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Dietary Iron Intake in Relation to Age of Menarche: A Perspective Cohort Study in Chilean Girls

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Dietary Iron Intake in Relation to Age of Menarche: A Perspective Cohort Study in Chilean Girls Abstract

Background: The early onset of menarche is considered an important risk factor for a number of diseases in adulthood. Dietary iron intake may be related to pubertal timing due to its role in childhood growth, optimal cognitive development, and reproductive function.
Objective: We investigated the relation between dietary iron intake and age of menarche in a prospective cohort of Chilean girls.
Methods: A total of 602 Chilean girls are included in the Growth and Obesity Cohort Study. The subjects have been followed longitudinally since they were 3–4 y old (from 2006 to the present). Starting in 2013, diet was assessed every 6 months via a 24-h recall. The date of menarche was reported every 6 months. Our analysis included 435 girls with prospective data on diet and age of menarche. We used a multivariable Cox proportional hazards regression model to estimate hazard ratios (HRs) and 95% CIs for the association between cumulative average iron intake and age at menarche.
Results: The majority of girls in our cohort (99.5%) attained menarche with an average (standard deviation) age at menarche of 12.2 (0.9) years. The mean dietary iron intake among girls was 13.5 mg/day with a range of 4.0 – 30.6 mg/day. Total iron intake was not associated with age at menarche in calorie- or multivariable-adjusted models. After adjusting for calorie intake, mother's age of menarche, percent of calories from fat and protein, folate and vitamin B12 intake, and time spent watching television in childhood, the HRs (95% CIs) for menarche in increasing quartiles of iron intake were 0.83 (0.61, 1.1), 0.72 (0.52, 0.98), and 0.90 (0.62, 1.31), respectively, compared to girls in quartile 1 (p-trend 0.32). Similar associations were observed after further adjustment for BMI z-score and height before age of menarche.
Conclusion: Dietary iron intake was not associated with age at menarche in Chilean girls with adequate iron intake.
Keywords: diet, iron, puberty, development, adolescent, menarche

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INTRODUCTION

The mean age of menarche (AOM) in developed countries is typically between ages 12-13 years, and its onset is considered an important health marker, as an early AOM can serve as a risk factor for a number of diseases in adulthood, including hormone-related cancers (4, 5), cardiovascular disease (19), and all-cause mortality (8, 13). Particularly, an earlier onset of AOM is associated with an increased risk of developing breast cancer, possibly due to greater length of exposure to estrogens (12, 14, 18); further, early menarche may be associated with an increased risk of ovarian cancer and ischemic heart disease (6). Though there are numerous factors, including genetics, that contribute to the variation in onset of menarche, in the past century AOM has decreased by several years suggesting that environmental characteristics could play a significant role (11, 2).

Dietary iron intake may be of particular interest because it plays an essential role in childhood growth, optimal cognitive development, and reproductive function (27; 24). Physiologically, a deficiency of iron in the diet may detrimentally impact a range of biological functions throughout the body due to lower hemoglobin synthesis and less activity of iron-containing enzymes in the brain (27). Moreover, as a child grows, the demand for iron accelerates and the risk for iron deficiency increases, due to ongoing growth associated with puberty (27). Despite these increased demands, adequate iron intake is less common among young girls compared with boys, leading to higher prevalence of anemia and iron deficiency (28). Roughly 9-40% of adolescent girls are iron deficient, highlighting a public health concern and a purpose for further research among adolescent girls relating to dietary iron intake and its potential relation to age of menarche (27).

To date, the literature on the relationship between premenarchal dietary iron intake and AOM is mixed with one small study finding a positive association and a larger study finding no association (16, 29). An additional study found that plasma ferritin, a proxy for iron stores in the body, measured during mid-childhood was positively associated with AOM (30). Given the inconsistency of the evidence, we aimed to investigate the association between dietary iron intake and age of menarche in a prospective cohort of Chilean girls.

METHODS

Study Population, Recruitment, and Design.

The Growth and Obesity Cohort is an ongoing study that began in 2006 by recruiting 3 and 4-year-olds who were attending nursery schools of the National Nursery Schools Council Program in the Southeast area of Santiago, Chile. To be eligible for the study, children had to of been born at term (between 37 to 42 weeks), had a birthweight of greater than 2500 g and less than 4000 g, and not in a current health state that could affect their growth (i.e having allergies to certain foods). Between 2006 and 2010, participants in the Growth and Obesity Cohort paid a visit to the Nutrition and Food Technology Health Clinic at least once a year for a physical examination. Beginning in 2011, participants began visiting the clinic every six months. During these clinic visits participants provided several types of biological specimens and underwent standardized anthropometric, growth, and sexual maturation assessments throughout follow up. At each in-person or telephonic contact, girls were also asked if they had begun menstruation and, if they had, the date of their first menstrual period. Beginning in 2013, at the in-person clinic visits, diet assessments via 24-hr recall were conducted. Further details on in-depth recruitment procedures and study design have been previously published (17). The protocol for this study was approved by the Ethics Committee of the Institute of Nutrition and Food Technology, University of Chile. Parents and guardians of the participants gave informed consent before data collection began.

Inclusion/Exclusion.

Of the 602 girls participating in the original cohort, 60 girls were excluded due to lack of active follow-up. Out of the remaining 542 girls, 32 had no diet assessments and 75 were missing a diet assessment prior to menarche. This left a total of 435 girls with complete information to be included in our analysis.

Diet Assessment.

Diet was assessed using 24-hour recalls conducted by trained dietitians via the United States Department of Agriculture (USDA) multiple-mass method. This method allowed the participants to receive aid from the dieticians to help recall and properly describe foods that they consumed in the last 24 hours (20). A food atlas was validated in the Chilean National Dietary Survey, which showed serving sizes of common foods and beverages in glasses, mugs, bowls, and plates (9); participants were given the atlas to use. Participants were provided images displaying common condiments and oils in measuring cups, spoons, and dollops accompanied by size references. The portion sizes were determined via direct measurements of the portions that were shown in the pictures. The food reported was then converted to grams, via the food atlas. To calculate nutrient values, including dietary iron intake, the USDA Food and Nutrient Database for Dietary Studies was used (1). Chilean mixed dishes that were not found in the USDA Food and Nutrient Database were broken down into their ingredients for linkage and matched to the food with the closest nutrient profile, which was finalized by a trained dietitian. Throughout the dietary assessment, various quality assurance procedures were implemented to assure the quality of the data (3). To reduce random error, we calculated the cumulative average of iron intake, if a girl had multiple 24-hour recalls before she attained menarche or the end of follow-up. Nutrient intakes were adjusted for total energy intake using the nutrient residual method (32).

Covariates.

Weight and height were measured every six to twelve months during follow-up via standardized techniques by trained personnel (7). BMI (in kg/m^2) was calculated as weight in kilograms divided by squared height. We estimated BMI for age z-scores on the basis of WHO 2007 growth references (10). Mothers self-reported their own age at menarche at an in-clinic study visit. The mother's current height and weight were also measured by a trained dietician. Information on television (TV) watching after school and while eating during childhood was collected at age 6 by self-report with the following response options: no, sometimes, and yes.

Statistical Analysis.

The baseline demographics are shown as mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables. Cumulative average dietary iron intake was categorized into quartiles of intake for the primary analysis. We used the Kruskal-Wallis test to compare differences in continuous baseline demographic and dietary variables across quartiles of iron intake, and chi-square tests and an extended Fisher's exact test (when 1 or more cell counts were less than or equal to 5) for categorical variables.

For the analysis of cumulative averaged iron intake and age at menarche, we used a Cox proportional hazards regression model to account for differing durations of follow-up among girls and for the failure of some girls to achieve menarche during follow-up. In the analyses, we used calendar time in months from birth to age at menarche as the time scale. Failing was defined as attaining menarche. If a girl had not reached menarche by the end of follow-up she was censored. We evaluated the proportional hazards assumption with the use of a likelihood ratio test; proportional hazards assumptions were met in all of our analyses. Tests for trend were conducted using a variable with the median intake in each category.

Confounding was assessed using prior knowledge as well as descriptive statistics from our study population. Covariates in the final adjusted model included average calorie intake, percent of calories from fat and protein, folate intake, vitamin b12 intake, mother's age of menarche, and time spent watching television in childhood. Since changes in body weight may mediate the associations between dietary iron intake and AOM, we omitted BMI for age z-score from the initial multivariable model to avoid over-adjustment and associated biases (22). However, due to the strong associations between BMI and AOM, we also present multivariable models additionally adjusted for these variables. Sensitivity analyses were performed to address potential bias due to differential exposure misclassification using only the girl's first 24-hr recall to define exposure and restricting the analysis to only girls with at least two 24-hr recalls. All statistical tests were 2-sided and performed at the 0.05 level of significance. We used SAS 9.4 (Cary, NC) for all analyses.

RESULTS

Of the 435 girls in our analysis, the majority (99.5%) attained menarche by May 2019, with an average (standard deviation) age at menarche of 12.2 (0.9) years (Figure 1). The mean dietary iron intake among girls was 13.5 mg/day with a range of 4.0 – 30.6 mg/day (Figure 2). Only 3.7% of girls consumed below the recommended dietary allowance (RDA) of iron intake (8 mg/day). The mean BMI z-score prior to menarche in our cohort was 0.85 with 48.3% and 15.6% of the girls being considered overweight (BAZ>1) and obese (BAZ>2), respectively. Overall, 39.5% of girls completed one 24-h recall, 23.0% completed two 24-h recalls, 17.9% completed three 24-h recalls, and 18.5% of girls completed at least four 24-h recalls before reaching age of menarche (Table 1).

There were some significant differences in macro- and micro-nutrient intakes across quartiles of iron intake. On average, girls in the highest quartile of iron intake had a higher percentage of calories from protein and carbohydrate, higher folate and vitamin B12 intakes, and lower percentage of calories from fat (Table 1). The rest of the demographic and dietary variables were similar across quartiles. There was a statistically significant difference of number of 24-hour recalls across quartiles of iron intake (p-value: 0.02). Girls in the lowest quartile of iron intake were more likely to have only one 24-hour recall prior to menarche (55.6%) compared to girls in the highest quartile of iron intake (38.5%).

Total iron intake was not associated with age at menarche in calorie-adjusted or multivariable adjusted models (Table 2). After adjusting for average calorie intake, the HRs (95% CIs) for menarche in increasing quartiles of iron intake were 0.87 (0.66, 1.14), 0.88 (0.67, 1.15), and 0.99 (0.76, 1.29), respectively, compared to quartile 1 (p-trend 0.93). With further adjustment for mother's age of menarche, percent of calories from fat and protein, folate intake, vitamin B12 intake, and time spent watching television in childhood, the corresponding HRs (95% CIs) for menarche in increasing quartiles of iron intake were 0.77 (0.57, 1.103), 0.75 (0.55, 1.02), and 0.79 (0.55, 1.15), respectively, compared to quartile 1 (p-trend 0.32). Moreover, with further adjustment for BMI z-score and height before age of menarche, the corresponding HRs (95% CIs) for menarche in increasing quartiles of iron intake were 0.78 (0.57, 1.06), 0.68 (0.50, 0.93), and 0.81 (0.56, 1.18), respectively, compared to quartile 1 (p-trend 0.33). No significant associations were found when iron intake was modelled as a continuous linear variable (HR 0.97 per 5mg/day increase 95% CI 0.87, 1.08) or quadratic variable.

To address potential bias due to differential exposure misclassification we performed two sensitivity analyses- in the first we used only the girl's first 24-hr recall to define exposure (as opposed to a cumulative average of all available recalls) and in the second we restricted the analysis to only girls with at least two 24-hr recalls prior to menarche (n=263 girls). In both subanalyses, similar to the main results, there were no associations between iron intake quartiles and age at menarche (Supplemental Table 1).

DISCUSSION

In this prospective cohort of Chilean girls, total dietary iron intake was not associated with age at menarche in calorie-adjusted or multivariable adjusted models. Due to the low number of girls with iron intakes below the RDA, we were limited to evaluating the association between iron intake within the range of adequacy on the timing of menarche. Our results indicate that iron intake during late childhood is not an important determinant of pubertal timing.

The existing literature on dietary iron intake and age at menarche is mixed. The first study came from a prospective cohort of 230 non-Hispanic White girls from southern California aged 9-15 at baseline and followed for ~3 years (16). Diet was assessed via 24-hr dietary recalls that were administered unannounced 2 to 4 times during the school year until the girl experienced menarche (16). The average dietary iron intake was 14 mg/day. Overall, this study found that girls in the highest quartile of iron intake (16-40 mg/day) had, on average, a 7–8 month later AOM than girls in the lowest quartile of dietary iron intake (0-10 mg/day) (16); however these estimates were not adjusted for any other demographic, dietary, or lifestyle characteristics. The second study on this topic came from a prospective cohort of 5th grade girls in Quebec City, Canada (n=2,299) where diet data was collected on three occasions using a 3-day diet record and AOM data was collected via letters sent to the mothers of the at the end of follow-up (29). The average dietary iron intake was 11.1 mg/day among the 911 girls reaching menarche and 10.9 mg/day among the 1,388 girls who did not reach menarche (29). The authors found no association between iron intake and age at menarche with incidence density ratios for menarche (95% CI) of 1.1 (0.9-1.4), 1.1 (1.0 -1.4), and 1.2 (1.0-1.4) for quartiles 2, 3, and 4 of iron intake, respectively, compared to quartile 1 after adjustment for age at baseline and mothers age at menarche (29). Finally, the third study on this topic came from a prospective cohort of Columbian girls aged 5-12 years (n=3,202) (30). Micronutrient biomarkers including plasma ferritin, a marker of iron status, were assessed in blood samples collected at baseline and menarche was assessed at home or school visits or over the phone. Over a median follow-up time of 5.7 years, 80.9% (n=1,184) of the cohort reached menarche. The HRs (95% CIs) for menarche in increasing quartiles of plasma ferritin were 1.12 (0.94, 1.33), 1.06 (0.89, 1.26), and 0.86 (0.72, 1.02), respectively, compared to quartile 1 (p-trend 0.06). The HR of menarche for every 1 SD increase in plasma ferritin (23.2 μg/l) was 0.94 (95 % CI 0.88, 0.99). In a sub-analysis, the association between ferritin and AOM was found to be statistically significant only in girls who were between 9 and 10 years of age at the time of ferritin assessment. The authors concluded that higher iron status in middle childhood was related to later age at menarche (30).

Taken together, two out of the three past studies suggest that girls with higher iron status (reflected by higher dietary intake or higher plasma ferritin levels) tend to experience later menarches. While our HRs for obtaining menarche were all below 1 for iron intake quartiles 2, 3, and 4, compared with quartile 1, suggesting a later age at menarche with higher iron intakes, these results were not significant. Given our modest sample size, it could be that we were underpowered to detect small differences in AOM. For example, in the Columbian study, the difference in AOM between girls in the highest and lowest quartiles of plasma ferritin concentrations was only 1.2 months. It could also be that due to our fairly narrow distribution of iron intake we had too few girls with very low or very high iron intakes where we might have observed larger differences. Since plasma ferritin and dietary iron intake are not directly interchangeable, it is hard to compare average iron levels between our two cohorts. Finally, plasma ferritin is considered a more objective marker of iron status compared to self-reported dietary intake and may represent iron intake over a longer time frame which may have enhanced their ability to detect an association.

There were limitations to our study as alluded to above. One noteworthy limitation is that all dietary assessments, including 24-hr recalls, are prone to measurement error. However, given that most of the girls in our study had multiple 24-hr diet recalls and we used a cumulative average of them, the probability of dietary misclassification is decreased. Moreover, due to the prospective nature of our cohort, this dietary misclassification would most likely result in bias towards the null. In regards to our outcome assessment, since the girls were prospectively asked about their date of menarche every 6 months, we do not expect large errors in the reporting of age of menarche. Due to the observational design of our study, residual confounding by other diet and lifestyle factors is possible despite our adjustment for a variety of factors. Finally, our study may lack external validity. Since all the girls in our cohort were Chilean and of low to middle socioeconomic status the generalizability of our results to girls of other populations with varying ethnicities and socioeconomic status is unclear. Among girls participating in the NHANES 2003-2012 cohorts, 26.9% (CI: 20.5, 33.3) had a dietary iron intake below RDA, compared to only 4% of girls in our cohort (25). On the other hand, our study had several strengths including its prospective design that included multiple 24-hr recalls per girl and consistent tracking of age at menarche every 6 months.

In conclusion, total iron intake was not associated with age at menarche in our prospective cohort of Chilean girls. Given the inconsistency of observed associations between iron intake and age at menarche across populations, further investigation is needed.

Table 1: Demographics of Chilean girls in the Growth and Obesity Cohort Study who were included in the prospective analysis of iron intake and age at menarche (2006–2019).

	Quartiles of Iron Intake				
	Q1	**Q2**	**Q3**	**Q4**	
Iron Intake, mg/day, median (min-max)	10.0 (4.0-11.2)	12.3 (11.3-13.2)	14.0 (13.3-15.3)	17.41 (15.3-30.6)	**P-Value***
Height before menarche, cm	147.6 (5.9)	149.1 (6.9)	148.7 (6.1)	148.0 (6.3)	0.35
Weight before menarche, kg	44.3 (8.4)	46.0 (10.0)	46.1 (9.8)	43.6 (8.0)	0.35
BMI-for-age z score before menarche	0.9 (1.1)	0.88 (1.1)	0.92 (1.1)	0.7 (1.0)	0.66
Mother's BMI, kg/m^2	27.1 (5.5)	26.4 (5.0)	27.9 (5.6)	26.4 (44.1)	0.52
Mother's age at menarche, yr	12.8 (1.7)	12.7 (1.6)	12.7 (1.6)	13.1 (1.8)	0.58
Total calories, kcal/day	1874.3 (572.7)	1811.5 (471.5)	1851.4 (443.8)	1924.2 (616.9)	0.76
Fat intake, % of energy	32.6 (7.8)	29.8 (5.7)	29.2 (5.3)	27.2 (5.2)	<0.0001
Protein intake, % of energy	13.4 (3.6)	13.8 (2.7)	14.7 (2.8)	14.5 (3.3)	0.0008
Carbohydrate intake, % of energy	55.2 (7.7)	57.8 (6.5)	57.4 (5.6)	59.7 (6.0)	<.0001
Vitamin A intake, mg/day	4765.9 (3453.6)	5174.6 (3527.3)	6527.9 (6935.6)	10424.9 (47493.3)	0.09
Vitamin C intake, mg/day	70.8 (58.9)	135.5 (406.3)	91.9 (223.7)	81.9 (106.3)	0.80
Vitamin E intake, mg/day	6.4 (3.1)	6.2 (2.5)	6.7 (2.9)	6.7 (3.2)	0.87
Folate intake, mcg/day,	251.4 (65.3)	320.0 (80.3)	366.7 (77.5)	451.1 (154.6)	<.0001
Vitamin B12 intake, mcg/d	3.4 (2.0)	3.31 (1.7)	5.4 (8.7)	5.8 (10.4)	0.05
Television Watching Time, n					0.89
(%)	93 (86.1)	92 (84.4)	94 (86.2)	92 (84.4)	
No	4 (3.7)	7 (6.4)	7 (6.4)	3 (2.8)	
Sometimes	6 (5.6)	6 (5.5)	4 (3.7)	8 (7.3)	
Yes	5 (4.6)	4 (3.7)	4 (3.7)	6 (5.5)	
Missing					
24-Hour Recalls, n (%)					0.02
1	60 (55.6)	39 (35.8)	31 (28.4)	42 (38.5)	
2	22 (20.4)	24 (22.0)	28 (25.7)	26 (23.9)	
3	13 (12.0)	23 (21.1)	22 (20.2)	20 (18.4)	
4-6	13 (12.0)	23 (21.1)	28 (25.7)	21 (19.3)	
Girl's age at menarche, n (%)					0.52
≤ 11	11 (10.2)	10 (9.2)	3 (2.8)	11 (10.1)	
11.1- 12	43 (39.8)	40 (36.7)	43 (39.5)	38 (34.9)	
12.1- 13	37 (34.3)	35 (32.1)	41 (37.6)	46 (42.2)	
13.1-14	13 (12.0)	19 (17.4)	18 (16.5)	10 (9.2)	
>14	4 (3.7)	5 (4.6)	4 (3.7)	4 (3.7)	

Data are presented as mean (standard deviation) or n (%) unless otherwise noted.

*p-value was calculated using Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables.

Table 2: Association between cumulative average dietary iron intake and age at menarche in 435 girls in the Growth and Obesity Cohort Study

	Number of Girls	Calorie Adjusted[1] HR (95% CI)	Model 2[2] HR (95% CI)	Model 3[3] HR (95% CI)
Quartile 1 (≤ 11.2 mg/day)	108	1.00 (REF)	1.00 (REF)	1.00 (REF)
Quartile 2 (11.23-13.2 mg/d)	109	0.87 (0.66, 1.14)	0.77 (0.57, 1.03)	0.78 (0.57, 1.06)
Quartile 3 (13.3-15.3 mg/day)	109	0.88 (0.67, 1.15)	0.75 (0.55, 1.02)	0.68 (0.50, 0.93)
Quartile 4 (≥ 15.4 mg/day)	109	0.99 (0.76, 1.29)	0.79 (0.55, 1.15)	0.81 (0.56, 1.18)
P trend		0.93	0.32	0.33

[1] Hazard ratios were estimated from a Cox proportional hazards model adjusted for average calorie intake.

[2] Hazard ratios were estimated from a Cox proportional hazards model adjusted for average calorie intake, percent of calories from fat and protein, folate intake, vitamin b12 intake, mother's age of menarche, and time spent watching television in childhood.

[3] Hazard ratios were estimated from a Cox proportional hazards model adjusted for all the variables in Model 2 as well as body mass index z-score and height before age of menarche.

Supplemental Table 1: Sensitivity analyses for the association between dietary iron intake and age at menarche in girls in the Growth and Obesity Cohort Study.

Multivariable HR[1] (95% CI)	Quartiles of Iron Intake				
	Q1	**Q2**	**Q3**	**Q4**	
Range, mg/day	≤ 11.2	11.3-13.2	13.3-15.3	≥ 15.4	p-trend
Main Analysis (n=435)	1.00 (REF)	0.77 (0.57, 1.03)	0.75 (0.55, 1.02)	0.79 (0.55, 1.15)	0.32
Only first recall per girl (n=435)	1.00 (REF)	1.03 (0.78, 1.37)	0.99 (0.73, 1.33)	0.97 (0.70, 1.35)	0.82
Only girls with ≥2 recalls (n=236)	1.00 (REF)	0.77 (0.57, 1.03)	0.75 (0.55, 1.02)	0.79 (0.55, 1.15)	0.32

[1]Hazard ratios were estimated from a Cox proportional hazards model adjusted for average calorie intake, percent of calories from fat and protein, folate intake, vitamin b12 intake, mother's age of menarche, and time spent watching television in childhood.

Figure 1: Cumulative Incidence of Menarche in 435 girls in the Growth and Obesity Cohort Study

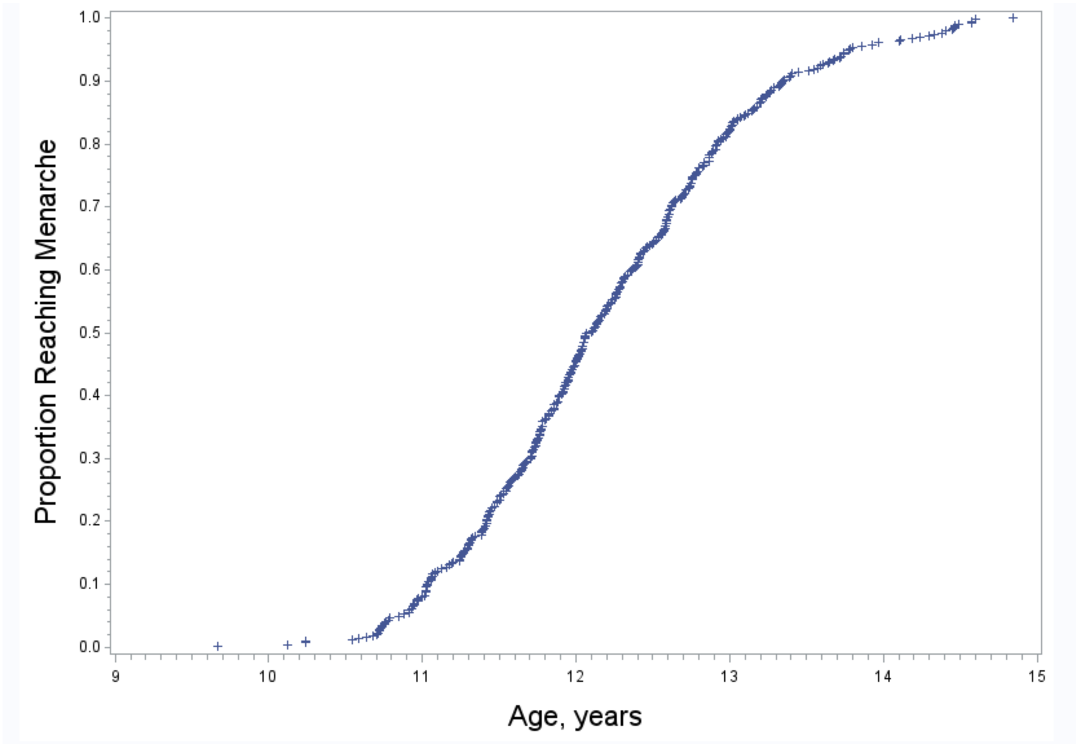
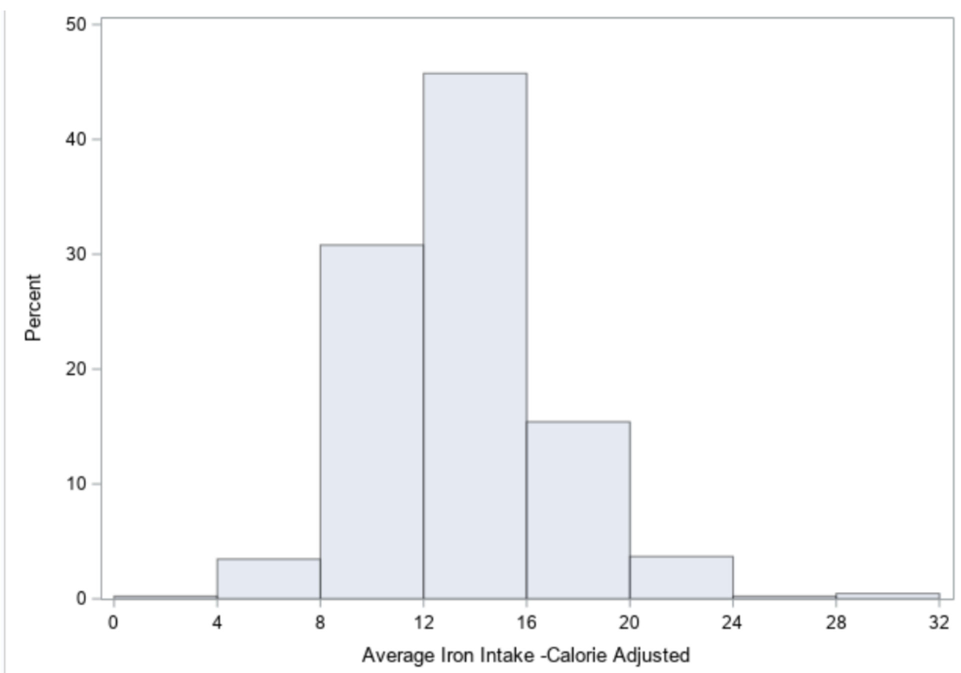


Figure 2: Distribution of Average Iron Intake (mg/day) in 435 girls in the Growth and Obesity Cohort Study



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