**Appendix 5.** Evidence table for protein intake and outcome puberty (4 cohort studies)

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| **Author (alphabetical order), year (ref.nr)**  **Country**  **Study design**  (RCT, CT, cohort, case control etc.) | **Population,**  **subject characteristics,** Inclusion/exclusion criteria  Setting,  No at baseline, Male/Female, Age, Ethnicity of the subjects, Anthropometry, Location | **Outcome measures**  Disease, biological measures | **Intervention/exposure** | **Time between baseline exposure and outcome assessment** | **Dietary assessment method**  FFQ, food record  **Internal validation** y/n  see separate table below for more details | **No of subjects analysed** | **Intervention**  Intervention (I)  (dose interval, duration)  Control (C) (active, placebo, usual care etc), compliance, achieved dietary change, adherence to dietary targets, actual dietary change | **Follow- up period, drop-out rate**  (from baseline to follow-up, or from end of intervention to follow-up)  Drop out (%) | **Results**  Results (I, C)  (Absolute difference, RR, OR, p-value, confidence interval, sensitivity, specificity, observer reliability?, etc) | **Confounders adjusted for** | **Study quality and relevance, Comments**  A-C |
| Berkey,  2000  (53)  USA  The Harvard Longitudinal Cohort Study (started 1930s-1940s) | Inclusion: Caucasian, likely to remain near Boston, committed to longterm study.  Exclusion: gross defects, premature.  67 girls followed from utero to 18 y.  Weight, height semi-annually 0-11y, annually 11-18 y. | Age at menarche, peak height growth velocity, peak growth velocity. | Dietary intake (kcal/d, animal protein g/d, vegetable protein g/d, total fat g/d, BMI) averaged over multiyear periods [1-2, 3-5, 6-8 y + 1 and 2 y before peak growth. | Mean age at menarche 12.83±1.09 y | Dietary history covering previous 6 mo. (This group at Harvard invented the method and they have at least one paper where they thoroughly discuss the pros and cons of the method) | 67 F  (+ 50 not included due to not complete data until 18 y) | NA | 0%  Only those with complete data included (the 50 with incomplete data had similar birth, length, and head- and chest circumference. | For *peak growth velocity* the same three factors emerged in all age periods; more calories, more animal protein and lower BMI were consistently associated with higher peak growth velocity (factors closer to puberty more important).  Timing of puberty was predicted by protein intake and height. Higher animal protein (energy adjusted) intake and less vegetable protein at 3-5 y had earlier menarche (+1 SD animal protein intake gave 0.63 y earlier menarche than -1SD, and peak height growth was +0.6 cm/y)  Higher dietary fat intake at 1-2 y associated with earlier peak growth (+1 SD fat intake gave 0.63 y earlier peak growth velocity than -1SD) and higher calorie intake at 1-2 y gave higher peak height velocity (+1 SD calorie intake gave +1.11 cm/y than -1SD)  and higher animal protein intake at 6-8 y had earlier peak growth.  Later age at menarche associated with lower age at peak growth (r=0.81, p<0.05) and lower peak growth velocity (r=-0.41, p<0.05) | Dietary intake and growth at younger ages were included in all models. (No other confounders included as far as I can tell) | B  Drop-out rate high (43%) |
| Günther,  2010  (54) Germany/ DONALD  Prospective cohort | Inclusion basic: Gestational age 37-42 w, singleton, birth weight ≥2500g  Inclusion this study:  1) Height measurments from 6 and 13 y + ≥ 5 measurments between these ages  2) Plausible dietary records from 12 mo, 18-24 mo (at least 1 of 2), 3-4 y (at least 1 of 2), 5-6 y (at least 1 of 2).  3) info on all relevant confounders  Setting Dortmund, Germany  N=112 (55 M, 57 F)  12 mo through puberty  White Germans.  Weight, length/height, BMI (all converted to SD-scores), skinfold thickness (biceps, triceps, subscapular, suprailiac), body density, % body fat (%BF), fat mass index (FMI), FMI SD-score, | Timing of puberty;  1) Age at take-off of pubertal growth spurt (ATO)  2) Age at peak height velocity  3) Menarche/voice break | Protein intake at 12 mo, 18-24 mo, 3-4 y, 5-6 y.  Q1. Is protein intake at 12 and/or 18-24 mo related to pubertal timing, or is diet in later childhood more important?  Q2. Can the protein source (animal, vegetable) be of importance? | Outcome at mean 13 y | 3-d weighed records at 12 mo, 18 mo, 24 mo, 3y, 4y, 5y, 6 y.  Semiquantitative recording allowed if weighing not possible.  Shofield equation (not describer here, but in previous study light PAL used as standard, cut-off 0.97 as suggested as suitable for 1-5y) | 112 (55 M, 57 F)  57 children had collected urine samples for urinary nitrogen excretion (for validating dietary data)  92 had data on age at menarche/voice break | NA | 0%  Only those with complete data included | Higher animal protein intake (E%) at 5-6 y was associated with earlier puberty. The highest tertile of intake had ATO 0.6 y earlier than the lowest tertile (p=0.048). Similar tendency at 3-4 y.  Vegetable protein intake (E%) was associated with a later ATO.  Protein intake from cow milk and dairy products at age 5-6 y (but not meat) was associated with an earlier ATO [mean ATO, 95% CI; tertile 1: 9.5, 9.2-9.8; tertile 2: 9.5, 9.3-9.8; tertile 3: 9.1, 8.8-9.4y; p-trend=0.04]  Children with higher animal protein intake E%) at 3-4 and 5-6 y had earlier APHV [3-4 y: tertile 1: 12.5, 12.2-12.9; tertile 3: 12.0, 11.7-12.3y; p<0.05], [5-6 y: tertile 1: 12.8, 12.5-13.1; tertile 3: 12.0, 11.7-12.3y; p<0.05], while those with high vegetable protein intake had later APHV [3-4 y: tertile 1: 12.1, 11.8-12.5; tertile 3: 12.6, 12.3-13.0 y; p-trend=0.02], [5-6 y: tertile 1: 12.2, 11.8-13.6; tertile 3: 12.6, 12.2-13.0y; p=0.04]  Higher animal protein intake (especially milk) at 3-4 y tended (p=0.06) to be (and at 5-6 y was (p=0.02), associated to earlier menarche/voice break and later for high vegetable protein intake (0.02 and 0.03 respectively)  Adjustment for confounders did not change all these associations. | Sex, birth year, birth weight (<3000 g, yes/no), breastfeeding (full brf ≥4 mo, yes/no), rapid weight gain between birth and age 2 (increase in weight SDS >0.67, yes/no), maternal overweight (BMI ≥25, yes/no), parental education university entrance qualification; yes/no, university degree; yes/no),  +  Mean energy from 1 to 6 y, total energy, fat intake (E%), fiber intake  +  ATO and prepubertal FMI SDS (1 y before ATO) | A |
| Remer, 2010  (55) Germany/ DONALD  Prospective cohort | healthy free-living Caucasian  children (n = 109)  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. | Timing of puberty;  1) Age at take-off of pubertal growth spurt (ATO)  2) Age at peak height velocity  3) Menarche/voice | Energy and animal protein intake at 1 and 2 years before puberty onset | Outcome at puberty ? | 3-d weighed dietary record | 109 | **NA** | Children return for three more visits in the first year, two in the second, and then once annually until early adulthood: 109 prepubertal healthy children (54 boys). information on age at menarche or voice break were available only for 100 (49 boys) a total of 376 subjects of the cohort had sufficient height measurements to allow plausible estimation of the puberty marker. 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). In two children not all information on potential confounding variables (birth weight, gestational age, breast-feeding, maternal overweight) | 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 intakewasindependently negatively associated withATOandAPHV(*P*<0.05 each) and tended to be negatively associated with age at menarche/voice break (*P* <0.07). | 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  [BMI＞25]. | B.  No information on physical activity, statistical power, follow-up period and time-exposure variable not totally clear. |
| Shi,  2009,  (56)  Germany/ DONALD  Prospective cohort | **1.**Inclusion/exclusion criteria: Healthy pre-pubertal GA: 3-12 yrs/ Existing characteristics growth, diet 2. Setting: Giessen, Germany 3. No at baseline: 186 🡪137eligible 4. Male/Female: 50%/50% 5. Age: 3-12 yrs 6. Ethnicity of the subjects: Caucasian? 7. Anthropometry Birth, Wt, Ht, BMI, Fat Mass (FM), Fat Free Mass (FFM) 8. Location: Giessen, Germany | Adrenachal androgen status (AA) | Anthropometry & intake (protein), Body com (FM) | Not clear | 3 days food-record | 73% i.e. n=137 (of 186) | NA | “Dropout” 27%  Subjects chosen for participation from a cohort depending on earlier existing data | Table 3 & Fig.2. AA depending on FM and protein intake | Yes | B.  No information on physical activity, statistical power, time of baseline or exposure variable not totally clear. |

**Evidence table: Dietary information/Background diet\***

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| **Author (alphabetical order), year, (ref.nr)** | **Expo-sure** | **Dietary Assessment Method\*\*** | **Food Composition Database\*\*\***  **Definition of relevant nutrient \*\*\*\*** | **Internal Calibration (or Validity) of Dietary Assessment? (**y/n**). If Yes, Provide Data** | **Biomarker Assay\*\*\*\*\*** | **Analytical Validity of Biomarker Data Reported? (**y/n**).**  **If Yes, Provide Data** | **Time between Biomarker Sampling and Analysis** | **Season/Date when biomarker samples were drawn** | **Background exposure data** |
| Berkey,  2000  (53) | Dietary intake (kcal/d, animal protein g/d, vegetable protein g/d, total fat g/d, BMI) averaged over multiyear periods [1-2, 3-5, 6-8 y + 1 and 2 y before peak growth. | Dietary history covering previous 6 mo. (This group at Harvard invented the method and they have at least one paper where they thoroughly discuss the pros and cons of the method) | Standard food composition tables was used (in 1930s and 40s). kcal/d, animal protein g/d, vegetable protein g/d, total fat g/d | In a previous paper (ref 19) the validity of the dietary history method was scrutinized (they invented the method). In the present paper it is stated that the reliability of method was estimated to 71 % for protein intake. Evidence of validity was provided by a strong correlation between daily protein intake and the child´s rate of growth of muscle in lower leg (r=0.46 for girls, 0.68 for boys). | nd | nd | nd | nd | nd |
| Günther,  2010  (54) | Protein intake at 12 mo, 18-24 mo, 3-4 y, 5-6 y.  Q1. Is protein intake at 12 and/or 18-24 mo related to pubertal timing, or is diet in later childhood more important?  Q2. Can the protein source (animal, vegetable, dairy (excl infant formula), cereal) be of importance? | 3-d weighed records at 12 mo, 18 mo, 24 mo, 3y, 4y, 5y, 6 y.  Semiquantitative recording allowed if weighing not possible. | LEBTAB  kJ/d  Protein g/d, | Yes  3% excluded  Do not give any details in this paper – gives ref 24 (Shofield) and 25 (report on under-reporting from DONALD study)  In previous publication (which they don´t refer to) they used Goldberg cut-off, BMR calculated Shofield equation (light PAL used as standard)  Cut-off 0.97 used in the present study (suggested as suitable for 1-5y)  1.9% excluded | nd | nd | nd | nd | nd |
| Remer, 2010 (55) | Energy and animal protein intake at 1 and 2 years before puberty onset | 3-d weighed dietary record | Method not stated or referred to elsewhere but energy as well as vegetable and animal protein intake are reported as in the DONALD studies from 2007, i.e., g/day and percent energy. | Reasonably reliable data for protein intake as previously published by the authors(Gunter et al 2010, J Nutr 140:565-571), | nd | nd | nd | nd | nd |
| Shi, 2009, (56) | Anthropometry & intake (protein), Body com (FM)?? | 3 days food-record  Note: refer to previous papers for validation or exact description of dietary methods - it was our thought that we should not go to every background study | Authors refer here to description of dietary methods (Kroke et al 2004, Eur J Nutr 43:45-54; Remer et al 2002, Am J Clin Nutr 75:561-9) | Authors refer to previous papers for validation , see next column. | nd | nd | nd | nd | nd |

\* Write “nd” if there was no data reported. Please do not leave blank

\*\*Please refer to brief name indicated in dietary assessment method table. If other method was used, please describe the detail.

\*\*\* Specify database used to calculate nutrient intakes. Other nutrient analysis, please specify.

\*\*\*\*Eg. are carbohydrates expressed as available carbohydrates or carbohydrates by difference, is fibre included in the carbohydrates or not, retinol equivalent or retinol activity etc. Chemical form of the nutrient.

\*\*\*\*\*ONLY biomarker of interest for outcome