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Higher Childhood Red Meat Intake Frequency Is Associated with Earlier Age at Menarche[1–3]

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Abstract

Background: Early age at menarche is associated with increased breast cancer risk. Red meat consumption in adolescence predicts breast cancer risk, but it is unknown whether it is also related to earlier menarche.

Objective: We studied the association between intake of red meat at ages 5–12 y and age at menarche in a prospective study.

Methods: We assessed usual diets with a food-frequency questionnaire in a group of 456 girls aged 8.4 ± 1.7 y and followed them for a median 5.6 y in Bogotá, Colombia. Girls were asked periodically about the occurrence and date of menarche. Median age at menarche was estimated with use of Kaplan-Meier survival probabilities by categories of red meat intake frequency. Cox proportional hazards models were used to compare the incidence of menarche by red meat intake frequency, adjusting for potential sociodemographic and dietary confounders including total energy intake and intake frequency of other animal food groups (dairy, poultry, freshwater fish, tuna/sardines, eggs, and innards).

Results: Median age at menarche was 12.4 y. After adjustment for total energy intake, maternal parity, and socioeconomic status, red meat intake frequency was inversely associated with age at menarche. When compared with girls with red meat intake <4 times/wk, those consuming it ≥2 times/d had a significantly earlier age at menarche (HR: 1.64; 95% CI: 1.11, 2.41; *P*-trend = 0.0009). Incidentally, we found that girls with tuna/sardine intake >1 time/wk had a significantly later age at menarche (HR: 0.62; 95% CI: 0.42, 0.90; *P* = 0.01) than those with intake <1 time/mo. Intake frequency of other animal food groups was not significantly associated with age at menarche.

Conclusion: Higher red meat intake frequency during childhood is associated with an earlier age at menarche, whereas greater fatty fish intake frequency is associated with a later menarcheal age. *J Nutr* 2016;146:792–8.

Keywords: red meat, animal foods, fatty fish, menarche, puberty

Introduction

Menarche, the first menstrual period, is a recognizable marker of puberty. An early age at menarche is associated with breast (1) and endometrial (2) cancers, obesity (3), type II diabetes (4), cardiovascular disease, and all-cause mortality (5). In addition, early menarche has been related to risk factors during adolescence including alcohol and tobacco use, early sexual debut, and teenage pregnancy (6, 7).

There is substantial variability in the timing of menarche across populations and a secular trend toward earlier menarche within countries (8–10). This suggests that the timing of puberty may be responsive to changes in environmental conditions. Although the exact nature of these changes remains uncertain, epidemiological studies have found associations between high intake of animal protein during childhood and earlier puberty (11–17). For example, total animal protein intake between ages 3 and 8 y was related to earlier menarche in US (11) and German (12) girls. Intake of protein from dairy was related to earlier puberty in girls from Germany (12), Iran (15), and the United States (16), whereas higher meat intake in childhood was associated with earlier menarche in British (13), Korean (14), and US (17) girls. There is some heterogeneity in this evidence, however, because a recent study in a large group of US girls found no association between dairy or meat intake at ages 9–14 y and age at menarche (18). One possible explanation for this discrepancy is differences in the timing of dietary assessments (13, 18), because intake around the time of puberty onset may be less relevant than earlier childhood diet. Relatively few studies, to our knowledge, have examined childhood intake of specific animal food groups in relation to menarche.

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[3] Supplemental Tables 1–3 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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A recent investigation found that red meat intake during adolescence was related to higher breast cancer risk (19). Considering that early menarche is a predictor of breast cancer (1), we hypothesized that red meat intake during childhood could be associated with an earlier menarche.

We evaluated the association between frequency of red meat intake at 5–12 y with age at menarche in a prospective study of premenarcheal school-age girls from Bogotá, Colombia.

Methods

Study population. The study was conducted in the context of the Bogotá School Children Cohort, an ongoing longitudinal investigation of nutrition and health in school-age children. Details concerning recruitment and data collection are published elsewhere (20). Briefly, in February 2006 we randomly sampled children from all public primary schools in Bogotá. Inclusion criteria involved being enrolled in 1 of the city's 361 public primary schools at the time by the beginning of the school year and being between 5 and 12 y of age. Four thousand children were invited to participate, of whom 3202 agreed and were enrolled in the cohort. Because most children in the public school system were from low- and middle-income families, the cohort represents these socioeconomic strata. Information collected at the time of enrollment included diet, anthropometry, and sociodemographic characteristics and health status.

Dietary assessment. Between May and June 2006, trained dietitians administered a 38-item FFQ to a sample of 1027 mothers to 531 girls and 496 boys who attended parents' meetings at schools in order to obtain information on their children's usual intake. Details of the FFQ have been described previously (21). The FFQ was based on the most frequently consumed foods in this population according to the Colombian National Nutrition Survey 2005 and included all major sources of energy in the population. For each item, we described reference portion sizes in natural units or standard measures for commonly consumed servings among children in this population, and inquired about frequency of intake with a scale comprising 9 options: 4–5 times/d, 2–3 times/d, 1 time/d, 5–6 times/wk, 2–4 times/wk, 1 time/wk, 1–3 times/mo, <1 time/mo, or never. Energy intakes were estimated by multiplying the consumption frequency of each food by the energy contents of the specific portion using composition values from the USDA's Standard Reference food composition database, supplemented with data from manufacturers and published reports (Food Processor software; http://www.esha.com) and the Food Composition Table of Colombian Foods by the Colombian Institute of Family Welfare (22). A team composed of Colombian and US dietitians worked together on the nutrient composition analyses to ensure that the foods chosen from the USDA database resembled the closest to the local foods.

Although the FFQ has not been formally validated, a previous investigation that included the girls in the current study provided strong evidence for its validity to measure intake frequency of animal food sources (21). In that study, principal component analyses of the FFQ clearly identified an "animal foods" dietary pattern that included frequent intake of beef, chicken, and dairy. Adherence to this pattern was strongly, positively, and linearly related to serum concentrations of vitamin B-12, a nutrient that is naturally found in animal foods only. In addition, meat intake frequency alone, as measured by the FFQ, was linearly and monotonically related to serum vitamin B-12. Among children reporting meat intake ≥2 times/d, serum vitamin B-12 was 24 pmol/L higher than in children with meat intake <4 times/wk (*P*-trend = 0.04), after adjustment for sex and intake frequency of dairy, fish, cow liver, and supplements. Because measurement errors in the FFQ and the serum vitamin B-12 values are uncorrelated, the findings indicate that the validity of the FFQ to measure animal foods is adequate.

Anthropometry. In the weeks following enrollment, trained research assistants visited the schools to obtain anthropometric measurements from the children. Weight was measured in light clothing to the nearest 0.1 kg on Tanita HS301 electronic scales (Tanita Arlington Heights, Illinois), and height was measured without shoes to the nearest 1 mm with wall-mounted Seca 202 stadiometers (Seca, Hanover, Maryland). We calculated height-for-age and BMI (in kg/m^2)-for-age z scores, with the use of the WHO sex-specific growth references for children aged 5–19 (23).

Parental and household characteristics. Information on parental and household characteristics was obtained with the use of a self-administered questionnaire that was sent to each child's home during the week of enrollment. The questionnaire inquired about parental age, education level, mother's parity and age at menarche, and the child's health habits, including time watching television or playing video games and playing outdoors. Socioeconomic status was determined from the child's home address, as the score assigned by the local government to establish the cost of public services. Maternal BMI was calculated from height and weight values that the research team measured at the time of FFQ administration, using the same methods described for the children. Measured values were available in 80% of mothers; self-reported values were used in the rest. Covariates were categorized as presented in **Table 1**.

Follow-up. Follow-up visits occurred in June and November 2006 and once yearly thereafter. If children were absent from school on the day of the visit, they were assessed at home. At each assessment, girls were asked if they had begun menstruation and, if they had, the date of their first menstrual period. Major holidays and school vacation time were used to aid the girls' recall of the date of menarche, if needed.

Written informed consent from parents or primary caregivers of all children was obtained before enrollment. The study protocol was approved by the Ethics Committee of the National University of Colombia Medical School. The Institutional Review Board at the University of Michigan approved the use of data from the study.

Data analysis. Sixty-six girls who had >5 missing responses in the FFQ were excluded. Of the remaining 465 girls with valid FFQ data, 9 had experienced menarche by the time of enrollment and were excluded. Thus, the analytic sample consisted of 456 girls.

The primary exposure was frequency of red meat intake. This was the sum of weighted intake frequencies of 3 separate items in the FFQ: beef/pork/veal/lamb, cold cuts (sausages/ham/bologna), and hamburger/hot dog. The frequency was categorized as <4 times/wk, 4–6 times/wk, 1 time/d, and ≥2 times/d. Because red meat is eaten as part of an animal food source pattern in this population (21), intake of other animal foods could confound the association with age at menarche; therefore, we also considered intake frequency of dairy (milk, cheese, and yogurt), poultry, freshwater fish, canned tuna or sardines (main sources of fatty fish in the population, asked together in a single FFQ item), eggs, and innards (cow's liver, spleen, lung, and tripe). Innards were treated separately because the items in the red meat category are typically more highly processed than innards. Furthermore, innards are not consumed as frequently as items in the red meat group, and as a result their intake may be measured with more error than other meats.

The outcome, age at menarche, was estimated in decimal years as date of menarche minus date of birth. Among 75 girls (16.5%) who did not recall the day of the month when menarche occurred, the 15th was imputed. In another 33 girls (7.2%) who only remembered the year of menarche, July 1 was imputed for the calculation of age at menarche. Each girl's mother was the primary informant of the date of menarche in 67 participants (14.7%). Twenty-six percent of girls (n = 119) did not have menarche during follow-up and were censored at the last interview date.

To identify potential confounders, we examined the associations of baseline sociodemographic characteristics and age at menarche using time-to-event analyses. Time-to-event analytic techniques including the Kaplan-Meier method and Cox regression properly account for right censoring in the data because some girls did not reach menarche during follow-up. These methods allow for information on menarcheal age from post-menarcheal girls to be combined with the last known age during follow-up when menarche had not occurred ("censoring") from premenarcheal girls. We estimated median ages at menarche by categories of sociodemographic correlates using Kaplan-Meier cumulative probabilities. Values in the text are medians (IQRs). Cox proportional hazards models were used to estimate HRs and 95% CIs, with decimal age as the time scale.

TABLE 1 Age at menarche in 456 Colombian schoolgirls according to sociodemographic characteristics

Baseline characteristics	n[1]	Median age at menarche,[2] y	HR (95% CI)[3]
Girl's birth, y			
1993–1996	165	12.4	1.00
1997–1998	176	12.4	1.21 (0.96, 1.54)
1999–2001	115	12.2	1.28 (0.94, 1.74)
P-trend[4]			0.07
Girl born in Bogotá			
Yes	382	12.4	1.00
No	51	12.2	1.22 (0.85, 1.76)
P			0.28
Girl's height-for-age *z* score[5]			
<−2	42	13.4	0.36 (0.24, 0.55)
−2 to <−1	132	12.8	0.45 (0.33, 0.61)
−1 to <0	157	12.2	0.82 (0.62, 1.09)
0 to <1	97	12.0	1.00
≥1	21	11.7	1.34 (0.76, 2.37)
P-trend			<0.0001
Girl's BMI-for-age *z* score[5]			
<−1	64	12.9	0.72 (0.51, 1.00)
−1 to <0	150	12.6	0.91 (0.70, 1.19)
0 to <1	160	12.3	1.00
≥1	74	11.9	1.82 (1.33, 2.49)
P-trend			<0.0001
Time viewing television/playing games, h/wk			
<10.0	129	12.6	1.00
10.0–19.9	112	12.3	1.08 (0.81, 1.44)
20.0–29.9	90	12.3	1.05 (0.77, 1.43)
≥30.0	52	12.5	0.95 (0.65, 1.38)
P-trend			0.88
Time playing outdoors, h/wk			
<1.5	104	12.5	1.00
1.5–4.4	112	12.2	0.97 (0.71, 1.33)
4.5–9.9	87	12.5	0.92 (0.66, 1.30)
≥10	72	12.3	1.03 (0.72, 1.48)
P-trend			0.99
Mother's age at girl's birth, y			
<20	58	12.3	1.00
20 to <25	120	12.3	0.98 (0.68, 1.43)
25 to <30	117	12.3	0.92 (0.64, 1.34)
30 to <35	85	12.6	0.90 (0.61, 1.33)
≥35	53	12.9	0.77 (0.50, 1.21)
P-trend			0.21
Mother's age at menarche, y			
<11.5	40	11.9	1.00
11.5 to <12.5	89	12.3	0.80 (0.52, 1.25)
12.5 to <13.5	110	12.5	0.66 (0.43, 1.00)
13.5 to <14.5	90	12.5	0.65 (0.42, 1.01)
≥14.5	82	13.0	0.48 (0.31, 0.76)
P-trend			0.0006
Mother's education, y			
Incomplete primary (1–4)	32	12.2	1.18 (0.72, 1.94)
Complete primary (5)	86	12.3	0.95 (0.70, 1.28)
Incomplete secondary (6–10)	107	12.5	0.85 (0.65, 1.11)
Complete secondary (11)	187	12.4	1.00
University (≥12)	19	13.2	0.76 (0.41, 1.40)
P-trend			0.80
Mother's parity			
1	50	11.9	1.00
2	154	12.4	0.65 (0.46, 0.93)
3	148	12.5	0.54 (0.38, 0.78)
4	41	12.1	0.59 (0.37, 0.95)
≥5	36	12.9	0.53 (0.33, 0.85)
P-trend			0.01
Mother's BMI, kg/m^2			
<18.5	17	12.2	1.29 (0.70, 2.37)
18.5–24.9	261	12.4	1.00
25.0–29.9	119	12.6	0.96 (0.75, 1.23)
≥30.0	40	12.3	1.41 (0.96, 2.06)
P-trend			0.47
Socioeconomic status[6]			
1	29	12.8	1.00
2	162	12.5	1.41 (0.86, 2.31)
3	223	12.2	1.79 (1.10, 2.93)
4	39	12.0	2.27 (1.26, 4.07)
P-trend			0.0006

[1] *n* ranges from 375 to 456 due to missing values.
[2] From Kaplan-Meier survival probabilities.
[3] From Cox proportional hazards models with age at menarche as the outcome and each predictor as the covariate.
[4] From a Wald test of a covariate representing ordinal categories of the predictor, introduced into the model as continuous.
[5] According to the WHO (23).
[6] According to the city's classification of neighborhoods' public services fees.

We next examined the distribution of the sociodemographic correlates across levels of red meat intake frequency. For continuous correlates, we estimated tests for linear trend from linear regression models in which a variable representing ordinal categories of red meat intake was introduced as a continuous predictor. We used robust estimates of the variance in these models to overcome potential deviations from the multivariate normality assumption (24). For categorical correlates, we used Cochran-Armitage tests. We also estimated the correlations (Spearman) of red meat intake frequency with that of other animal food groups.

In bivariate analysis, we estimated median ages of menarche and HRs with 95% CI by intake frequency categories of red meat and other animal food groups using Kaplan-Meier and Cox regression analyses. We conducted tests of linear trend for the associations between intake frequency of each food group and menarche by introducing into each Cox model a variable representing ordinal categories of intake frequency as a continuous predictor and performing a Wald test.

Finally, we conducted multivariable analyses of the association between red meat intake frequency and age at menarche by fitting a Cox proportional hazards model that included as covariates sociodemographic characteristics that were independent predictors of menarche, other relevant animal food groups, and total energy intake. Height-for-age and BMI-for-age *z* scores were deliberately excluded from the multivariable model because they could be mediators on the causal pathway between red meat intake and age at menarche (25). The proportional hazards assumption was verified with the use of terms for the interaction between time and covariates. This assumption was met in all models.

Because there were missing values in some covariates, we conducted supplemental analyses in which these missing values were estimated using a Markov Chain Monte Carlo multiple imputation technique (26) before their inclusion in the multivariable models. Results from 10 multiple imputation cycles were combined with the use of the PROC MIANALYZE routine of SAS Software version 9.3 (SAS Institute, Cary, North Carolina).

Although our primary aim was to estimate the total effect of red meat intake on age at menarche, we conducted additional supplemental analyses to ascertain the potential mediating effect of BMI-for-age by introducing it as a covariate into the multivariable models. These analyses assumed a lack of interaction between frequency of intake and BMI-for-age on age at menarche and no unmeasured confounding of the association between BMI-for-age and age at menarche (27). Results were considered statistically significant when $P < 0.05$.

Results

The mean age ± SD of girls at recruitment was 8.4 ± 1.7 y. Their mean ± SD red meat intake frequency was 1.3 ± 1.2 times/d. Mean total energy intake was 1478 ± 718 kcal/d. The median length of follow-up was 5.6 y (IQR 2.6–6.8 y). It was shorter for girls who were censored (median 2.5 y; IQR 1.7–4.0 y) than for those who had menarche during follow-up (median 6.2 y; IQR 5.2–6.8 y). Nevertheless, the 2 groups did not differ from each other with respect to frequency of red meat intake, age, socioeconomic status, or BMI-for-age.

The estimated median age at menarche was 12.4 y (IQR 12.2 to 12.5 y). Girls' baseline height-for-age *z* scores, BMI-for-age *z* scores, and socioeconomic status were inversely associated with age at menarche, whereas maternal age at menarche and parity were positively associated with age at menarche (Table 1).

Red meat intake frequency was positively related to height-for-age and BMI-for-age *z* scores, total energy intake, and socioeconomic status and inversely associated with maternal parity (**Table 2**). Red meat intake frequency also correlated with dairy ($\rho = 0.45$, $P < 0.0001$) and poultry ($\rho = 0.44$, $P < 0.0001$) frequency of intake (**Supplemental Table 1**).

In bivariate analysis, red meat intake frequency was inversely associated with age at menarche (**Table 3**). When compared with girls with red meat intake <4 times/wk, those with intake ≥2 times/d had a significantly earlier age at menarche (HR: 1.41; 95% CI: 1.02, 1.95; *P*-trend = 0.04). We also noted that intake frequency of canned tuna/sardines was positively related to age at menarche in a nonlinear manner; girls with intake ≥1 time/mo had a significantly later age at menarche than girls with tuna/sardine intake <1 time/mo (HR: 0.78; 95% CI: 0.62, 0.98; $P = 0.03$).

After adjustment for total energy intake, maternal parity, and socioeconomic status, red meat intake frequency remained linearly associated with age at menarche (Table 3). When compared with girls with red meat intake <4 times/wk, those consuming it ≥2 times/d had a significantly earlier age at menarche (HR: 1.64; 95% CI: 1.11, 2.41; *P*-trend = 0.0009). On the other hand, when compared with girls with tuna/sardine intake <1 time/mo, those with intake >1 time/wk had a later age at menarche (HR = 0.62; 95% CI: 0.42, 0.90; $P = 0.01$). Results remained essentially unchanged after additional adjustment for dairy and poultry intake frequencies, maternal age at menarche, and time spent playing outdoors. Similarly, using multiple imputation of missing covariate values did not change the results.

In supplemental mediation analyses, introducing BMI-for-age *z* scores at baseline into the multivariable imputed model resulted in an attenuation of the estimates for red meat. When compared to that of an intake frequency <4 times/wk, the adjusted HRs (95% CI) for categories 4–6 times/wk, 1 time/d, and ≥2 times/d were, respectively, 1.16 (0.85, 1.60), 1.17 (0.85, 1.62), and 1.33 (0.90, 1.96) (*P*-trend = 0.17). The estimates for intake frequency of tuna/sardines did not change.

Intake frequency of other animal food groups including dairy, poultry, eggs, freshwater fish, or innards was not significantly associated with age at menarche in bivariate (**Supplemental Table 2**) or multivariable adjusted (**Supplemental Table 3**) analyses.

Discussion

In this cohort study of school-age girls, childhood intake frequency of red meat was inversely related to age at menarche

TABLE 2 Sociodemographic characteristics of 456 Colombian schoolgirls according to frequency of red meat intake in childhood[1]

Baseline characteristics	Frequency of intake				
	<4 times/wk	4–6 times/wk	1 time/d	≥2 times/d[2]	*P*-trend[3]
n	120	129	109	81	
Girls					
Age, y	8.5 ± 1.7	8.3 ± 1.6	8.2 ± 1.7	8.5 ± 1.7	0.59
Born in Bogotá, %	87.3	88.1	89.1	87.3	0.90
Height-for-age *z* score[4]	−0.84 ± 0.92	−0.71 ± 1.03	−0.76 ± 1.05	−0.49 ± 0.97	0.03
BMI-for-age *z* score[4]	−0.07 ± 0.93	0.01 ± 0.99	0.14 ± 0.94	0.24 ± 0.93	0.01
Time viewing television/playing games, h/wk	17.5 ± 14.9	18.2 ± 14.4	16.2 ± 11.8	16.6 ± 10.5	0.44
Time playing outdoors, h/wk	7.1 ± 11.8	5.7 ± 7.9	6.0 ± 7.0	7.0 ± 7.3	0.89
Total energy intake, kcal/d	1175 ± 524	1314 ± 470	1471 ± 590	2147 ± 984	<0.0001
Mothers					
Age at girl's birth, y	27.5 ± 6.6	26.8 ± 6.0	26.6 ± 6.0	27.8 ± 6.8	0.96
Age at menarche, y	13.3 ± 1.4	13.4 ± 1.5	13.1 ± 1.4	13.3 ± 1.5	0.54
Education, y	8.4 ± 3.3	8.9 ± 3.0	8.3 ± 3.0	8.9 ± 3.1	0.73
Parity, *n* live births	2.9 ± 1.2	2.6 ± 1.0	2.6 ± 1.1	2.5 ± 1.0	0.04
BMI, kg/m^2	24.7 ± 3.8	24.1 ± 3.9	24.4 ± 4.2	24.2 ± 3.6	0.38
Socioeconomic status[5]	2.4 ± 0.8	2.8 ± 0.6	2.7 ± 0.7	2.6 ± 0.7	0.02

[1] Values are means ± SDs, unless noted otherwise.
[2] Totals <456 due to missing responses to specific food items in the FFQ.
[3] For continuous variables, a Wald test was conducted when a variable representing ordinal categories of red meat intake was introduced in a linear regression model as a continuous predictor. Robust estimates of the variance were specified in each model. For place of birth, the Cochran-Armitage test was used.
[4] According to the WHO (23).
[5] According to the city's classification of household's public services fees.

TABLE 3 Multivariable-adjusted HRs for age at menarche in Colombian schoolgirls according to red meat and fatty fish intake frequencies during childhood

Frequency of intake	n^1	Median age at menarche,[2] y	Unadjusted HR (95% CI)[3]	Adjusted HR (95% CI)[4]	Adjusted HR (95% CI)[5]
Red meat					
<4 times/wk	120	12.7	1.00	1.00	1.00
4–6 times/wk	129	12.3	1.23 (0.91, 1.65)	1.16 (0.84, 1.60)	1.20 (0.87, 1.64)
1 time/d	109	12.3	1.24 (0.92, 1.69)	1.32 (0.95, 1.83)	1.28 (0.93, 1.76)
≥2 times/d	81	12.3	1.41 (1.02, 1.95)	1.64 (1.11, 2.41)	1.59 (1.09, 2.32)
P-trend[6]			0.04	0.009	0.02
Tuna/sardines					
<1 time/mo	144	12.2	1.00	1.00	1.00
1–3 times/mo	122	12.6	0.77 (0.58, 1.02)	0.70 (0.52, 0.94)	0.70 (0.53, 0.93)
1 time/wk	110	12.4	0.79 (0.59, 1.06)	0.67 (0.49, 0.92)	0.66 (0.48, 0.90)
>1 time/wk	75	12.5	0.79 (0.57, 1.10)	0.62 (0.42, 0.90)	0.69 (0.48, 0.98)
P-trend[6]			0.11	0.008	0.01

[1] *n* ranges from 439 to 451 due to missing values.
[2] From Kaplan-Meier survival probabilities.
[3] From Cox proportional hazards models with age at menarche as the outcome and indicator variables for intake frequency of each food as predictors.
[4] Complete case analysis (*n* = 407). Estimates are from Cox proportional hazards models with age at menarche as the outcome and predictors that included indicator variables for red meat and tuna/sardine intake frequencies, total energy intake, maternal parity, and socioeconomic status.
[5] Multiple imputation analysis (*n* = 456). Estimates are from multivariable Cox proportional hazards models including imputed data for missing values.
[6] From a Wald test when a variable representing ordinal categories of each food group was introduced in the Cox model as a continuous covariate.

in a linear fashion. This relation was independent of potential sociodemographic and dietary confounders. Unexpectedly, we also found that canned tuna/sardine intake frequency was positively associated with age at menarche.

Although earlier studies had reported associations between intake of animal foods and age at menarche (11–17), our study substantially extends previous findings by examining intake frequency of specific categories of animal food sources in midchildhood. The association of red meat intake frequency and age at menarche is generally consistent with previous studies that linked prepubertal consumption of animal protein and food sources to earlier puberty (11–17). Nevertheless, it is opposite to findings from an investigation of US girls in which intake of red meat at ages 9–14 y was not associated with age at menarche (18). One reason for the discrepancy could be related to the timing of the dietary assessment. Whereas dietary assessment in our study occurred in girls with a mean age of 8 y, girls in the US study had a mean age of 11 y at baseline and were much closer to the timing of menarche. Some authors have proposed that diet at younger ages may be more important for the timing of puberty than peripubertal intake (13). In fact, some argue that the timing of puberty is determined by environmental conditions acting as early as around the time of birth (28). This is not necessarily contrary to our findings if we assume that diet tracks during childhood. Additionally, reverse causation bias could have affected the results of the US study because the dietary exposures were assessed within a short period with respect to the outcome. Because menarche is a relatively late event during sexual development, girls may have initiated puberty at the time of dietary assessment, and their diet might have changed as a consequence (18).

One pathway to explain potential effects of animal foods, particularly dairy, on the timing of puberty has been related to a protein-mediated stimulation of insulin-like growth factor I secretion (29, 30). However, meat intake has not been related to insulin-like growth factor I secretion, and there could be other plausible mechanisms. Availability of micronutrients found in red meat such as iron and zinc could signal the body to initiate puberty because these nutrients are essential for sustaining pregnancy and for the offspring's survival and development. In a small randomized trial, zinc supplementation resulted in earlier age at menarche (31). Another mechanism could be through the development of adiposity. Childhood obesity is related to early menarche (25), and intake of some foods in the red meats group, including hot dogs and hamburgers, was related to children overweight in this population (32). In supplemental mediation analyses, the association of red meat intake frequency with age at menarche was attenuated after adjustment for BMI-for-age z score at baseline, suggesting that part of the effect of red meat on age at menarche could be mediated through body size. There are also other potential mechanisms related to substances ingested with red meat, including estrogenic mycotoxins such as zearalenone (33, 34) or heterocyclic amines produced during preparation of red meat at high temperatures (34).

Previous investigations have consistently shown an association between early onset of menses and breast cancer in adulthood (1). Also, red meat intake during adolescence has been related to premenopausal breast cancer risk (19). Our finding suggests a potential mechanism to explain these relations, in that early age at menarche could be a mediator on the causal pathway between adolescent red meat intake during childhood and breast cancer risk.

Of note, we incidentally found that tuna/sardine intake frequency was related to a later age at menarche. Two previous studies that examined associations between fish intake and age at menarche found no relation (35, 36). Although the finding in our study could have been due to chance, there are potential underlying mechanisms to explain it. Tuna and sardines are rich in long-chain PUFAs, which are related to more favorable cardiovascular profiles in adolescents (37), including slower weight gain (38).

Our study has several strengths. First, its longitudinal design precluded bias due to reverse causation. The prospective collection of data on age at menarche prevented recall bias. Any misclassification of the outcome is unlikely to be differential with respect to exposure status. In addition, follow-up was high. Other strengths include the ability to examine different animal food groups and the possibility to adjust for important confounders. Adjusted HRs for red meat intake frequency became stronger than the unadjusted estimates after controlling for negative confounding by tuna/sardine intake. The association of tuna/sardine intake frequency was strengthened in multivariable analyses after controlling for negative confounding by red meat intake and maternal parity. We assessed intake using an FFQ that measures animal food intake validly (21). Our data were internally consistent considering that the associations of age at menarche with known predictors of puberty were in the expected directions. Red meat intake was also positively associated with height, BMI, total energy intake, and socioeconomic status as expected in this population. Finally, we accounted for missing data in the analysis using state-of-the-art multiple imputation techniques.

The study also has some limitations. First, the results may not be generalizable to girls of the highest socioeconomic levels. Second, unmeasured intake of foods or nutrients that are associated with red meat intake and are independent predictors of age at menarche [e.g., fiber, isoflavones (39, 40)] could have introduced residual confounding. Third, misclassification of exposure due to measurement errors in the FFQ could have been a source of bias. Because the FFQ was administered to the girls' mothers, intake of foods eaten away from home may have been underestimated. Measurement error in the report of diet is likely not associated with the recall of age at menarche; thus, its potential consequence could be an attenuation of the true underlying effect. Fourth, some girls were lost to follow-up, and this could potentially introduce selection bias. Fifth, we could not account for potential changes in diet after the childhood measurement. Sixth, we were unable to examine associations of micronutrients present in red meat with age at menarche. Finally, although the short-term recall of age at menarche is highly reliable (41), the effects of diet on earlier events of puberty may differ from those on menarche. It is uncertain whether they represent effects on the onset rather than the duration of puberty.

In summary, red meat intake frequency during childhood was inversely associated with age at menarche, whereas fatty fish intake frequency was positively associated with age at onset of menses. Future studies should examine the role of particular components of red meat, including micronutrients and by-products of processing, on the timing of sexual maturation. Examining the role of fish intake and the timing of puberty in other populations is also warranted. Whether replacing red meat with other animal food sources influences age at menarche would need to be determined in intervention studies.

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EV designed the research; CM and MM-P conducted the research; ECJ and EV analyzed the data, wrote the paper, and had primary responsibility for final content. All authors read and approved the final manuscript.

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