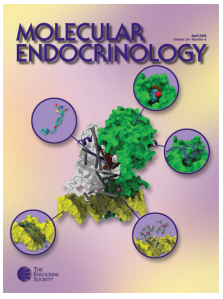
JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

Prepubertal Adrenarchal Androgens and Animal Protein Intake Independently and Differentially Influence Pubertal Timing

Thomas Remer, Lijie Shi, Anette E. Buyken, Christiane Maser-Gluth, Michaela F. Hartmann and Stefan A. Wudy

J. Clin. Endocrinol. Metab. 2010 95:3002-3009 originally published online Apr 6, 2010; , doi: 10.1210/jc.2009-2583



THE ENDOCRINE SOCIETY®

Prepubertal Adrenarchal Androgens and Animal Protein Intake Independently and Differentially Influence Pubertal Timing

Thomas Remer, Lijie Shi, Anette E. Buyken, Christiane Maser-Gluth, Michaela F. Hartmann, and Stefan A. Wudy

Research Institute of Child Nutrition (T.R., L.S., A.E.B.), 44225 Dortmund, Germany; Steroid Laboratory (C.M.-G.), Department of Pharmacology, University of Heidelberg, 69120 Heidelberg, Germany; and Steroid Research and Mass Spectrometry Unit (M.F.H., S.A.W.), Center of Child and Adolescent Medicine, Justus-Liebig-University, 35392 Giessen, Germany

Context: Whether adrenarche impacts on pubertal development is controversial.

Objective: The objective of the study was to examine the associations of adrenal androgen (AA) secretion with early and late pubertal markers, independent of potential influences of dietary animal protein intake.

Design and Participants: This was a prospective cohort study of healthy free-living Caucasian children (n = 109) who provided both 24-h urine samples and 3-d weighed dietary records 1 and 2 yr before the biological age at take-off of the pubertal growth spurt (ATO).

Measurements: Twenty-four-hour excretion rates of androgen (C19) metabolites quantified by gas chromatography-mass spectrometry were measured.

Main Outcomes: ATO, age at peak height velocity (APHV), age at menarche/voice break, duration of pubertal growth acceleration, and ages at Tanner stage 2 for breast (girls) and genital (boys) development (B2-G2) and pubic hair (PH2).

Results: Higher adrenarchal C19 steroids predicted earlier ages at Tanner stage 2 for pubic hair ($P < 0.0001$) and B2-G2 ($P = 0.009$) as well as a shorter pubertal growth acceleration period ($P = 0.001$), independently of animal protein intake. Children with a higher AA secretion had a 1.5-yr earlier beginning of pubarche and a 0.8-yr earlier beginning of B2-G2 than those with a lower AA excretion. Furthermore, animal protein intake was independently negatively associated with ATO and APHV ($P < 0.05$ each) and tended to be negatively associated with age at menarche/voice break ($P = 0.07$).

Conclusion: A higher animal protein intake may be involved in an earlier attainment of ATO and APHV, whereas a more intensive adrenarchal process may precipitate a shorter pubertal growth spurt and a notably earlier onset of breast and genital development in girls and boys, respectively. **(*J Clin Endocrinol Metab* 95: 3002–3009, 2010)**

Whether adrenarche, the increase in adrenal androgen (AA) secretion during midchildhood, may be implicated in the timing of puberty has not been longitudinally examined in healthy children until now. Based on available mostly cross-sectional clinical data, this question has been debated controversially for decades. A number of authors assume that this developmental rise of the principal adrenal 17-ketosteroids dehydroepiandrosterone

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-2583 Received December 4, 2009. Accepted March 10, 2010.
First Published Online April 6, 2010

Abbreviations: AA, Adrenal androgen; APHV, age at peak height velocity; ATO, age at take-off of the pubertal growth spurt; B2-G2, Tanner stage 2 for breast or genital development; BMI, body mass index; ΣC19, sum of major adrenal androgen metabolites; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; FMI, fat mass index; GABA, γ-aminobutyric acid; GC-MS, gas chromatography-mass spectrometry; PH, pubic hair; SDS, SD score.

(DHEA) and its sulfate ester (DHEAS) several years before puberty onset may rather represent a marker of body maturation than a factor influencing the beginning of puberty (1–3).

During normal growth, adrenarche and gonadarche are temporally closely linked, but in pathological situations, adrenarche can occur without subsequent gonadarche and gonadarche without preceding adrenarche (2). Despite this apparent causal independence of both developmental processes, the appearance of pubic hair in girls is usually attributed to the influence of adrenarchal C19 steroids on the androgen-dependent groin area (4). Accordingly, in normal girls, pubic hair occurs as one of the first indications of approaching puberty (5), and in girls with premature pubarche (premature pubic hair development) circulating DHEA or DHEAS levels are usually elevated for the respective chronological age (6). In normally developing boys, the appearance of pubic hair rarely precedes the beginning of genital development and appears to depend more closely on gonadal testosterone secretion (7). However, boys with precocious pubarche are, like girls, also characterized by an elevated DHEA or DHEAS level (8).

Recently Binder *et al.* (9) have shown that daily replacement with 25 mg DHEA orally to adolescent girls with central adrenal insufficiency induces a significant progress in pubic hair growth. These data together with the hormonal findings in children with precocious pubarche (4, 7, 8, 10) and infants with a combined occurrence of genital hair and elevated DHEAS (but without any other signs of androgen excess) (11) underscore that AAs are causally involved in the appearance and growth of pubic hair. However, information on the strength of the temporal acceleration of pubarche by a stronger *vs.* a less pronounced adrenarche in healthy children is lacking.

Intensity of adrenal androgen secretion appears to be influenced by a number of lifestyle-related and early life factors. Body fatness (12) and dietary protein intake (12) as well as the combination of low birth weight and a high current weight have been associated with a more pronounced adrenarche (13).

There is convincing evidence that exaggerated adrenarche with or without precocious pubarche in low birth weight girls can be followed by an early onset of puberty and early menarche (10, 14). In a recent review on adrenarche and the polycystic ovary syndrome, Nader (15) renewed the hypotheses that adrenarche is not only a promoter of pubarche but also gonadarche, suggesting that sexual maturation can occur earlier. Therefore, the first aim of the present paper was to examine this hypothesis in healthy children.

Recently animal protein intake in midchildhood has been reported to potentially trigger an earlier onset of certain, especially growth-related, puberty markers, *e.g.* the age at take-off of the pubertal growth spurt (ATO) (16). Animal protein appears also to modulate the intensity of AA secretion (12). Thus, our second aim was to examine whether dietary animal protein intake might independently (of adrenarchal influences) contribute to an altered timing of at least some pubertal markers.

Subjects and Methods

Study population and diet

The children examined are a subsample of healthy participants of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study, an ongoing, open cohort study conducted in Dortmund, Germany. The study protocol was approved by the Ethics Committee of the University of Bonn (Bonn, Germany). All assessments were performed with parental consent. Since recruitment began in 1985, detailed information on diet, growth, and development between infancy and early adulthood has been collected from more than 1100 children. Every year, an average of approximately 40 infants are newly recruited and first examined at the age of 3 months. Children return for three more visits in the first year, two in the second, and then once annually until early adulthood. Accomplishment of a 3-d weighed dietary record is scheduled in yearly intervals along with a 24-h urine collection (usually performed on the third day of diet recording). From the weighed diet records, reasonably reliable data on intakes of energy and nutrients (*e.g.* protein) can be derived (16). For the present study, we calculated the mean 3-d intakes of total energy (kilocalories per day) and animal protein (grams per day) at 2 and 1 yr before puberty onset (see below).

Dietary, developmental, and urine-related information available over time varies between the subjects. Therefore, the number of children included in this analysis was derived as follows: a total of 376 subjects of the DONALD cohort had sufficient height measurements to allow plausible estimation of the puberty marker ATO (17). Of these, 111 children, who had not refused regular assessment of Tanner stages, had also collected 24-h urine samples as well as dietary data at both time points (2 and 1 yr) before ATO. In two children not all information on potential confounding variables (birth weight, gestational age, breast-feeding, maternal overweight) were available. Hence, the subcohort analyzed herein included 109 prepubertal healthy children (54 boys). However, information on age at menarche or voice break were available only for 100 (49 boys).

Puberty outcomes and anthropometric measures

The growth-dependent puberty variable ATO was determined using the parametric model 1 of Preece and Baines (18), which also produces estimates of velocity at take-off, age at peak height velocity (APHV), and peak height velocity.

The model was fitted on various sex-specific age ranges of the height-for-age data, beginning at age 2 yr, to determine the optimal range for the data. ATO was defined as the age at minimal height velocity (zero acceleration) at the onset of the pubertal growth spurt. Goodness of fit was determined by graphical inspection of each child's individual growth curve, a comparison of the residual SDS (random error had to be smaller than the expected

measurement error for height), and considering the plausibility and distribution of the pubertal parameters estimated (17). Based on the criteria determined for best fit, in this analysis all measurements from age 5 and 6 yr onward in girls and boys, respectively, were selected and entered into Preece and Baines model 1.

From the derived data, we calculated the duration of pubertal growth acceleration (APHV minus ATO), which can be used as an index for growth spurt duration and puberty duration. Tanner stages for pubic hair (PH) and either breast (girls) or genital (external genitalia, boys) development (B-G) are assessed by one of the DONALD study pediatricians from age 5 yr onward. In addition, children and/or parents are asked at each visit whether menarche or voice break has already occurred, and the respective month and year are recorded. Pubertal staging is performed according to the standardized criteria published by Marshall and Tanner (19) and testis volume determined by palpation using the Prader orchidometer. We used age at testis volume of 4 ml or greater to define the onset of genital development in boys (Tanner stage G2). Herein we denoted Tanner stage 2 for breast and genital development in girls and boys, respectively as B2-G2.

DONALD study participants are anthropometrically assessed at each visit according to standard procedures. From the age of 2 yr onward, standing height is measured to the nearest 0.1 cm using a digital stadiometer. Weight is measured to the nearest 0.1 kg using an electronic scale (753 E; Seca, Hamburg, Germany). Skinfold thicknesses are measured from the age of 6 months onward on the right side of the body to the nearest 0.1 mm using a Holtain caliper. Information on birth weight, length, and gestational age are abstracted from the Mutterpass, a standardized document given to all pregnant women in Germany. Percentage body fat was determined by using the Slaughter equations for prepubertal children (20). Fat mass was calculated as body weight multiplied by percentage body fat and fat-free mass as body weight minus fat mass. From these, the fat mass index (FMI; fat mass/height^2) and the fat-free mass index (fat free mass/height^2) were derived.

Urinary measurements

Prepubertal 24-h urine collections (1 and 2 yr before ATO) were performed at home under standardized conditions (21), and samples were stored at −20 C or less until analyzed. Urinary steroid profiles were determined using gas chromatography-mass spectrometry (GC-MS) analysis (21). Total AA secretion was determined as the sum (ΣC19) of androsterone, etiocholanolone, 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol (androstenediol), DHEA, 16α-hydroxy-DHEA, and 5-androstene-3β,16α,17β-triol (12, 21). To assess overall daily cortisol secretion of the adrenals as an index of adrenocortical activity, the seven quantitatively most important urinary glucocorticoid metabolites were profiled and summed: tetrahydrocortisone, tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortolone, β-cortolone, α-cortol, and β-cortol (22).

Statistical analysis

All statistical tests were performed using SAS procedures (version 9.1; SAS Institute Inc., Cary, NC). Descriptive data are given as means ± SD or median with interquartile range when appropriate. Sex differences for pubertal, anthropometric, hormonal, and dietary characteristics were tested by using χ^2 or unpaired *t* test.

Multiple linear regression analyses were used to analyze the independent associations of hormone excretion rates and animal protein intake with pubertal variables. The hormonal and nutritional predictors were included as arithmetic mean values of the respective data from 1 yr and 2 yr before ATO. For this, 24-h excretion rates of ΣC19 (log transformed) were expressed as SD score (SDS) of published GC-MS reference values (21) and animal protein consumption as percent of daily energy intake. Analyses of covariance were performed to test for sex interactions. No sex-by-animal protein intake and no sex-by-ΣC19 interactions ($P > 0.1$) were observed for the outcomes ATO, APHV, duration of pubertal growth acceleration, age at B2-G2, age at PH2, and age at menarche/voice break. Accordingly, the respective multiple regression analyses were performed with boys and girls combined. To exemplify that the associations between C19 steroids and puberty onset were comparable in both sexes, a sex-stratified subanalysis was done for the puberty marker B2-G2. In this subanalysis two models were run, which differed only with regard to inclusion mode of FMI-SDS. In model 2, FMI-SDS was preadjusted for overall daily cortisol secretion (adrenocortical activity), *i.e.* residuals were obtained by regressing FMI-SDS on the sum of urinary glucocorticoid metabolites (for the glucocorticoid parameter, see *Urinary measurements*). This preadjustment of FMI-SDS accounts for the fact that cortisol secretion (adrenocortical activity) and body fatness are associated in healthy children (23). As a consequence, body fatness variation (via covariation with adrenocortical activity) partly reflects the potential C19 influence on the puberty outcomes (*i.e.* the part of C19 variation caused by adrenocortical activity). Removal of the latter influence from the FMI-SDS variable by preadjustment reduces inappropriate attribution of parts of the C19 influence to FMI and should hence improve specificity of the variation explained by C19.

Potential confounding factors considered in the fully adjusted regression models were sex, FMI, total energy intake-SDS, urine volume related to body surface area, gestational age, birth weight, breast-feeding 2 wk or more, and maternal overweight [body mass index (BMI) ≥25 kg/m^2]. FMI and total energy intake were expressed as SDS of the steroid GC-MS reference sample (21). Multicollinearity tests were performed to check for independence of the variables. For none of the covariate combinations, multicollinearity was observed.

Additionally, animal protein intake (percent energy) and ΣC19-SDS were each grouped into three categories: low (<25th percentile), medium (≥25th and ≤75th percentiles), and high (≥75th percentiles). Least-squares regression analyses, adjusted for sex, FMI-SDS, birth weight, and urine volume or total energy intake-SDS, were used to predict the adjusted means of puberty variables by categories of ΣC19-SDS or animal protein intake (percent energy). *P* for trend refers to the *P* values obtained in linear regression models with respective variables as continuous variables. In all statistical tests, $P < 0.05$ was considered as significant.

Results

Anthropometric, urinary, and nutritional characteristics of the study sample (at average growth curve derived biological age of 1.5 yr before ATO) are presented in Tables 1 and 2. For most variables, *e.g.* fat-free mass index, daily C19 excretion rates, and daily energy intake, the absolute values were significantly higher in boys, whereas the corresponding SDS or percent energy-related data, used in the regression analyses, no longer showed sex differences.

TABLE 1. Anthropometric and pubertal characteristics of the study sample (n = 109)

	Boys (n = 54)	Girls (n = 55)	*P* for difference[a]
Age (yr)[b]	8.8 ± 0.9[c]	7.3 ± 0.7	<0.0001
Anthropometric[b]			
Weight (kg)	30.9 ± 5.0	25.3 ± 4.8	<0.0001
Height (cm)	137.1 ± 7.0	126.2 ± 6.0	<0.0001
Body surface area (m^2)[d]	1.09 ± 0.11	0.94 ± 0.10	<0.0001
BMI (kg/m^2)	16.3 ± 1.6	15.7 ± 1.8	NS
BMI-SDS[e]	−0.16 ± 0.76	−0.15 ± 0.86	NS
FMI (kg/m^2)	2.2 (1.8, 2.8)[f]	2.2 (2.0, 2.8)	NS
FMI-SDS[g]	−0.46 ± 0.76	−0.17 ± 1.0	NS
Fat-free mass index (kg/m^2)	13.8 ± 0.9	13.1 ± 0.8	<0.0001
Fat-free mass index-SDS[g]	−0.06 ± 0.86	0.05 ± 0.77	NS
Pubertal			
ATO (yr)	10.4 ± 0.9	8.8 ± 0.6	<0.0001
APHV (yr)	13.6 ± 0.9	11.8 ± 0.7	<0.0001
Duration of pubertal growth acceleration (APHV-ATO) (yr)	3.2 ± 0.3	2.9 ± 0.4	<0.05
Age at Tanner (B2-G2) (yr)[h]	10.9 ± 1.0	10.6 ± 0.9	NS
Age at Tanner (PH2) (yr)[i]	11.6 ± 1.1	10.8 ± 1.1	<0.05
Age at menarche/voice break (yr)[j]	13.7 ± 1.1	13.1 ± 0.8	<0.05

NS, Not significant.

[a] Sex differences were tested with unpaired *t* test; [b] values were derived from each individual's arithmetic mean from 1 and 2 yr before ATO, *i.e.* data represent average values approximately 1.5 yr before ATO; [c] mean ± SD (all such values); [d] calculated according to the established Du Bois and Du Bois formula; [e] SDS according to the German reference values for BMI; [f] median: 25th and 75th percentile in *parentheses* (all such values); [g] calculation based on the data of children with C19 steroid GC-MS reference values (21); [h] age at Tanner stage 2 for breast (girls) and external genitalia (boys) development; [i] age at Tanner stage 2 for pubertal hair; [j] no information on age at menarche for four girls and age at voice break for five boys.

Boys showed a significantly longer pubertal growth acceleration than girls. ATO, APHV, ages at B2-G2, and PH2 occurred earlier in girls than boys. Only for B2-G2 the sex difference was not significant (Table 1).

In the fully adjusted model (allowing for birth weight and other covariates), higher adrenarchal C19 steroids predicted earlier ages at B2-G2 ($P = 0.009$) and PH2 ($P < 0.0001$) as well as shorter pubertal growth acceleration ($P =$

TABLE 2. Urinary, nutritional, and early life-related characteristics of the study sample (n = 109)

	Boys (n = 54)	Girls (n = 55)	*P* for difference[a]
Urinary[b]			
Urine volume (ml/d)	700 (579, 1017)[c]	617 (501, 734)	<0.05
ΣC19 (μg/d)	444 (313, 609)	243 (167, 346)	<0.05
ΣC19-SDS[d]	−0.20 ± 1.04[e]	−0.23 ± 0.91	NS
ΣC21 (μg/d)[f]	4220 (3646, 4725)	3421 (2864, 3783)	<0.0001
ΣC21-SDS[f]	0.30 ± 0.91	0.64 ± 0.87	NS
Nutritional[b]			
Energy intake (MJ/d)	7.3 ± 1.0	6.3 ± 0.9	<0.0001
Vegetable protein	20 ± 5	17 ± 3	<0.05
intake (g/d) (percent of energy)	(4.6 ± 0.8)	(4.6 ± 0.9)	NS
Animal protein intake (g/d)	37 ± 9	30 ± 8	<0.05
(percent of energy)	(8.5 ± 1.7)	(8.0 ± 1.7)	NS
Early life-related			
Birth weight (g)	3609 ± 466	3405 ± 355	<0.05
Gestational age (wk)	40 (40, 41)	40 (39, 40)	NS
Maternal overweight [n (%)]	17 (31.5)	15 (27.3)	NS
Full breast-feeding for ≥2 wk [n (%)]	43 (79.7)	47 (85.5)	NS

NS, Not significant.

[a] Sex differences were tested with unpaired *t* test for continuous variables and χ^2 test for categorical variables; [b] values were derived from each individual's arithmetic mean from 1 and 2 yr before ATO, *i.e.* data represent average values approximately 1.5 yr before ATO; [c] median: 25th and 75th percentile in *parentheses* (all such values); [d] calculation based on the data of children with C19 steroid GC-MS reference values (21); [e] mean ± SD (all such values); [f] sum of the seven quantitatively most important urinary glucocorticoid metabolites; SDS calculation based on the data of children with glucocorticoid reference values (22).

TABLE 3. Association of adrenal androgens (ΣC19-SDS) and animal protein intake (adjusted for each other) with pubertal variables in 109 healthy children[a]

Outcomes	Predictors	β	SE	P
ATO	C19-SDS	−0.002	0.09	1.0
	Animal protein intake (%)	−0.10	0.05	0.02
APHV	C19-SDS	−0.13	0.10	0.20
	Animal protein intake (%)	−0.10	0.05	0.04
Duration of pubertal growth acceleration	C19-SDS	−0.12	0.03	0.002
	Animal protein intake (%)	−0.01	0.02	0.7
Age at Tanner (B2-G2)[b]	C19-SDS	−0.29	0.11	0.009
	Animal protein intake (%)	−0.08	0.06	0.1
Age at Tanner (PH2)[c]	C19-SDS	−0.65	0.12	<0.0001
	Animal protein intake (%)	−0.07	0.06	0.3
Age at menarche/ voice break[d]	C19-SDS	−0.07	0.11	0.6
	Animal protein intake (%)	−0.12	0.06	0.07

[a] Final multiple regression model (adjusted for sex, FMI-SDS, body surface related urine volume, total energy intake-SDS, birth weight, gestational age, full breast-feeding ≥2 wk, and maternal overweight) showing the independent associations of both predictors C19-SDS and animal protein intake (as percent of energy intake); [b] age at Tanner stage 2 for breast (girls) and external genitalia (boys) development; [c] age at Tanner stage 2 for pubertal hair; [d] n = 100 (51 girls).

0.002) (Table 3). In a model not including animal protein intake, C19 showed comparable associations with pubertal variables (with closely corresponding *P* values, data not shown). An example for boys and girls with a pronounced adrenarche and a short pubertal growth acceleration period (as well as a weak adrenarche and the corresponding growth acceleration period) is given in Fig. 1. Animal protein intake was, independently of C19, negatively associated with ATO and APHV ($P < 0.05$, each) and (as a trend, $P = 0.07$) with menarche/voice break in the fully adjusted model (Table 3). The sex-stratified subanalysis given in Table 4 shows, as an example for B2-G2, that the C19-puberty associations were comparable in both sexes. Age at G2 and B2 was negatively associated with C19 in boys and girls, respectively, and this association was significant after FMI-SDS was preadjusted for overall daily cortisol secretion (adrenocortical activity) (for details see *Statistical analysis*).

The adjusted means of the ages at Tanner stage 2 (B-G and PH) for ΣC19-SDS categories and ages at takeoff and peak height velocity for animal protein intake (percent) categories are shown in Fig. 2. Children with a higher AA secretion level had a 0.8-yr earlier beginning of breast or genital development and a 1.5-yr earlier beginning of pubarche than those with a lower AA excretion. Additionally, peak height velocity and the beginning of the pubertal growth spurt occurred approximately 0.4 or 0.5 yr earlier in high animal protein consumers.

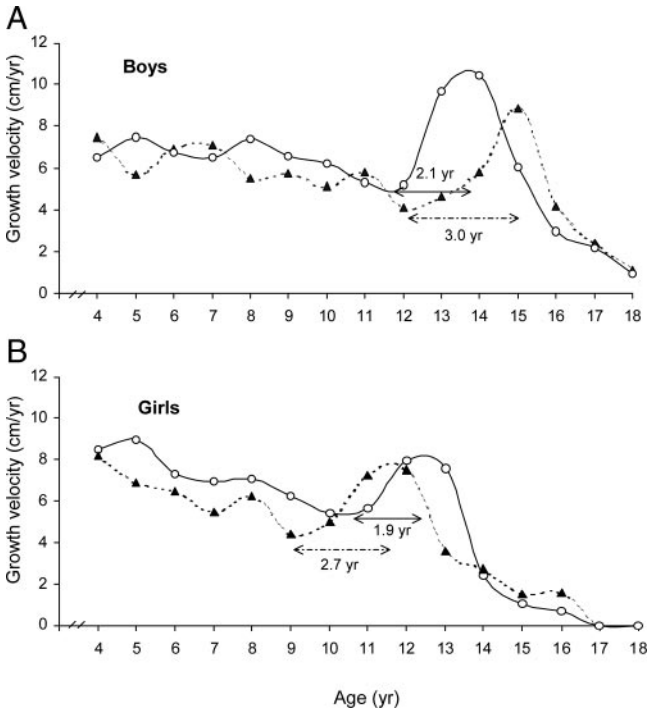


FIG. 1. Individual growth velocity curves for children (A, boys; B, girls) with strong (*solid line*) and weak (*dashed line*) adrenarche and corresponding durations of pubertal growth acceleration. ΣC19-SDS, boys: 1.01 *vs.* −1.92; girls: 1.73 *vs.* −1.53.

TABLE 4. Sex-stratified analysis for age at Tanner stage 2 for breast (girls, n = 55) and external genitalia (boys, n = 54) development[a]

Outcomes	Predictors	β	SE	P
Model 1[b]				
Age at B2	C19-SDS	−0.31	0.16	0.06
	Animal protein intake (%)	−0.02	0.08	0.8
Age at G2	C19-SDS	−0.32	0.16	0.06
	Animal protein intake (%)	−0.15	0.09	0.09
Model 2[c]				
Age at B2	C19-SDS	−0.32	0.15	0.03
	Animal protein intake (%)	−0.02	0.08	0.8
Age at G2	C19-SDS	−0.33	0.15	0.03
	Animal protein intake (%)	−0.15	0.09	0.1

[a] Final multiple regression model (adjusted for FMI-SDS, body surface related urine volume, total energy intake-SDS, birth weight, gestational age, maternal overweight, and full breast-feeding ≥2 wk) showing the independent associations of both predictors C19-SDS and animal protein intake (as percent of energy intake).

[b] Conventional adjustment for the FMI-SDS.

[c] FMI-SDS preadjusted for overall daily cortisol secretion (adrenocortical activity; for further details see *Statistical analysis*).

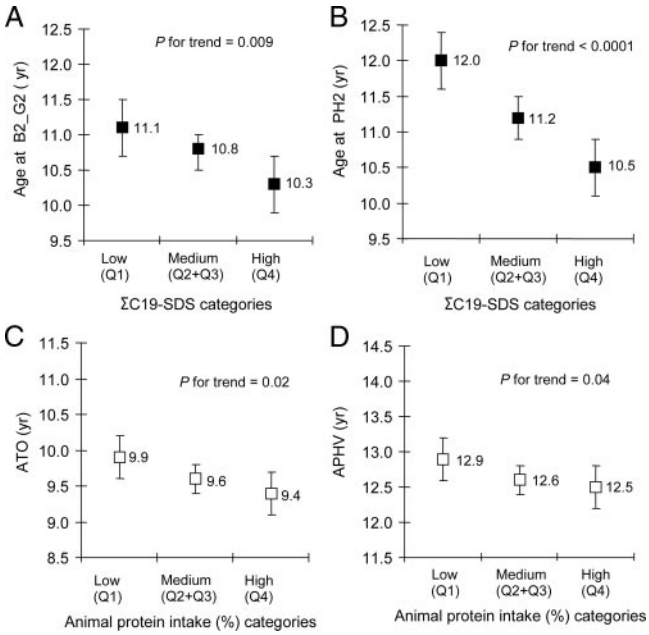


FIG. 2. Ages at B2-G2 (A) and PH2 (B) by categories of SDS of urinary 24-h C19 steroid excretion rates; data are means (95% confidence intervals) adjusted for animal protein intake, FMI, birth weight, and sex). ATO (C) and APHV (D) by categories of animal protein intake are shown; data are means (95% confidence intervals) adjusted for C19-SDS, FMI, birth weight, and sex. *P* for continuous trend refers to the *P* values obtained in linear regression models with C19-SDS and animal protein intake as continuous variables (n = 109). Q, Quartile.

Discussion

Our prospective analysis of adrenarchal and nutritional influences on the timing of early and late pubertal markers provides observational evidence that adrenarche and dietary animal protein intake are independently and differentially involved in the modulation of pubertal development in healthy nonobese children. Higher prepubertal AAs may precipitate a shorter pubertal growth acceleration period and an earlier onset of breast and genital development in girls and boys, respectively.

In line with our earlier observation that the midgrowth spurt (onset) around age 6–7 yr is obviously not due to a preceding (or parallel) increase in AA secretion (24), AAs did not show a predictive association with the start of the pubertal growth spurt (ATO) either. However, children in the highest quartile of animal protein intake appear to experience ATO 0.5 yr and APHV 0.4 yr earlier than children with the lowest animal protein intake (Fig. 2). These results extend our previous analyses (16) in that the associations of protein intake with the pubertal markers are obviously independent, *i.e.* not mediated by AAs.

Although dietary animal protein intake may increase adrenarchal androgen secretion in children (12) and urinary DHEAS and 3α-androstanediol glucuronide excretion in adults (25), it appears evident that the significant associations of 24-h AA excretion rates with duration of pubertal growth acceleration and ages at B2-G2 and PH2 constitute independent AA influences and no pathway effects arising from higher dietary protein intakes. This can be deduced because the association between animal protein intake and AAs is only modest (12) and animal protein intake was not (neither alone nor together with C19) significant in the respective regression models for the outcomes: duration of pubertal growth acceleration, age at B2-G2, and age at PH2.

It is generally assumed that adrenarchal androgens impact on pubic hair (4, 8, 26) and that premature pubarche is usually due to premature adrenarche, *i.e.* due to prepubertally markedly elevated AA levels (10, 27). In accord herewith, oral DHEAS or DHEA substitution therapy in patients with atrichia pubis led to accelerated pubic hair growth (9, 28). However, to our knowledge, the impact of physiological adrenarche on the timing of pubarche has not yet been examined in healthy children. Accordingly, our present findings provide novel physiologically based information on the effect strength of AAs regarding the approximate time span between earlier and later onset of pubarche due to the influence of AAs. Children with a more pronounced adrenarche experience their first pubic hair growth, on average, 1.5 yr earlier than children with only a moderate adrenarche.

The timing of puberty is highly variable, and apart from genetic influences (29), environmental factors including nutrition also add to this variability. As explicitly discussed by Veldhuis *et al.* (30), among the nutritionally influenceable hormones, appropriate concentrations of IGF-I (and GH) are required for a normal pace of maturation of at least some of the puberty variables. Because animal protein intake stimulates the growth-promoting peptide IGF-I (31) in circulation, it is thus intelligible why this dietary component may be associated particularly with the growth-related puberty markers ATO and APHV.

Some of the authors who have favored the hypothesis that a higher level of AA may precipitate an earlier sexual maturation have proposed that here an increased peripheral aromatization of adrenal androgens to estrogens could be involved (32). Actually, in the male (pubertal and adult), as in the female, estrogens constitute the primary determinant of the sex steroid-negative feedback to the hypothalamic-pituitary-gonadal axis. Accordingly, for an appropriate gonadal function in men, a preceding conversion of testosterone to estradiol is essential (33). These data suggest that also before puberty onset and peripubertally, androgens, including AAs, are aromatized to estrone and estradiol in both sexes. How these estrogens and/or adrenarchal secretion products might interact with the neurobiological machinery that controls pulsatile GnRH release and thereby accelerate puberty onset is not known.

There is compelling evidence that various neuropeptides and neurotransmitters like kisspeptin and γ-aminobutyric

acid (GABA) are involved in the neurobiological mechanism holding and/or releasing the check on GnRH pulsatility during juvenile development (34). The GABA synapses and their receptors on GnRH neurons principally exert an inhibitory action on hypothalamic GnRH release (35). Thus, the peripubertal activation of the hypothalamic-pituitary-gonadal axis requires attenuation of the GABA signal to allow for an increase in pulsatile LHRH release. In this regard the neurosteroid effects reported for DHEAS (the quantitatively most important adrenarchal secretion product) may also play a relevant role for the GnRH neuronal network. DHEAS has been shown to act as an noncompetitive antagonist of the GABA receptor complex in a number of studies summarized by Campbell (36). Campbell (36) put forward the hypothesis that this inhibitory action on the GABA system may be one cause for the changes in social behavior, anxiety, and learning abilities seen from childhood onward. We would like to extend this hypothesis by suggesting that the physiological adrenarchal increase in secretion of weak sex steroids, especially DHEAS, may provide one of the extrahypothalamic signals that, probably via successive GABA inhibition, could accelerate puberty onset and modulate puberty duration.

Our findings that higher AAs predict an earlier pubertal development in healthy children are in agreement with the reports of a higher risk for early puberty onset in girls with precocious pubarche (14, 37), patients with nonclassical 21-hydroxylase deficiency (38), and patients with classical congenital adrenal hyperplasia (39). The fact that we found a significant variation of puberty timing due to physiologically varying AA levels in healthy children, who attained the pubertal milestones at normal pace (in accordance with other European studies (40–44), underscores the role of adrenarche as one physiological modulator that fine-tunes pubertal development.

It appears that this fine-tuning is primarily related to breast or genital development and pubarche and to a lesser degree to growth-related puberty markers. Unfortunately, only few studies, largely focusing on children with premature adrenarche (45), have examined the relationship between adrenarche and pubertal growth. Also, studies directly examining the association between ATO and the onset of breast or genital development are widely lacking. However, analyses of longitudinal data from birth cohort groups of the Fels Longitudinal Study revealed an almost constant ATO in girls born 1929–1983 (46), whereas a convincing trend toward an earlier breast development onset has been reported for the corresponding time interval (47). Our findings of possible differential effects of AA and dietary protein intake on these developmental landmarks could be part of the explanation for the above probable dissociation between ATO and breast development.

A major strength of our present analysis is that we could examine prospectively collected 24-h urine samples and weighed dietary records (regarded as the most accurate technique for assessing dietary intake) that refer to a biological (1 and 2 yr before ATO) and not a chronological age. A limitation may be the relatively small sample size, but it was sufficient to detect differences of 0.4 yr in pubertal timing between the two groups (high *vs.* low level of the respective predictor). The clinical relevance of smaller effect sizes may be questionable.

In conclusion, nutrition and the intensity of the adrenarchal process appear to be independently and differentially involved in the modulation of pubertal timing. Although higher animal protein intake may be involved in an earlier attainment of ATO and APHV, higher AA levels may precipitate a shorter pubertal growth spurt and a notably earlier onset of breast and genital development in girls and boys, respectively.

Acknowledgments

Address all correspondence and requests for reprints to: Dr. Thomas Remer, Research Institute of Child Nutrition, Department of Nutrition and Health, Heinstueck 11, 44225 Dortmund, Germany. E-mail: remer@fke-do.de.

This work was supported by a research grant from the Long-Range Research Initiative of the European Chemical Industry Council (to T.R. and S.A.W.). The DONALD study is funded by the Ministry of Science and Research of North Rhine Westphalia, Germany.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Forest MG, de Peretti E, David M, Sempe M** 1982 [Does adrenarche really play a determining role in pubertal development? A study of the dissociations between adrenarche and gonadarche. The failure of dehydroepiandrosterone sulfate treatment in delayed adrenarche]. Ann Endocrinol (Paris) 43:465–495
2. **Cutler Jr GB, Loriaux DL** 1980 Andrenarche and its relationship to the onset of puberty. Fed Proc 39:2384–2390
3. **Grumbach MM, Styne DM** 1998 Puberty: ontogeny, neuroendocrinology, physiology, and disorders. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. Williams textbook of endocrinology. 9 ed. Philadelphia: Saunders; 1509–1625
4. **Auchus RJ, Rainey WE** 2004 Adrenarche—physiology, biochemistry and human disease. Clin Endocrinol (Oxf) 60:288–296
5. **Marshall WA** 1981. Normal puberty. In: Brook CGD, ed. Clinical paediatric endocrinology. Oxford, London: Blackwell Scientific Publications; 193–206
6. **Utriainen P, Voutilainen R, Jääskeläinen J** 2009 Continuum of phenotypes and sympathoadrenal function in premature adrenarche. Eur J Endocrinol 160:657–665
7. **Tanner JM** 1981. Endocrinology of puberty. In: Brook CGD, ed. Clinical paediatric endocrinology. Oxford, London: Blackwell Scientific Publications; 207–223

8. **Rosenfield RL, Rich BH, Lucky AW** 1982 Adrenarche as a cause of benign pseudopuberty in boys. J Pediatr 101:1005–1009
9. **Binder G, Weber S, Ehrismann M, Zaiser N, Meisner C, Ranke MB, Maier L, Wudy SA, Hartmann MF, Heinrich U, Bettendorf M, Doerr HG, Pfaeffle RW, Keller E** 2009 Effects of dehydroepiandrosterone therapy on pubic hair growth and psychological well-being in adolescent girls and young women with central adrenal insufficiency: a double-blind, randomized, placebo-controlled phase III trial. J Clin Endocrinol Metab 94:1182–1190
10. **Ibáñez L, Jiménez R, de Zegher F** 2006 Early puberty-menarche after precocious pubarche: relation to prenatal growth. Pediatrics 117:117–121
11. **Kaplowitz P, Soldin SJ** 2007 Steroid profiles in serum by liquid chromatography-tandem mass spectrometry in infants with genital hair. J Pediatr Endocrinol Metab 20:597–605
12. **Shi L, Wudy SA, Buyken AE, Hartmann MF, Remer T** 2009 Body fat and animal protein intakes are associated with adrenal androgen secretion in children. Am J Clin Nutr 90:1321–1328
13. **Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, de Zegher F, Ibáñez L, Dunger DB** 2004 Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. J Clin Endocrinol Metab 89:2647–2651
14. **Ibáñez L, Ong K, Valls C, Marcos MV, Dunger DB, de Zegher F** 2006 Metformin treatment to prevent early puberty in girls with precocious pubarche. J Clin Endocrinol Metab 91:2888–2891
15. **Nader S** 2007 Adrenarche and polycystic ovary syndrome: a tale of two hypotheses. J Pediatr Adolesc Gynecol 20:353–360
16. **Günther AL, Karaolis-Danckert N, Kroke A, Remer T, Buyken AE** 2010 Dietary protein intake throughout childhood is associated with the timing of puberty. J Nutr 140:565–571
17. **Buyken AE, Karaolis-Danckert N, Remer T** 2009 Association of prepubertal body composition in healthy girls and boys with the timing of early and late pubertal markers. Am J Clin Nutr 89:221–230
18. **Preece MA, Baines MJ** 1978 A new family of mathematical models describing the human growth curve. Ann Hum Biol 5:1–24
19. **Marshall WA, Tanner JM** 1986. Puberty. In: Falkner F, Tanner JM, eds. Human growth: a comprehensive treatise. 2nd ed, vol 2. New York: Plenum Press; 171–203
20. **Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, Bemben DA** 1988 Skinfold equations for estimation of body fatness in children and youth. Hum Biol 60:709–723
21. **Remer T, Boye KR, Hartmann MF, Wudy SA** 2005 Urinary markers of adrenarche: reference values in healthy subjects aged 3–18 years. J Clin Endocrinol Metab 90:2015–2021
22. **Wudy SA, Hartmann MF, Remer T** 2007 Sexual dimorphism in cortisol secretion starts after age 10 in healthy children: urinary cortisol metabolite excretion rates during growth. Am J Physiol Endocrinol Metab 293:E970–E976
23. **Dimitriou T, Maser-Gluth C, Remer T** 2003 Adrenocortical activity in healthy children is associated with fat mass. Am J Clin Nutr 77:731–736
24. **Remer T, Manz F** 2001 The midgrowth spurt in healthy children is not caused by adrenarche. J Clin Endocrinol Metab 86:4183–4186
25. **Remer T, Pietrzik K, Manz F** 1998 Short-term impact of a lactovegetarian diet on adrenocortical activity and adrenal androgens. J Clin Endocrinol Metab 83:2132–2137
26. **Rosenfield RL** 1991 Puberty and its disorders in girls. Endocrinol Metab Clin North Am 20:15–42
27. **Ibáñez L, Dimartino-Nardi J, Potau N, Saenger P** 2000 Premature adrenarche—normal variant or forerunner of adult disease? Endocr Rev 21:671–696
28. **Wit JM, Langenhorst VJ, Jansen M, Oostdijk WA, van Doorn J** 2001 Dehydroepiandrosterone sulfate treatment for atrichia pubis. Horm Res 56:134–139
29. **Ong KK, Elks CE, Li S, Zhao JH, Luan J, Andersen LB, Bingham SA, Brage S, Smith GD, Ekelund U, Gillson CJ, Glaser B, Golding J, Hardy R, Khaw KT, Kuh D, Luben R, Marcus M, McGeehin MA, Ness AR, Northstone K, Ring SM, Rubin C, Sims MA, Song K, Strachan DP, Vollenweider P, Waeber G, Waterworth DM, Wong A, Deloukas P, Barroso I, Mooser V, Loos RJ, Wareham NJ** 2009 Genetic variation in LIN28B is associated with the timing of puberty. Nat Genet 41:729–733
30. **Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC, Sheffield-Moore M, Mauras N, Bowers CY** 2005 Endocrine control of body composition in infancy, childhood, and puberty. Endocr Rev 26:114–146
31. **Hoppe C, Udam TR, Lauritzen L, Mølgaard C, Juul A, Michaelsen KF** 2004 Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. Am J Clin Nutr 80:447–452
32. **Katz SH, Hediger ML, Zemel BS, Parks JS** 1985 Adrenal androgens, body fat and advanced skeletal age in puberty: new evidence for the relations of adrenarche and gonadarche in males. Hum Biol 57:401–413
33. **Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley Jr WF, Hayes FJ** 2008 Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab 93:784–791
34. **Plant TM, Ramaswamy S** 2009 Kisspeptin and the regulation of the hypothalamic-pituitary-gonadal axis in the rhesus monkey (*Macaca mulatta*). Peptides 30:67–75
35. **Zhang C, Bosch MA, Rønnekleiv OK, Kelly MJ** 2009 γ-Aminobutyric acid B receptor mediated inhibition of gonadotropin-releasing hormone neurons is suppressed by kisspeptin-G protein-coupled receptor 54 signaling. Endocrinology 150:2388–2394
36. **Campbell B** 2006 Adrenarche and the evolution of human life history. Am J Hum Biol 18:569–589
37. **Ibáñez L, Diaz R, López-Bermejo A, Marcos MV** 2009 Clinical spectrum of premature pubarche: links to metabolic syndrome and ovarian hyperandrogenism. Rev Endocr Metab Disord 10:63–76
38. **New MI** 2006 Extensive clinical experience: nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab 91:4205–4214
39. **Bonfig W, Pozza SB, Schmidt H, Pagel P, Knorr D, Schwarz HP** 2009 Hydrocortisone dosing during puberty in patients with classical congenital adrenal hyperplasia: an evidence-based recommendation. J Clin Endocrinol Metab 94:3882–3888
40. **Aksglaede L, Olsen LW, Sørensen TI, Juul A** 2008 Forty years trends in timing of pubertal growth spurt in 157,000 Danish school children. PLoS One 3:e2728
41. **Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, Main KM, Skakkebaek NE** 2006 Pubertal development in Danish children: comparison of recent European and U.S. data. Int J Androl 29:247–255; discussion 286–290
42. **Kahl H, Schaffrath Rosario A, Schlaud M** 2007 [Sexual maturation of children and adolescents in Germany. Results of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS)]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 50:677–685
43. **Rubin C, Maisonet M, Kieszak S, Monteilh C, Holmes A, Flanders D, Heron J, Golding J, McGeehin M, Marcus M** 2009 Timing of maturation and predictors of menarche in girls enrolled in a contemporary British cohort. Paediatr Perinat Epidemiol 23:492–504
44. **Silventoinen K, Haukka J, Dunkel L, Tynelius P, Rasmussen F** 2008 Genetics of pubertal timing and its associations with relative weight in childhood and adult height: the Swedish Young Male Twins Study. Pediatrics 121:e885–e891
45. **Pere A, Perheentupa J, Peter M, Voutilainen R** 1995 Follow up of growth and steroids in premature adrenarche. Eur J Pediatr 154: 346–352
46. **Demerath EW, Li J, Sun SS, Chumlea WC, Remsberg KE, Czerwinski SA, Towne B, Siervogel RM** 2004 Fifty-year trends in serial body mass index during adolescence in girls: the Fels Longitudinal Study. Am J Clin Nutr 80:441–446
47. **Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sorensen TI, Dunkel L, Himes JH, Teilmann G, Swan SH** 2008 Examination of U.S. puberty-timing data from 1940 to 1994 for secular trends: panel findings. Pediatrics 121(Suppl 3):S172–S191