Previous evidence suggests that variants in the fat mass and obesity-associated gene (FTO) affect adiposity in an age-dependent fashion in children, and nutritional factors may modify genotype effects. We assessed the effect of FTO rs9939609 on BMI and BMI-for-age Z score changes during childhood in a population-based longitudinal study in the Brazilian Amazon and investigated whether these effects were modified by vitamin D status, an important nutritional factor related to adiposity. At baseline, 1,088 children aged <10 years had complete genotypic and anthropometric data; 796 were followed up over a median 4.6 years. Baseline vitamin D insufficiency was defined as <75 nmol/L. We observed a 0.07 kg/m²/year increase in BMI and a 0.03 Z/year increase in BMI-for-age Z score per rs9939609 risk allele over follow-up (P = 0.01). Vitamin D status significantly modified FTO effects (P for interaction = 0.02). The rs9939609 risk allele was associated with a 0.05 Z/year increase in BMI-for-age Z score among vitamin D–insufficient children (P = 0.003), while no significant genetic effects were observed among vitamin D–sufficient children. Our data suggest that FTO rs9939609 affects child weight gain, and genotype effects are more pronounced among children with insufficient vitamin D levels.

Genome-wide association studies have reliably identified variation at the fat mass and obesity-associated gene (FTO) locus as the strongest genetic effect for obesity risk (1). Age-dependent effects of FTO on BMI have been proposed in studies conducted among children mainly in developed regions worldwide. This locus was associated with lower BMI at the first years of life and earlier adiposity rebound, followed by subsequent greater BMI gain from the end of infancy through childhood (2). Few studies have assessed FTO genotype effects on longitudinal weight gain in children. In addition, there is evidence that physical activity and diet might modify FTO effects in adult populations (3,4). However, such research is still scarce for children.

Vitamin D concentrations have been inversely associated with adiposity indicators mainly in cross-sectional reports. Lower 25-hydroxyvitamin D [25(OH)D] levels are prevalent among overweight and obese children and adolescents aged 6–18 years, according to recent nationally representative analyses conducted in the U.S. (5). Among Mexican boys and girls aged 6–12 years, higher percentages of body fat, as measured by bioimpedance, BMI, triceps skinfold, and waist circumference, were associated with decreased vitamin D levels (6). Decreasing quartiles of 25(OH)D levels were associated with higher odds ratios for obesity and metabolic syndrome indicators among Korean children 9 years of age (7). In a prospective investigation with Colombian school children...
Excessive weight gain is an emerging public health problem in low- to middle-income countries, where rapid increases in weight during childhood, specifically after 2 years of age, have been consistently associated with unfavorable metabolic outcomes in later years (9). In the current study, we aimed to assess the effect of FTO variation on changes in BMI during childhood in a population-based longitudinal study in the Brazilian Amazon area and particularly investigated whether vitamin D status might modify these genetic effects.

RESEARCH DESIGN AND METHODS
A baseline population-based cross-sectional study on child health and nutrition was conducted in December 2007 in the urban area of Acrelândia (9°49'S, 66°53'W), Western Brazilian Amazon area. A total of 1,225 children aged <10 years (98.0% of those eligible) were initially enrolled for household interviews, blood sample collection, and anthropometric measurements (10). Follow-up assessments were carried out in December 2009 for all participants included at baseline and in July 2012 for children aged >6 years at the time of this last visit. We identified 909 children (74.2%) in 2009 and 514 children of 714 eligible participants (72.0%) in 2012. Written informed consent for participation was obtained before enrollment. The study was approved by the ethics review board of the School of Public Health, University of São Paulo.

Field Procedures
In December 2007, household interviews with each participant’s mother or guardian collected information on child’s sex, age, race/ethnicity, nutritional history, and recent morbidity; the presence of 12 household assets was determined to generate a wealth index, as previously detailed (10). A sample (5 mL) of fasting venous blood was collected from children at baseline; serum and plasma samples were shipped to São Paulo on dry ice and frozen at −70°C until further analysis. Children’s anthropometric measurements were obtained directly by trained research assistants using standardized procedures and calibrated equipment at all study assessments. BMI (kg/m²) was used to calculate BMI-for-age Z scores using the World Health Organization (WHO) Child Growth Standards (11) for children ≤5 years old and the WHO Growth Reference Data for children >5 years old (12). Pubertal development according to Tanner stages was ascertained during follow-up examinations conducted in 2009 and 2012 (13).

Genotyping
DNA was extracted from EDTA-containing whole blood aliquots using DNA kits (Qiagen, Hilden, Germany). The single nucleotide polymorphism rs9939609 near the FTO gene was genotyped by allele-specific PCR with molecular beacons (Prevention Genetics, Marshfield, WI). Quality-control samples (10%) were typed in duplicate with >99% agreement.

Vitamin D Status Assessment
Serum 25-hydroxivitamin D₃ concentrations were measured using high-performance liquid chromatography (14) in a separate aliquot stored away from light. The detection limit was 6.2 nmol/L; intra- and interassay variation was <7%. Baseline vitamin D insufficiency was defined as <75 nmol/L (15).

Statistical Analyses
Complete data on FTO rs9939609 genotype and anthropometric measurements were available for 1,088 children (88.8%) at baseline. Characteristics of children across each study assessment were compared using χ² tests for categorical variables and ANOVA for continuous variables. Cross-sectional effects of genetic variation on mean BMI and BMI-for-age Z scores at each assessment were estimated from linear regression models for each A allele of the rs9939609 variant, assuming an additive genetic model. Main genetic effects and potential interactions with baseline vitamin D status on mean changes in BMI and BMI-for-age Z scores per year were estimated from mixed-effect linear regression models. Random-effects included subject-specific intercepts for the 2007–2009 follow-up period and subject-specific intercepts and slopes for the 2007–2012 follow-up period. All models were initially adjusted for a child’s age, sex, and race/ethnicity (model 1) and further adjusted for a child’s pubertal stage at the last follow-up visit and baseline household wealth (model 2). Missing observations (<8%) were included in the multiple models by creating missing-value categories. We used STATA 11.2 (Stata, College Station, TX) for all analyses.

RESULTS
At baseline, the minor allele frequency of FTO rs9939609 (A allele) was 0.384 among all 1,088 children with complete genotypic and anthropometric data; genotype distribution fitted the Hardy-Weinberg equilibrium (P = 0.23). In 2007, there were no significant differences in genotype distribution according to sex, age, and vitamin D status, but differences were observed among ethnicity groups (P < 0.001) and household wealth quartiles (P = 0.01). Self-reported race/ethnicity, on the other hand, was not associated with either children’s BMI or vitamin D status.

A total of 796 children with baseline data were evaluated in 2009, of whom 436 were assessed again in 2012. Children lost to follow-up were not different from those included in analyses, except for household wealth; 30.8% of children lost to follow-up were in the lowest quartile of household wealth compared with 22.4% of children successfully followed up. The median follow-up period was 4.6 years (range 1.7–4.7). Table 1 presents the main characteristics across the study assessments.
No statistically significant increase in the proportion of children at risk for overweight was detected during follow-up from 2007 to 2012.

In cross-sectional analyses (Table 2), association of FTO with children’s BMI was not significant in 2007 (mean [SD] age 5.2 [2.8] years) and 2009 (7.2 [2.8] years), but effect size increased over follow-up (Fig. 1). At the last assessment in 2012 (10.5 [2.3]), each A allele was significantly associated with a 0.57 kg/m² higher mean BMI and 0.25 $Z$ higher mean BMI-for-age $Z$ score ($P$ = 0.01) after adjustment for age, sex, ethnicity, pubertal stage, and baseline household wealth.

We then performed mixed-effect linear regression models to evaluate the main effect of rs9939609 on the annual change in children’s BMI (Table 3). In adjusted models, each A allele was positively associated with a mean larger annual gain of 0.08 kg/m²/year in BMI ($P$ = 0.002) and 0.03 $Z$/year in BMI-for-age $Z$ score ($P$ = 0.03) from 2007 to 2009. These associations were maintained when extending the follow-up period to 2012, with a mean 0.07 kg/m²/year higher increase in BMI and a mean 0.03 $Z$/year higher increase in BMI-for-age $Z$ score per A allele ($P$ = 0.01).

In addition, we observed that baseline vitamin D status significantly modified the effects of FTO rs9939609 on changes in children’s BMI during follow-up (Table 3). From 2007 to 2009, among those who presented with insufficient 25(OH)D levels at baseline ($n$ = 217; 32.0%), the A allele in an additive model was positively associated with a mean 0.13 kg/m²/year higher increase per year in

### Table 1—Characteristics of urban Amazonian children at each study assessment

|                        | 2007        | 2009        | 2012        | $P^*$ |
|------------------------|-------------|-------------|-------------|------|
| $n$                    | 1,088       | 796         | 436         |      |
| Sex, $n$ (%)           |             |             |             | 0.76 |
| Female                 | 546 (50.2)  | 411 (51.6)  | 226 (51.8)  |      |
| Male                   | 542 (49.8)  | 385 (48.4)  | 210 (48.2)  |      |
| Age (years), mean (SD) | 5.2 (2.8)   | 7.2 (2.8)   | 10.5 (2.3)  | <0.001|
| Race/ethnicity, $n$ (%)|             |             |             | 0.84 |
| White                  | 95 (9.4)    | 69 (9.4)    | 38 (9.3)    |      |
| Mulatto/mixed race     | 864 (85.5)  | 638 (86.7)  | 351 (86.0)  |      |
| Black                  | 52 (5.1)    | 29 (3.9)    | 19 (4.7)    |      |
| BMI (kg/m²), mean (SD) | 15.8 (1.7)  | 16.3 (2.2)  | 17.7 (3.3)  | <0.001|
| BMI-for-age $Z$ score, mean (SD)† | -0.02 (1.02) | 0.12 (1.11) | 0.11 (1.25) | <0.001|
| Risk for overweight, $n$ (%) |            |             |             | 0.24 |
| No                     | 926 (85.1)  | 659 (82.8)  | 358 (82.1)  |      |
| Yes                    | 162 (14.9)  | 137 (17.2)  | 78 (17.9)   |      |
| Height (cm), mean (SD) | 106.5 (20.3)| 119.7 (17.6)| 139.9 (14.5)| <0.001|
| Height-for-age $Z$ score, mean (SD)† | -0.34 (1.05) | -0.40 (0.97) | -0.21 (0.97) | 0.004|
| Stunting, $n$ (%)       |             |             |             | 0.07 |
| No                     | 1,029 (94.6)| 762 (95.7)  | 424 (97.2)  |      |
| Yes                    | 59 (5.4)    | 34 (4.3)    | 12 (2.8)    |      |
| FTO rs9939609 genotype, $n$ (%) |           |             |             | 0.99 |
| TT                     | 422 (38.8)  | 315 (39.6)  | 172 (39.4)  |      |
| TA                     | 496 (45.6)  | 357 (44.8)  | 197 (45.2)  |      |
| AA                     | 170 (15.6)  | 124 (15.8)  | 67 (15.4)   |      |
| Baseline vitamin D status (nmol/L), $n$ (%) | | | | 0.34 |
| <75                    | 297 (32.1)  | 224 (32.0)  | 138 (35.9)  |      |
| ≥75                    | 629 (67.9)  | 477 (68.0)  | 246 (64.1)  |      |
| Tanner stage, $n$ (%)  |             |             |             | <0.001|
| Prepubertal            | —           | 610 (81.1)  | 222 (51.0)  |      |
| Pubertal               | —           | 142 (18.9)  | 213 (49.0)  |      |
| Baseline household wealth, $n$ (%) |           |             |             | 0.38 |
| 1st quartile (lowest)  | 268 (24.7)  | 178 (22.4)  | 94 (21.6)   |      |
| 2nd quartile           | 272 (25.0)  | 195 (24.5)  | 95 (21.8)   |      |
| 3rd quartile           | 286 (26.3)  | 215 (27.0)  | 121 (27.7)  |      |
| 4th quartile (highest) | 261 (24.0)  | 208 (26.1)  | 126 (28.9)  |      |

Total may be different from 1,088 because of missing data. *$P$ values were calculated by $x^2$ test for categorical variables and ANOVA for continuous variables. †BMI-for-age and height-for-age $Z$ scores were calculated according to the WHO growth references (11,12).
BMI-for-age Z score (P = 0.01; P for interaction = 0.03). The genetic interaction with baseline vitamin D status remained significant up to the last follow-up visit in 2012 when the annual change in BMI-for-age Z score is considered (P for interaction = 0.02), and each A allele of rs9939609 was associated with a mean larger increase of 0.05 Z/year in vitamin D–insufficient children (P = 0.003). No significant genetic effects were observed among participants with sufficient 25(OH)D levels at baseline (n = 461).

DISCUSSION

In this population-based longitudinal study in the Brazilian Amazon area, the A allele of FTO rs9939609 was significantly associated with higher BMI gain during childhood. The genotype effects were modified by baseline vitamin D status—children with insufficient 25(OH)D levels presented larger increases in BMI for each A allele compared with those with normal 25(OH)D concentrations.

Our findings of the longitudinal effects of FTO genotype on child weight gain are in line with previous reports. A large meta-analysis including data from eight European cohorts with 9,143 children on average per age stratum found an additive effect of rs9939609 A allele on BMI starting at 5.5 years of age (2). FTO variants have also been associated with higher BMI among African American subjects as young as 10 years old (16) and Brazilian children since age 4 years (17). There is indication that variation at the FTO locus may impact developmental age in relation to BMI in childhood by accelerating adiposity rebound and fat mass deposits (2), which may have potential implications for later metabolic disease risk (18).

### Table 2—Cross-sectional associations of BMI at each assessment with FTO rs9939609 genotype among urban Amazonian children

| rs9939609 genotype | 2007 (n = 1,088) | 2009 (n = 796) | 2012 (n = 436) |
|--------------------|----------------|----------------|----------------|
|                    | β (SE)* | P     | β (SE)* | P     | β (SE)* | P     |
| BMI (kg/m²)        |         |       |         |       |         |       |
| Model 1            | 0.04 (0.07) | 0.61  | 0.14 (0.11) | 0.21  | 0.55 (0.21) | 0.008 |
| Model 2            | 0.04 (0.07) | 0.57  | 0.15 (0.11) | 0.17  | 0.57 (0.22) | 0.008 |
| BMI-for-age Z score† |         |       |         |       |         |       |
| Model 1            | 0.03 (0.04) | 0.52  | 0.06 (0.06) | 0.31  | 0.24 (0.09) | 0.006 |
| Model 2            | 0.03 (0.04) | 0.46  | 0.06 (0.06) | 0.30  | 0.25 (0.09) | 0.005 |

Model 1 was adjusted for child’s age, sex, and race/ethnicity, and model 2 was further adjusted for child’s pubertal stage at the last follow-up visit and baseline household wealth. *β-Coefficients and their SE were estimated from linear regression models for each A allele of the FTO rs9939609 genotype. †BMI-for-age Z scores were calculated according to the WHO growth references (11,12).

Figure 1—Mean BMI among urban Amazonian children according to FTO rs9939609 genotype at each study assessment (2007: n = 1,088; 2009: n = 796; 2012: n = 436). Estimates were adjusted for a child’s age, sex, race/ethnicity, pubertal stage at the last follow-up visit, and baseline household wealth. TT, rs9939609 TT genotype; TA/AA, rs9939609 TA/AA genotypes.
Lower 25(OH)D levels have been associated with higher adiposity measures in children in several cross-sectional reports (5–7), and there is also indication from a longitudinal study that insufficient levels of vitamin D could be related to increases in BMI during childhood (8). In the present analysis, prevalence of vitamin D insufficiency was lower than that observed among children in the U.S. (5), which is conceivable considering that circulating levels of vitamin D depend mainly on sunlight exposure (15) and which is conceivable considering that circulating levels of vitamin D depend mainly on sunlight exposure (15) and therefore, vitamin D levels. Our present findings still need to be replicated in other populations, although attrition is common in prospective studies, its implications are difficult to assess and caution should be taken when extrapolating our findings to the general population. While we could not completely rule out possible confounding by genetic ancestry, we adjusted all analyses by self-reported race/ethnicity, which in turn was not significantly associated with BMI, BMI-for-age Z score, or vitamin D status at baseline. Also, we were not able to adjust the present analyses by children's physical activity, which could influence weight status as well as exposure to sunlight and, therefore, vitamin D levels. Our present findings still need to be replicated in other populations, and underlying mechanisms involved in the interaction between FTO genotype and vitamin D status still need to be clarified.

In conclusion, FTO rs9939609 is positively associated with weight gain among children residing in the Brazilian Amazon. We found evidence that vitamin D status might modify FTO genetic effects, which were
more pronounced among children with insufficient vitamin D levels.

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Appendix

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