) | 0.01 (0.01; 0.01) | −0.00 (−0.00; −0.00) | −0.01 (−0.02; −0.01) | <0.001 |
| %FM | Reference | 0.72 (0.51; 0.94) | 2.17 (1.95; 2.38) | 0.09 (−0.14; 0.32) | −1.15 (−3.36; 1.05) | 0.160 |
| FMI z-score | Reference | 0.41 (0.18; 0.64) | 0.52 (0.39; 0.66) | 0.21 (0.02; 0.40) | 0.02 (−0.53; 0.56) | 0.242 |

**Association of Dietary Flavonoids From Cocoa-Based Products With Adiposity Parameters**

The results from the multivariate-adjusted linear regression analyses showed that a higher intake of flavonoids from cocoa-based products was associated with lower values of BMI z-score (P-for trend < 0.001); however, no significant difference was observed between the highest and lowest quintiles. Moreover, participants with highest intake of flavonoids from cocoa-based products had lower values of WC z-score [B = −0.04, 95% CI (−0.07; −0.01), P-for trend = 0.045] and WHtR [B = −0.01,
95% CI (−0.02; −0.01), P-for trend < 0.001] (Table 4). However, quartiles 2 and 3 had higher values of BMI z-score, %FM, and FMI z-score compared to quartile 1. No interaction with gender was found in the regression analysis.

Table 5 shows the association of flavonoids from each cocoa-based product and anthropometric parameters. Inverse associations were observed between flavonoids from cocoa powder and BMI z-score [B = −0.08, 95% CI (−0.12; −0.05), P < 0.001], WC z-score [B = −0.06, 95% CI [−0.11; −0.02], P = 0.003], WHtR [B = −0.01, 95% CI (−0.01; −0.00), P < 0.001], %FM [B = −1.11, 95% CI (−1.48; −0.75), P < 0.001], and FMI z-score [B = −0.18, 95% CI (−0.20; −0.17), P < 0.001]. Regarding dark chocolate, an inverse association only with WC z-score [B = −0.06, 95% CI (−0.08; −0.05), P < 0.001] was found. However, no association was observed between flavonoids from milk chocolate intake and anthropometric parameters.

Multivariate-adjusted logistic regression analyses revealed a tendency of having less probability of having overweight (BMI at or above the 85th percentile to less than the 95th percentile) and high WHtR (≥0.5 thresholds) in participants with higher flavonoid intake from cocoa-based products (Table 6). In addition, participants in the highest quintile had less probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), P-for trend = 0.001] compared to the lowest quintile (Figure 2). However, participants in quintiles 4 and 3 had a higher probability of obesity (BMI at or above 95th percentile) compared to quintile 1.

Finally, in the correlation analysis between individual flavonoids from cocoa-based products and adiposity parameters, weak inverse correlations between WHtR and catechins (R = −0.08, FDR value = 0.027), epicatechins (R = −0.09, FDR value = 0.014), and proanthocyanidins (R = −0.08, FDR value = 0.021) were observed (Data not shown).

**DISCUSSION**

In the present study, a higher intake of flavonoids from cocoa-based products was inversely associated with individual adiposity parameters and abdominal obesity in adolescents. To our knowledge, this is one of the first studies to explore these associations in this target population.
Cocoa Flavonoid Intake in Adolescents

Cocoa-based products are an important dietary source of flavonoids. In our study, adolescents consumed a mean of 57.4 mg/day of flavonoids from cocoa-based products, representing 11% of the total dietary flavonoid intake (mean 530.1 mg/day). A lower intake of flavonoids from cocoa-based products was reported by Bawaked et al. in Spanish children aged 6 to 11 years, who consumed 10.9 mg/day of flavonoids from cocoa powder and chocolate, which provided 23.5% of the total flavonoid intake (mean 70.7 mg/day) (36). In the Healthy Lifestyle in Europe by Nutrition in Adolescence study, chocolate products were once the major source of dietary (poly)phenols and flavonoids in European adolescents (37).

Flavonoids From Cocoa-Based Products and Adiposity Parameters

The inverse association of flavonoids from cocoa-based products with adiposity parameters is in accordance with previous studies, although most of them were conducted in adults. The fact that the results remained consistent when using different adiposity parameters (BMI z-score, WC z-score, WHtR, %FM, and FMI z-score) further strengthens the study findings. In addition, in our cross-sectional, multivariate-adjusted model, clinical relevance was observed between extremes of quintiles of flavonoids from cocoa-based products (Q5 vs. Q1) and less probability of having abdominal obesity. These results were independent of physical activity, puberty development, parental education, intake of energy, sweetened products, meat, and processed meat, as well as other (poly)phenol-rich foods intake such as fruits, vegetables, legumes, nuts, and extra olive oil. Fruits and vegetables represent the main food source of flavonoids in the diet of adolescents and their consumption could influence the association of flavonoids from cocoa-based products with adiposity parameters (36, 38–40). According to the results of a cross-sectional study of European adolescents, consumption of energy-dense foods is associated with a higher probability of obesity (41). In our study, a tendency to consume less energy-dense and sugary foods was observed in participants with a higher intake of flavonoids from cocoa-based products.

In agreement with our findings, Cuenca-García et al. recently reported that higher chocolate consumption was associated with lower BMI, body fat, and WC in European adolescents (40). Similar results were obtained in a large cross-sectional analysis in non-diabetic US adults, where the BMI and WC of individuals who reported chocolate intake were lower by 0.92 Kg/m² and 2.07 cm, respectively, compared to the non-reporters, a difference that could be attributed to the intake of cocoa flavonoids (42). However, the authors did not define the type of chocolate being consumed (for example, dark or milk), and its flavonoid content was not calculated. In our study, inverse associations between flavonoids from cocoa powder and BMI z-score, WC z-score, WHtR, %FM, and FMI z-score were observed, but WC z-score was inversely associated only with dark chocolate. No association between anthropometric parameters and milk chocolate was found. These results could be attributed to the highest concentration of flavonoids in cocoa powder compared to dark or milk chocolate (17, 43). In addition to this, it could be also explained by the fact that cocoa powder was consumed by most participants (75%), with fewer consuming milk (68%) and dark chocolates (31%).
Evidence between cocoa flavonoid consumption and adiposity in adults is conflicting. The results of a meta-analysis based on randomized clinical trials suggested that the consumption of at least 30 g/day of cocoa/dark chocolate for 4–8 weeks decreases BMI in adults (12). Similarly, weight reduction in overweight/obese adults (BMI ≥ 25 kg/m²) was related to the consumption of flavanol-containing products such as tea, cocoa, and apple in a subgroup meta-analysis (13). In contrast, a meta-analysis of short-term trials did not find significant associations between flavonoid intake from cocoa-based products and BMI in adults, although this could have been due to the short-term nature of the studies (44). Longer-term randomized controlled clinical trials are needed to examine the magnitude of the effect of flavonoids from cocoa-based products on adiposity parameters. Instead, the limitations of BMI as an adiposity indicator are well-known, because it does not provide information on adiposity distribution, and therefore additional anthropometric measurements are required, such as WC, WHtR, %FM, and FMI (24, 45).

Cocoa-based products also contain other bioactive compounds like theobromine, a methylxanthine highly associated with body weight, lipid, and glucose metabolism (46). In our study, theobromine was not quantified so their possible association with adiposity parameters has been not determined.

The effect of cocoa-based product intake on body fat and obesity could be explained by an associated reduction in plasma adipokine (leptin and adiponectin) concentrations, although the mechanisms involved still need to be clarified (8, 47). Leptin and adiponectin are hormones mainly secreted by adipose tissue and delivered into the systemic circulation to modify glucose and lipid metabolism, insulin sensitivity, and cardiovascular function (48). Leptin promotes fatty acid oxidation and reduces lipogenesis by regulating peripheral metabolic pathways in skeletal muscle, adipose tissue, the liver, and the pancreas (49). Meanwhile, plasma adiponectin improves insulin sensitivity, activates muscle utilization of glucose, induces muscle and hepatic fatty acid oxidation, and reduces hepatic glucose production (48). In the present study, adipokine levels were not analyzed and their possible relationship with the intake of flavonoids from cocoa-based products was not determined.

The relationship between the consumption of cocoa-based products and adiposity parameters has been attributed to their flavanol (flavan-3-ol) content. Flavanols from cocoa include mainly monomers and polymers of catechin and epicatechin (7, 9, 10, 43). In our exploratory analysis, we observed a negative correlation between catechins, epicatechins, proanthocyanidins (polymers of flavanols), and WHtR. Catechins and epicatechins, both flavanols monomers, are rapidly absorbed from the upper portion of the small intestine and could influence metabolic pathways related to body weight (50, 51). Gutiérrez-Salméan et al. suggested that epicatechin decreases the expression of proteins associated with mitochondrial function and increases the expression of protein-induced thermogenesis (51). Instead, although proanthocyanidins are the most abundant (poly)phenols in cocoa-based products, they are poorly absorbed in the small intestine due to their large number of hydrophilic hydroxyl groups (9, 50). Most proanthocyanidins reach the colon and are transformed by the gut microbiota into phenylvalerolactones and phenolic acids, such as hydroxyphenylpropionic acid, hydroxyphenylacetic acid, and benzoic acid (43, 50, 52, 53). These microbial metabolites might be responsible in part for health beneficial effects of proanthocyanidins and could be implicated in adipogenesis and lipogenesis mechanisms (6).

Results from a cross-sectional study, based on 2,734 women twins aged 18–83 years, revealed that women with a higher dietary intake of proanthocyanidins-rich foods, which included apples and cocoa drinks, had lower fat mass and central fat mass, both measured by dual-energy-X-ray-absorptiometry (54). In another way, according to the results shown by Lee et al., 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone, a microbial flavanols metabolite, reduces lipid accumulation in 3T3-L1 mature adipocytes regulating free fatty acids metabolism through the suppression of the expression of lipogenic proteins (6). However, evidence for the effect of flavanols from cocoa-based products and their microbial metabolites on adipogenesis and lipogenesis metabolic pathways is yet inconclusive and further studies are needed to better understand the mechanisms of action implicated in weight maintenance.

Although cocoa-based products are an important source of flavonoids that might contribute to the improvement of adiposity parameters, their consumption should be promoted with caution, considering that most commercial formulations are high in calories, sugars, and fats (7). Thus, from a public health perspective, cocoa-based products low in fats and sugars might be recommended.

**Limitations and Strengths**

A limitation of the present study is its cross-sectional design, which precludes causal assumptions about flavonoid intake from cocoa-based products and differences in adiposity parameters. In addition, data derived from food frequency questionnaires are prone to bias because misreporting is common in dietary self-assessment in adolescents (55). Misreporting in adolescents is associated with several factors, specifically weight status, weight loss or weight maintenance, body image dissatisfaction, and skipping breakfast (56, 57). Adolescents with high values of BMI tend to report a lower consumption of food rich in energy, fats, and sugars, like cocoa-based products. Misreporting may reflect socially desirable answers where adolescents with self-image dissatisfaction are more likely to under-report the consumption of high fat/high sugar foods. Another plausible reason could be that under-eating is the result of a dietary regimen to lose or maintain weight, so there could be a control in the intake of cocoa-based products. Instead, adolescents with normal weight status could real over-eating to reflect higher intakes due to a growth spurt. Regarding the dietary flavonoids assessment, although our validated food frequency questionnaire specifies portion size, measurement error will be present with any assessment of the flavonoid content of cocoa-based commercial products because they depend on the manufacturing process like alkalinization treatment (58). Furthermore, a limitation of using a food frequency questionnaire is that there is no possible
way to determine the exact content of flavonoids from specific cocoa-based products since the percentage of cacao varies for each commercial product. In addition to this, flavonoid intake was estimated through a database, which may not reflect the true concentration of compounds reaching the target organs after digestion, absorption, and metabolism. Therefore, the association between flavonoids from cocoa-based products and adiposity parameters might be distorted by the dietary data bias, so these results should be interpreted with caution. Further longitudinal analyses will be necessary to clarify the true direction of these associations.

Strengths of the present study include the large sample size ($n = 944$) of well-characterized participants, the standardization of measures performed in the SI! Program for Secondary Schools trial, and the inclusion of a range of anthropometric variables, not only BMI, to evaluate adiposity.

In conclusion, a higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and less probability of abdominal obesity. These findings are relevant for hypothesis generation regarding mechanisms underlying potential therapeutic effects of cocoa flavonoids against obesity and should stimulate further prospective studies and clinical trials to determine the health beneficial effects of cocoa flavonoids on adolescents.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this article are not readily available because there are restrictions on the availability of the data for the SI! Program study, due to signed consent agreements around data sharing, which only allow access to external researcher for studies following project purposes. Requestor wishing to access the database used in this study can make a request to the Steering Committee (SC) chair. For the present study, the database was requested from the SC on 24 February 2022. Requests to access the datasets should be directed to gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, restruch@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, and vfuster@cnic.es.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Ethics Committee of Instituto de Salud Carlos III in Madrid (CEI PI 35_2016), the Fundació Unió Catalana d’Hospitals (CEI 16/41), and the University of Barcelona (IRB00003099). Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

**AUTHOR CONTRIBUTIONS**

RL-R: conceptualization. EL-S, AT-R, and RL-R: methodology. EL-S and CA-R: formal analysis. EL-S, CA-R, and RL-R: investigation. AC-G: data curation. EL-S, CA-R, AT-R, and RL-R: writing—original draft preparation. AT-R, RF-J, JF-A, GS-B, MM, PB, AC-G, CR, AR-L, SC-B, RC, RE, and RL-R: writing—review and editing. EL-S: visualization. AT-R and RL-R: supervision. RF-J, JF-A, GS-B, MM, PB, AC-G, JM-G, AT-R, RE, and RL-R: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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