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Relevance of fruits, vegetables and flavonoids from fruits and vegetables during early life, mid-childhood and adolescence for levels of insulin-like growth factor (IGF-1) and its binding proteins IGFBP-2 and IGFBP-3 in young adulthood

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Abstract

The growth hormone (GH) insulin-like growth factor (IGF) axis has been linked to insulin metabolism and cancer risk. Experimental evidence indicates that the GH–IGF axis itself can be influenced by dietary flavonoids. As fruit and vegetable (FV) intake is a major source of flavonoid consumption, FV's beneficial health effects may be explained via flavonoids' influence on the GH–IGF axis, but observational evidence is currently rare. We used data from Dortmund Nutritional and Anthropometric Longitudinally Designed Study participants to analyse prospective associations between FV, fruit intake and flavonoid intake from FV (FlavFV) with IGF-1 and its binding proteins IGFBP-2 and IGFBP-3. Subjects needed to provide a fasting blood sample in adulthood (18–39 years) and at least two 3-d weighed dietary records in early life (0·5–2 years, *n* 191), mid-childhood (3–7 years, *n* 265) or adolescence (girls: 9–15 years, boys: 10–16 years, *n* 261). Additional analyses were conducted among those providing at least three 24-h urine samples in adolescence (*n* 236) to address the predictor urinary hippuric acid (HA), a biomarker of polyphenol intake. Higher fruit intake in mid-childhood and adolescence was related to higher IGFBP-2 in adulthood ($P=0·03$ and $P=0·045$). Comparable trends ($P=0·045$–$0·09$) were discernable for FV intake (but not FlavFV) in all three time windows. Similarly, higher adolescent HA excretion tended to be related ($P=0·06$) to higher adult IGFBP-2 levels. Regarding IGFBP-3, a marginal ($P=0·08$) positive association was observed with FlavFV in mid-childhood only. None of the investigated dietary factors was related to IGF-1. In conclusion, higher fruit and FV intakes during growth may be relevant for adult IGFBP-2, but probably not for IGFBP-3 or IGF-1.

Key words: Fruits and vegetables: Flavonoids: Children: Insulin-like growth factor

A high fruit and vegetable (FV) intake has been associated with a number of health benefits, including reduced risk for CVD and different types of cancer[1,2]. Importantly, several observational studies have demonstrated that a higher fruit intake in childhood and adolescence might already be related to reduced cancer risk later in life[3,4]. However, more recent data do not fully support the evidence for inverse FV–cancer relationships, which may to some extent be attributable to imprecise exposure assessment[5,6]. Another possible reason for this discrepancy may be that health benefits arise mainly from one component of FV, which is unevenly distributed across different FV subgroups. Polyphenols may represent this relevant component, as certain fruit polyphenols have been demonstrated to restrict cancer growth *in vitro* and *in vivo*[7,8].

Considering the long latency period between lifestyle (e.g. dietary) exposures and cancer diagnosis, intermediate markers related to cancer risk are particularly valuable in long-term observational studies. Components of the growth hormone (GH) insulin-like growth factor (IGF) axis, a major regulator of human growth, may represent such intermediate markers[9,10]. Specifically, whereas higher IGF-1 levels seem to be associated with an increased cancer risk[9], the antiproliferative and pro-apoptotic actions of its major binding protein IGFBP-3 suggest that higher IGFBP-3 levels might be related to lower

Abbreviations: DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; FlavFV, flavonoid intake from FV; FV, fruit and vegetable; GH, growth hormone; GI, glycaemic index; HA, hippuric acid; IGF, insulin-like growth factor; IGFBP, IGF-1 and its binding protein; USDA, US Department of Agriculture.

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cancer risk[10]. The GH–IGF axis is also susceptible to nutritional influences[9], and we and others have shown that diet during critical periods of early life may relevantly influence the GH–IGF axis in the longer term[11,12], thereby possibly explaining associations between childhood diet and later cancer risk. Thus far, epidemiological evidence for the possible longer-term relevance of dietary polyphenols, or their major subgroup flavonoids, for the GH–IGF axis is missing. Several *in vitro*[13,14] and animal studies[15,16] have, however, demonstrated that administration of (fruit) polyphenols can reduce IGF-1 and/or elevate IGFBP-3 with a concurrent inhibition of tumour growth. Thus, higher IGFBP-3 and lower IGF-1 levels, attributable to a higher dietary polyphenol intake (from fruit and vegetables), may represent a plausible mechanism linking higher FV consumption to lower cancer risk. The GH–IGF axis is also closely linked to the metabolism of insulin, with higher IGFBP-2 concentrations potentially reflecting better long-term insulin sensitivity at lower insulin levels[17]. Higher flavonoid consumption from FV may improve insulin sensitivity[18], thereby decreasing insulin levels, which could in turn contribute to decreased cancer risk[19]. The cancer-protective role of FV may therefore also be explained by their influence on the regulation of insulin and IGFBP-2, but further research is needed.

Thus, the aim of the present study was to investigate whether FV intake, fruit intake or dietary flavonoid intake from FV (FlavFV) during three distinct periods of growth (i.e. early life, adiposity rebound in mid-childhood and adolescence) is related to the GH–IGF axis in young adulthood in a general healthy population. To investigate these relationships in depth, exposure assessment was based on both the dietary intake data and the urinary biomarker hippuric acid (HA).

Methods

Study population

Data for the prospective analysis of dietary influences on the GH–IGF axis came from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study, an open-cohort study that was initiated at the Research Institute of Child Nutrition in Dortmund, Germany, in 1985. The DONALD Study investigates relationships between diet, metabolism, growth and development from infancy until adulthood[20]. To this end, thirty-five to forty infants are newly recruited every year and are first examined at the age of 3 months. Three further visits are scheduled in the 1st and two visits in the 2nd year of life. Afterwards, annual assessments take place that generally include 3-d weighed dietary records, medical and anthropometric examinations as well as interviews on lifestyle. Beginning at the age of 3–4 years, 24-h urine samples are usually collected in parallel with the dietary records. In addition, since 2005, adult participants are invited for subsequent examinations including a fasting blood withdrawal. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the University of Bonn (Germany). All assessments were performed with parental and, later on, with the children's written consent.

Thus far, >1500 children have participated in the DONALD Study, but the ages of the children initially recruited were quite variable, resulting in the fact that information on the first few years of life is not always available. Moreover, many participants have not yet reached adult age. The present analysis was based on a sample of 382 DONALD participants who were term (36–43-week gestation) singletons with a birth weight ≥2500 g and had provided a fasting blood sample for measurements of IGF-1, IGFBP-2 and IGFBP-3 between 18 and 39 years of age. Analyses in relation to FV intakes and flavonoid intakes estimated from dietary records were based on those who had additionally provided at least two plausible 3-d weighed dietary records to describe habitual diet in the age range of interest (i.e. early life: 0·5–2 years; approximate age of adiposity rebound: 3–7 years; adolescence: girls, 9–15 years; boys, 10–16 years). Dietary records were considered implausible if the ratio of reported total energy intake:predicted BMR was below age- and sex-specific cut-off values[21]. Participants who had consistently under-reported energy intake (i.e. all dietary records implausible or more implausible than plausible records) were not considered for the present analysis (n 15 in the adolescent data set). Furthermore, participants had to provide information on relevant covariates such as early life and socio-economic factors. Applying these criteria, study populations for the dietary analyses consisted of 191, 265 and 261 participants for prediction from the time frames of early life, adiposity rebound and adolescence, respectively.

Regarding the period of adolescence, additional analyses were based on the urinary biomarker HA. To address this aim, participants had to provide ≥3 complete 24-h urine samples in adolescence, resulting in a study population of 236 participants. Urine samples were considered as complete if body weight-related 24-h creatinine excretion was ≥0·1 mmol/kg[22].

Preliminary power calculations indicated that available sample sizes were sufficient to detect correlations of 0·17–0·21 between dietary intakes and adult outcomes with a power of >80 %.

Dietary intake

Dietary intake was assessed using 3-d weighed dietary records. During 3 consecutive days, all foods and beverages consumed (including leftovers) are weighed and recorded to the nearest 1 g with the help of electronic food scales (initially Soehnle Digita 8000; Leifheit; now WEDO digi 2000; Werner Dorsch). If weighing is not possible, semi-quantitative recording (e.g. number of spoons) is allowed. For the present analysis, intakes of foods and nutrients were calculated as the individual means of the 3 recording days using our continuously updated in-house nutrient database LEBTAB (LEBensmittel TABelle)[23], which is based on German standard food tables and data obtained from commercial food products. The food group characterising general FV intake consisted of fruits and vegetables (including fresh, frozen and canned products) as well as fruit and vegetable juices, and is referred to as FV. Intake was calculated as the sum of (unprocessed) separately ingested fruits, vegetables or juices and ingredients of processed or prepared foods.

To estimate flavonoid consumption from FV, all composite foods were broken down to the ingredients and flavonoid

assignment was performed on the recipe level. Aggregated values for flavonoids[24], proanthocyanidins[25] and isoflavones[26] were taken from databases of the US Department of Agriculture (USDA). For all fruits, vegetables and juices consumed by DONALD participants included in the present analyses, available values from these three databases were assigned. If a consumed food item was not available in the USDA databases, a value for a similar food item (or the mean value of several similar food items) was assigned. In these cases, criteria for similarity included the botanical family as well as appearance (e.g. colour, size, texture). If data for a food item were only available in a different preparation (e.g. values for cooked pears or pear juice were needed, but the USDA database contains only values for raw pears), the value for the raw item was multiplied with published retention and/or yield factors[27]. Flavonoid intake in the present study was calculated as the sum of flavonoids, isoflavones and proanthocyanidins reported in the three USDA databases. However, as proanthocyanidin monomers in the proanthocyanidin database[25] and flavan-3-ols in the flavonoid database[24] indicate the same compounds, the values for proanthocyanidin monomers were not considered to avoid data duplicity, as has been described previously[28].

Laboratory measurements

Annual 24-h urine collections are usually performed at the 3rd day of the 3-d dietary records. During the 24-h collection period, all micturitions are immediately (i.e. at home) stored frozen ≤ −12°C in Extran-cleaned (Extran MA03; Merck), preservative-free 1 litre plastic containers before being transferred to the Research Institute where they are further stored at ≤ −20°C until analysed. After thawing and stirring, urine volume is documented and creatinine excretion is determined by the kinetic Jaffé's method on a creatinine analyser (Beckman-2; Beckman Instruments) in all urine samples. Urea was analysed photometrically using the Urease-Berthelot method (Randox Laboratories Ltd). Urinary HA, used as a biomarker for polyphenol consumption from fruits and vegetables[29,30], was quantified colourimetrically according to Tomokuni & Ogata[31] with minor modifications[30]. HA excretion has been reported to increase markedly after the ingestion of foods rich in flavonoids and phenolic acids[29,32]; moreover, although HA excretion may also be influenced by dietary intakes of protein and the food preservative benzoic acid as well as by the individual intestinal microbiota[30], robust correlations with calculated FlavFV were observed in the DONALD cohort[33].

Venous blood samples were drawn after an overnight fast and were separated by centrifugation at 4°C for 15 min. Blood samples were stored frozen at −80°C until shipped to the Laboratory for Translational Hormone Analytics in Paediatric Endocrinology at the University of Giessen, where IGF-1 and IGFBP-3 were analysed by RIA[34] and IGFBP-2 was determined using an ELISA.

Anthropometric measurements and calculations

Anthropometric measurements were performed during each visit at the study centre according to standard procedures by trained nurses undergoing annual quality control. For determination of height and weight, participants were dressed in underwear only with no shoes. Until 2 years of age, recumbent length was measured using a Harpenden stadiometer (Holtain Ltd). For children older than 2 years, standing height was determined to the nearest 0·1 cm with a digital telescopic wall-mounted stadiometer (Harpenden; Holtain Ltd). Body weight was measured to the nearest 0·1 kg with the help of an electronic scale (Seca 753E; Seca Weighing and Measuring Systems). Skinfold thickness was measured at four different sites (biceps, triceps, suprailiacal, subscapular) at the right side of the body to the nearest 0·1 mm using a Holtain caliper (Holtain Ltd).

Body surface area (BSA) in m^2 was calculated according to the formula of DuBois & DuBois[35]: $0{\cdot}007184 \times \text{height (cm)}^{0{\cdot}725} \times \text{weight (kg)}^{0{\cdot}425}$. Body weight and height were also used to calculate participants' BMI (kg/m^2), and age- and sex-independent standard deviation scores for BMI were determined according to German reference curves[36]. Calculation of body fat percentage (BF%) during early life and adiposity rebound was based on all four skinfolds[37], whereas pubertal BF % was estimated using the equations of Slaughter *et al.*[38] for pubescent children, which consider triceps and subscapular skinfolds. Fat mass index (FMI, kg/m^2) was then calculated using the following equation: $\text{FMI} = \text{weight} \times \text{BF\%}/\text{height}^2$.

Parental characteristics and additional information

On admission of their children to the DONALD Study, parents were interviewed about familial characteristics (e.g. educational status, number of smokers in the household), and anthropometric measurements were performed with the same equipment as used for the children. Information on the child's birth characteristics was abstracted from the 'Mutterpass', a standardised document given to all pregnant women in Germany. The duration of full breast-feeding (i.e. no solid foods or liquids except breast milk, tea or water) was enquired during the first visit until complementary feeding was initiated.

Statistical analyses

SAS procedures (version 9.2; SAS Institute) were used for all analyses and a P value <0·05 was considered significant in all statistical tests.

For prospective analyses of potential relationships between dietary intakes of FV, fruits, FlavFV and HA excretion and IGF-1, IGFBP-2 and IGFBP-3, multiple linear regression models were used. Nutritional variables were energy adjusted using the residual method[39] and standardised by age group and sex (mean = 0 (SD 1)) to account for age-dependent changes in intake. Similarly, because no information on adolescent energy intake in the urinary data set was available and because of the close correlation between BSA and energy intake (r 0·58 in our adolescent data set with dietary data), urinary HA was calculated as residuals on individual BSA and standardised by age group and sex. Dietary and urinary predictors were included in the regression analyses as individual arithmetic means of the repeated measurements during the respective time frames (i.e. early life, adiposity rebound and adolescence) to provide

estimates on habitual intake or excretion levels. To achieve normal distribution, all outcome variables except for IGF-1 were log transformed before analyses. Initial models (model A in Tables 2–5) included the respective dietary or urinary predictor, sex, age at outcome assessment and a dummy variable for year of blood measurement. This dummy variable was assigned because measurements of the GH–IGF axis were conducted in two separate series (2011 and 2014). Interaction analyses in these initial models indicated no differences in the predictor–outcome relationships between males and females.

For adjusted models (model B in Tables 2–5), the following covariates were additionally considered as potential confounders: gestational age, birth weight, full breast-feeding (>2 weeks, yes/no), maternal overweight (BMI ≥25 kg/m^2, yes/no), high maternal educational status (≥12 years of schooling, yes/no), any smokers in the household (yes/no) and BMI or FMI at baseline (i.e. the first measurement in the respective time window). Dietary intakes of energy, protein (animal), SFA and fibre from sources other than FV were additionally considered in models with dietary predictors. Urine volume, 24-h creatinine excretion and 24-h urea excretion (as a biomarker of protein intake[40]) were tested in the models with the predictor 24-h HA excretion. Covariates were only included in the final models if they modified the regression coefficient of the main predictor by ≥10 %. Adjusted means (i.e. the least square means of IGF-1, IGFBP-2 and IGFBP-3 predicted by the model when the other variables were held constant) are presented with their 95 % CI by tertiles of the respective predictors in Tables 2–5. For reasons of comparability, the same adjustment was used for all dietary predictors within the same period for a given outcome. This adjustment was usually derived from the regression analyses with the predictor FV.

Results

Socio-economic, dietary (or urinary) and anthropometric characteristics of the study samples available for early life, adiposity rebound and adolescence (dietary and urinary data set) are presented in Table 1, together with information on relevant early life factors and characteristics in young adulthood. Participants obtained a higher percentage of energy from fat and SFA in early life compared with mid-childhood and adolescence, whereas percentage energy consumption from carbohydrates was highest in adolescence. Although total energy intake was more than twice as high in the pubertal sample compared with the early life sample, absolute consumption of FV and FlavFV differed less markedly with age, with 69 % higher FV intake and 83 % higher median FlavFV in adolescence compared with early life. Absolute median fruit intake was almost constant across the age groups. In those subjects providing dietary intake data in all three growth periods (n 150), FV intake and fruit intake correlated moderately between the different age ranges (r 0·34–0·55, data not shown).

During early life, adjusted linear regression models (Table 2, model B) revealed no associations of FV, fruits or FlavFV with IGF-1 or IGFBP-3, but higher intakes of FV in this age group tended to be related to higher IGFBP-2 concentrations in young adulthood (P = 0·07).

In models adjusted for dietary and early life factors (Table 3, model B), higher FV as well as fruit consumption around adiposity rebound were significantly related to higher adult IGFBP-2 levels (P = 0·045 and P = 0·03, respectively), whereas no similar associations were observed for FlavFV. However, a higher FlavFV was in trend (P = 0·08) related to higher IGFBP-3 in young adulthood in the adjusted model (Table 3, model B). Moreover, FlavFV showed a significant inverse association with the IGF-1:IGFBP-3 ratio (P = 0·04 in the adjusted model; data not shown), thought to reflect (biologically active) free IGF-1 concentrations[41]. Nevertheless, also for the age range around adiposity rebound, none of the investigated dietary predictors was associated with IGF-1.

With respect to dietary intakes during adolescence, a trend for an association with higher adult IGFBP-2 was observed for higher FV intake (P = 0·09) and a significant association for higher fruit intake (P = 0·045) (Table 4, model B). Again, no associations were observed between FlavFV and IGFBP-2 as well as between any of the dietary predictors and adult IGF-1 or IGFBP-3. For the biomarker 24-h HA excretion in adolescence (Table 5), a trend (P = 0·056) for a direct association was detected for adult IGFBP-2, but not for IGFBP-3 or IGF-1. Sensitivity analyses in a subgroup providing at least two dietary records with parallelly collected 24-h urine samples during adolescence (n 224) indicated that HA-IGFBP-2 associations remained stable after adjustment for intakes of energy, protein (animal), SFA or fibre from other sources than FV (data not shown).

Repeating the analyses using FV intake without juices as a predictor yielded results that were very similar to those reported in Tables 2–4 (FV intake including juices). Moreover, additional adjustment for vegetable intake in the models with fruit intake as the predictor changed the results only marginally (data not shown).

To examine whether the observed associations between fruit or FV intake in childhood and adolescence with the adult GH–IGF axis are independent of adult intake levels, we repeated our analyses in smaller data sets (n 150 in early life, n 203 around adiposity rebound, n 196 in adolescence) of subjects who had also provided 3-d dietary records at the time of blood sampling (see online Supplementary Tables S1–S3). Although the prospective association between fruit intake during mid-childhood and adult IGFBP-2 levels was attenuated by adjustment for fruit intake in young adulthood, trends for higher IGFBP-2 levels associated with higher FV intakes in early life or mid-childhood were independent of adult intake levels. In the smaller adolescent sample (with consequently reduced statistical power), relations of FV or fruit intakes to adult IGFBP-2 levels were no longer discernable, regardless of adult intake levels.

As a lower dietary glycaemic index (GI), which has been related to higher fruit consumption[42], might be one explanation for the observed relations between fruit or FV and an improved insulin sensitivity (as indicated by higher levels of IGFBP-2), we considered dietary GI as an additional covariate in our analyses. In the adjusted models including GI, the associations of fruit intake and FV intake around adiposity rebound with IGFBP-2 in young adulthood were attenuated

Table 1. Characteristics of the study samples during early life (0·5–2 years), adiposity rebound (3–7 years) and adolescence (boys: 10–16 years, girls: 9–15 years) (Dortmund Nutritional and Anthropometric Longitudinally Designed Study, Germany)
(Medians and interquartile ranges (IQR); frequencies and percentages)

	Early life		Adiposity rebound		Adolescence (diet)		Adolescence (urine)	
Characteristics	Median	IQR	Median	IQR	Median	IQR	Median	IQR
n	191		265		261		236	
Female (%)	51·8		52·1		55·2		53·4	
Age (years)	1·3	1·2–1·6	5·0	5·0–5·1	12·1	12·0–13·0	12·3	11·8–13·0
Birth and infancy								
Gestational age (weeks)	40	39–41	40	39–41	40	39–41	40	39–41
Birth weight (g)	3500	3170–3800	3490	3150–3810	3450	3130–3800	3455	3130–3825
Fully breast-fed*								
n	149		202		190		173	
%	78·0		76·2		72·8		73·3	
Family								
Maternal overweight†								
n	56		75		79		73	
%	29·3		28·3		30·3		30·9	
Maternal schooling ≥12 years								
n	114		144		129		121	
%	59·7		54·3		49·4		51·3	
Any smokers in the household								
n	59		90		84		78	
%	30·9		34·0		32·2		33·1	
Diet‡								
Total energy (MJ/d)	3·6	3·3–3·9	5·5	5·0–6·0	8·0	6·8–9·0	–	
Fat (%en)	37·6	34·7–40·0	37·1	34·9–39·6	36·0	33·1–38·2	–	
SFA (%en)	17·4	16·0–18·9	16·9	15·6–18·1	15·9	14·2–17·2	–	
Carbohydrate (%en)	49·2	46·4–52·3	49·9	47·5–52·8	50·8	48·2–54·1	–	
Protein (%en)	13·2	12·3–14·3	12·7	11·8–13·6	13·0	12·0–14·1	–	
Fibre (g/d)	9·4	7·9–11·4	13·3	11·4–15·8	18·9	16·7–22·2	–	
FV (g/d)	255	205–317	338	257–426	430	328–571	–	
Fruit (g/d)	107	74–136	116	77–155	122	78–171	–	
FlavFV (mg/d)	69	51–98	102	75–145	126	86–181	–	
Urine‡								
Volume (ml/d)	–		–		–		952	783–1194
Creatinine excretion (mmol/d)	–		–		–		8·2	7·0–9·6
Hippuric acid excretion (mg/d)	–		–		–		496	429–610
Anthropometry‡								
BMI (kg/m^2)	16·8	16·1–17·7	15·6	14·8–16·6	18·4	16·8–20·1	18·8	16·9–20·6
BMI-SDS§	0·22	−0·34–0·74	0·02	−0·60–0·57	−0·15	−0·87–0·35	−0·06	−0·84–0·48
Adulthood								
Age at outcome assessment (years)	18·2	18·1–21·2	19·4	18·1–22·7	21·4	18·1–24·2	21·2	18·1–24·0
Overweight								
n	37		60		56		57	
%	19·4		22·6		21·5		24·2	

FV, fruits and vegetables including juices; FlavFV, flavonoid intake from FV; SDS, standard deviation score.
* Defined as full breast-feeding >2 weeks.
† BMI ≥25 kg/m^2.
‡ Dietary, urinary and anthropometric data are presented as the arithmetic means of ≥2 repeated measurements (or ≥3 repeated measurements for urinary data) for each individual in the respective time frame.
§ SDS derived from German reference values for children and adolescents[36].

($P=0·06$ and $P=0·1$), whereas GI adjustment did not relevantly affect the fruit IGFBP-2 associations for the adolescent sample (data not shown).

Discussion

Our findings suggest that a habitually higher FV intake during critical periods of childhood and adolescence may be related to higher levels of IGFBP-2 in young, healthy adults. These were additionally supported by our analyses based on the urinary polyphenol biomarker HA during adolescence, but not by the results for estimated FlavFV. In contrast to our findings for IGFBP-2, we did not observe any associations of the investigated dietary (and urinary) predictors during growth with adult IGF-1 levels, and a direct relation with adult IGFBP-3 concentrations was only observed in trend for FlavFV in mid-childhood.

With respect to previous evidence on the relevance of FV intake for IGFBP-2 concentrations, a few observational studies have been conducted, but it has been reported that levels of IGFBP-2 (and IGFBP-1) were substantially higher in British women consuming a vegan diet compared with those eating meat or following a vegetarian diet[43]. As the partial attenuation of FV–IGFBP-2 associations in our pathway analyses indicated, a lower dietary GI may be one relevant aspect of FV intake contributing to its beneficial effect on IGFBP-2. Alternatively,

Table 2. Prospective associations of fruits and vegetables including juices (FV), fruit and flavonoid intake from FV (FlavFV) during early life (0·5–2 years) and insulin-like growth factor (IGF-1) and its binding proteins (IGFBP-2 and IGFBP-3) in young adulthood*
(Mean values and 95 % confidence intervals; medians and interquartile ranges (IQR); *n* 191)

	T1		T2		T3		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	P_{trend}
FV intake (g/d)							
Median	184		251		348		
IQR	160–217		225–279		303–429		
IGFBP-3 (mg/l)							
Model A†	3·8	3·5, 4·1	3·9	3·6, 4·2	3·9	3·6, 4·2	0·5
Model B‡	3·8	3·5, 4·1	3·9	3·6, 4·2	3·8	3·5, 4·2	0·6
IGFBP-2 (μg/l)§							
Model A	128	111, 148	153	133, 177	138	120, 160	0·2
Model B‖	127	110, 148	153	132, 176	140	121, 162	0·07
IGF-1 (μg/l)							
Model A	317	292, 343	307	282, 332	307	283, 332	0·6
Model B¶	323	296, 349	307	281, 334	314	286, 341	0·7
Fruit intake (g/d)							
Median	63		103		162		
IQR	50–74		90–113		135–189		
IGFBP-3 (mg/l)							
Model A	3·6	3·4, 4·0	3·9	3·6, 4·2	4·0	3·7, 4·3	0·4
Model B	3·6	3·3, 4·0	3·9	3·6, 4·2	4·0	3·7, 4·3	0·4
IGFBP-2 (μg/l)							
Model A	138	119, 160	134	116, 154	148	128, 171	0·3
Model B	137	118, 159	133	116, 154	149	129, 173	0·2
IGF-1 (μg/l)							
Model A	318	293, 344	306	281, 330	308	283, 333	0·8
Model B	321	295, 347	307	281, 333	317	289, 344	0·9
FlavFV (mg/d)							
Median	46		67		111		
IQR	36–53		59–74		92–145		
IGFBP-3 (mg/l)							
Model A	3·8	3·5, 4·1	3·8	3·5, 4·1	3·9	3·6, 4·2	0·2
Model B	3·8	3·5, 4·1	3·8	3·5, 4·1	3·9	3·6, 4·2	0·3
IGFBP-2 (μg/l)							
Model A	144	124, 166	132	114, 153	143	124, 165	0·8
Model B	143	123, 166	131	114, 152	145	125, 167	0·7
IGF-1 (μg/l)							
Model A	333	308, 357	302	277, 326	297	273, 322	0·7
Model B	337	312, 363	304	277, 331	300	273, 326	0·5

T, tertile.
* Dietary predictors were included in the models as residuals on energy intake, standardised by age group and sex.
† Adjusted for sex, adult age and dummy variable for year of blood measurement.
‡ Model B for IGFBP-3: model A additionally adjusted for baseline BMI.
§ *n* 190 for IGFBP-2.
‖ Model B for IGFBP-2: model A additionally adjusted for intake of SFA.
¶ Model B for IGF-1: model A additionally adjusted for intakes of SFA and fibre from other sources than FV, for gestational age, high maternal education and full breast-feeding.

the high flavonoid content of FV could probably also explain their associations with IGFBP-2 and the extent to which it reflects insulin sensitivity, because a recent intervention study demonstrated that long-term flavonoid administration was able to reduce insulin resistance in post-menopausal diabetic women[44].

Apart from these mechanistic considerations, it is possible that the FV–IGFBP-2 associations observed in our study reflect shorter-term influences of current intake rather than longer-term adaptations of the IGF axis to intake levels during growth. We thus performed sensitivity analyses adjusting for adult FV or fruit intake levels in young adulthood, which indeed suggest that the benefits associated with fruit intake in mid-childhood may be partly attributable to tracking of fruit intake into young adulthood. However, interpretation is hampered by the reduced power in the smaller subsamples available for these analyses. Yet, it is of interest that these sensitivity analyses did not refute the potential protective link between FV intake in early life or mid-childhood and adult IGFBP-2 levels.

In our analyses, we found a direct association between the polyphenol biomarker HA in adolescence and adult IGFBP-2 levels. However, these findings were not corroborated with respect to estimated flavonoid consumption from FV. These conflicting results may be partly due to methodological problems in flavonoid estimation. As has been previously stated, estimation of flavonoid intake from dietary protocols is difficult due to the great variation of flavonoid content in natural products, differing bioavailability of the ingested compounds and incomplete or missing information in food composition databases[45,46]. Although several prospective studies calculating flavonoid intakes from the USDA databases observed meaningful associations of these dietary compounds with

Table 3. Prospective associations of fruits and vegetables including juices (FV), fruit and flavonoid intake from FV (FlavFV) during adiposity rebound (3–7 years) and insulin-like growth factor (IGF-1) and its binding proteins (IGFBP-2 and IGFBP-3) in young adulthood*
(Mean values and 95 % confidence intervals; medians and interquartile ranges (IQR); *n* 265)

	T1		T2		T3		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	P_{trend}
FV intake (g/d)							
Median	230		338		478		
IQR	194–262		303–361		421–547		
IGFBP-3 (mg/l)							
Model A†	3·9	3·6, 4·2	4·0	3·7, 4·3	3·8	3·6, 4·1	0·9
Model B‡	3·8	3·5, 4·1	3·9	3·7, 4·2	3·8	3·5, 4·1	0·5
IGFBP-2 (μg/l)§							
Model A	131	114, 149	139	122, 157	146	129, 167	0·07
Model B‖	124	108, 143	131	114, 149	142	123, 164	0·045
IGF-1 (μg/l)							
Model A	298	275, 321	283	261, 304	296	274, 318	0·4
Model B¶	300	276, 324	283	260, 306	304	280, 328	0·7
Fruit intake (g/d)							
Median	63		116		174		
IQR	48–77		99–128		153–211		
IGFBP-3 (mg/l)							
Model A	3·8	3·5, 4·1	3·9	3·6, 4·2	4·0	3·7, 4·3	0·4
Model B	3·7	3·5, 4·0	3·8	3·6, 4·1	4·0	3·7, 4·3	0·3
IGFBP-2 (μg/l)							
Model A	132	116, 150	139	122, 158	146	128, 166	0·04
Model B	125	109, 143	129	113, 149	141	123, 162	0·03
IGF-1 (μg/l)							
Model A	301	279, 323	278	256, 300	296	274, 318	0·5
Model B	303	280, 326	279	255, 303	302	278, 325	0·6
FlavFV (mg/d)							
Median	63		102		162		
IQR	53–75		95–113		145–184		
IGFBP-3 (mg/l)							
Model A	3·7	3·5, 4·0	3·9	3·7, 4·2	4·0	3·8, 4·3	0·1
Model B	3·7	3·4, 4·0	3·9	3·6, 4·2	4·0	3·7, 4·3	0·08
IGFBP-2 (μg/l)							
Model A	135	119, 154	139	122, 158	142	125, 162	0·2
Model B	128	112, 147	131	114, 151	136	119, 157	0·1
IGF-1 (μg/l)							
Model A	302	280, 324	280	257, 302	292	270, 314	0·3
Model B	304	281, 327	283	259, 307	296	272, 320	0·4

T, tertile.
* Dietary predictors were included in the models as residuals on energy intake, standardised by age group and sex.
† Adjusted for sex, adult age and dummy variable for year of blood measurement.
‡ Model B for IGFBP-3: model A additionally adjusted for standardised energy intake, intake of SFA and maternal overweight (BMI ≥25 kg/m^2, yes/no).
§ *n* 264 for IGFBP-2.
‖ Model B for IGFBP-2: model A additionally adjusted for intake of SFA and full breast-feeding.
¶ Model B for IGF-1: model A additionally adjusted for intake of SFA, birth weight and full breast-feeding.

hypertension incidence[46] as well as CVD mortality[47], our findings strengthen the importance of combining intake data with biomarker analyses in epidemiological studies on diet–disease relationships.

The elevated IGFBP-2 concentrations related to higher FV intake may have distinct implications for future cancer risk, because unlike IGFBP-2's clearly favourable role in insulin metabolism[17], conflicting results have been reported regarding the relevance of circulating IGFBP-2 levels for cancer. On the one hand, two previous studies found a reduced risk of colorectal cancer[48] as well as post-menopausal breast cancer[49] in those individuals with higher IGFBP-2 concentrations, probably related to its inverse regulation by insulin and its role in restricting IGF-1 bioavailability. On the other hand, it has been reported that IGFBP-2 levels are frequently elevated in individuals with different types of cancer and that IGFBP-2 might be a marker of tumour differentiation[50]. These findings implicate that IGFBP-2 may exert a different role in cancer initiation compared with the already-established disease.

In contrast to the results for IGFBP-2 in our study populations, no consistent prospective associations were discernible between the investigated dietary predictors and IGF-1 or IGFBP-3. This is in contrast with *in vitro* and animal studies that have quite consistently reported up-regulation of IGFBP-3 and down-regulation of IGF-1 concurrently with diminished tumour growth upon administration of different plant polyphenols[13–16,51]. The effects of polyphenol-rich extracts or single polyphenolic compounds administered in pharmacological doses in these studies may, however, not be transferable to polyphenol levels achievable with normal human diets.

To our knowledge, long-term associations between flavonoid intakes and the GH–IGF system have not been investigated in

Table 4. Prospective associations of fruits and vegetables including juices (FV), fruit and flavonoid intake from FV (FlavFV) during adolescence (boys: 10–16 years, girls: 9–15 years) and insulin-like growth factor (IGF-1) and its binding proteins (IGFBP-2 and IGFBP-3) in young adulthood*
(Mean values and 95 % confidence intervals; medians and interquartile ranges (IQR); *n* 261)

	T1		T2		T3		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	P_{trend}
FV intake (g/d)							
Median	273		426		628		
IQR	216–336		379–474		566–735		
IGFBP-3 (mg/l)							
Model A†	4·0	3·7, 4·3	3·7	3·4, 4·0	3·9	3·6, 4·2	0·9
Model B‡	3·9	3·6, 4·2	3·7	3·4, 3·9	3·9	3·6, 4·2	0·5
IGFBP-2 (μg/l)§							
Model A	145	128, 166	141	123, 161	164	143, 187	0·2
Model B‖	140	122, 160	137	119, 157	162	141, 187	0·09
IGF-1 (μg/l)							
Model A	279	257, 301	276	253, 298	281	259, 303	0·7
Model B¶	273	251, 295	276	254, 298	287	264, 309	0·2
Fruit intake (g/d)							
Median	66		122		194		
IQR	41–79		103–146		171–229		
IGFBP-3 (mg/l)							
Model A	3·8	3·5, 4·1	3·9	3·7, 4·2	3·9	3·6, 4·2	>0·9
Model B	3·7	3·5, 4·0	3·9	3·6, 4·2	3·8	3·5, 4·2	0·9
IGFBP-2 (μg/l)							
Model A	147	129, 167	146	128, 168	156	136, 178	0·05
Model B	142	124, 162	143	125, 164	151	131, 174	0·045
IGF-1 (μg/l)							
Model A	276	254, 298	285	262, 307	275	253, 297	>0·9
Model B	273	251, 295	284	262, 306	278	256, 300	0·6
FlavFV (mg/d)							
Median	73		126		206		
IQR	57–88		110–147		179–232		
IGFBP-3 (mg/l)							
Model A	3·9	3·6, 4·2	3·8	3·5, 4·1	3·9	3·7, 4·2	0·8
Model B	3·8	3·5, 4·1	3·8	3·5, 4·1	3·9	3·6, 4·2	0·5
IGFBP-2 (μg/l)							
Model A	143	125, 163	154	135, 177	153	134, 174	0·3
Model B	138	120, 157	150	131, 173	150	130, 172	0·2
IGF-1 (μg/l)							
Model A	279	257, 301	273	250, 296	283	261, 305	0·7
Model B	274	252, 296	273	250, 295	287	266, 309	0·7

T, tertile.
* Dietary predictors were included in the models as residuals on energy intake, standardised by age group and sex.
† Adjusted for sex, adult age and dummy variable for year of blood measurement.
‡ Model B for IGFBP-3: model A additionally adjusted for intakes of SFA and protein, for birth weight and maternal overweight (BMI ≥25 kg/m^2, yes/no).
§ *n* 260 for IGFBP-2.
‖ Model B for IGFBP-2: model A additionally adjusted for intake of SFA, high maternal educational status and maternal overweight (BMI ≥25 kg/m^2, yes/no).
¶ Model B for IGF-1: model A additionally adjusted for intake of SFA.

epidemiological studies, but cross-sectional studies in adults on (biomarkers of) FV, as a flavonoid-rich food group, have reported inconsistent results, with some studies supporting[41,52] and other studies opposing[53] the findings from the above-mentioned *in vitro* and animal data. Regarding available evidence during growth, a cross-sectional analysis in 521 7–8-year-old children found that IGFBP-3 levels were not associated with the intakes of fruits, vegetables or tomatoes, whereas higher IGF-1 concentrations were unexpectedly observed in those boys with the highest fruit intake[54]. In addition to the difficulties of interpreting these contradictory results from studies examining different age groups with different FV intake levels as well as varying dietary assessment tools, no causal relations can be deduced from cross-sectional data.

Another factor that may have contributed to the inconsistent results regarding dietary influences on circulating components of the GH–IGF axis is the ability of these blood levels to reflect the biologically active concentrations: in rats, oral administration of the flavonoid genistein and the stilbene resveratrol effectively reduced tumour growth and tissue expression of IGF-1, whereas serum levels of IGF-1 were unaffected by the treatment[16]. In addition, a human study reported that IGFBP-3 expression in the colonic mucosa, but not plasma IGFBP-3 levels, was lower in patients with colorectal adenomas compared with healthy controls[55]. These studies[16,55] indicate that – at least in the short term – circulating levels of IGF-1 and its binding proteins may not reflect the relevant tissue levels. Furthermore, heterogeneous findings in epidemiological studies may arise from the assay used, as previous studies demonstrated that at least for IGF-1 and IGFBP-3 disease risk estimates can in great part depend on the method of measurement[56,57]. In our study, the same in-house RIA were used for both

Table 5. Associations of 24-h hippuric acid excretion during adolescence (boys: 10–16 years, girls: 9–15 years) and insulin-like growth factor (IGF-1) and its binding proteins (IGFBP-2 and IGFBP-3) in young adulthood*
(Mean values and 95 % confidence intervals; medians and interquartile ranges (IQR); *n* 236)

	T1		T2		T3		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	P_{trend}
Hippuric acid excretion (mg/d)							
Median	401		496		658		
IQR	345–453		449–545		580–773		
IGFBP-3 (mg/l)							
Model A†	4·1	3·8, 4·5	3·9	3·6, 4·2	4·0	3·7, 4·4	0·7
Model B‡	4·1	3·8, 4·4	3·8	3·5, 4·1	3·9	3·5, 4·2	>0·9
IGFBP-2 (μg/l)§							
Model A	135	117, 156	134	117, 153	157	136, 180	0·056
Model B‖		–		–		–	–
IGF-1 (μg/l)							
Model A	293	268, 317	272	249, 295	289	265, 313	0·7
Model B¶	295	271, 320	270	247, 292	287	262, 311	0·9

T, tertile.
* Hippuric acid excretion was included in the models as residuals on individual body surface area, standardised by age group and sex.
† Adjusted for sex, adult age and dummy variable for year of blood measurement.
‡ Model B for IGFBP-3: model A additionally adjusted for 24-h urine volume, 24-h urea excretion, maternal overweight (BMI ≥ 25 kg/m^2, yes/no) and smokers in the household (yes/no).
§ *n* 235 for IGFBP-2.
‖ Model B for IGFBP-2: see model A (no additional confounders identified).
¶ Model B for IGF-1: model A additionally adjusted for baseline fat mass index, 24-h urine volume, 24-h creatinine excretion and gestational age.

measurement series (2011 and 2014) of IGF-1 and IGFBP-3, which preclude bias due to a change in methodology. Nevertheless, subtle changes between the measurement series could have contributed to dilution of possible influences of fruit, FV and FlavFV on these outcomes.

Our study has several additional limitations including the comparatively small sample sizes as well as the fact that only one blood sample for each individual could be used for measurements of the GH–IGF axis in young adulthood. Despite the fact that we used the mean of at least two dietary records, which were also checked for plausibility, to obtain stable estimates of usual dietary intake in each age range, we cannot exclude the possibility that the higher percentage of dietary records filled in by the adolescents themselves affects the comparability of the data between the age groups. However, previous analyses indicated that the number of autonomously recorded dietary protocols did not differ between adolescents with plausible and implausible records[21]. Flavonoid intake in our analyses was only calculated from the food groups of fruits, vegetables and juices. Thus, relevant flavonoid intake from other foods might have influenced our results. However, non-FV food groups reported to relevantly contribute to flavonoid intake in other population-based studies, such as tea, red wine and coffee[28,58], were consumed in only minimal amounts by our DONALD children and adolescents. Moreover, additional adjustment for the consumption of coffee or tea as well as cocoa products yielded very similar results compared with those reported in Tables 2–4 (data not shown).

Strengths of our study include its prospective design covering different periods of childhood and adolescence to identify potentially vulnerable age ranges in which diet may influence the GH–IGF axis in the longer term. Furthermore, repeated detailed dietary data were available to reliably describe habitual diet in the time frames of interest. Although the DONALD cohort is characterised by a relatively high socio-economic status and extremes of dietary behaviour may not be represented[20], FV intake in DONALD children seems comparable with data from other German representative paediatric populations[59]. Finally, in our adolescent sample, comparative analyses could be performed using urinary HA excretion, a polyphenol biomarker that reflects potentially bioavailable polyphenols and does not share the same potential biases as dietary assessment[45]. We were, however, not able to control for the influence of benzoic acid added as a food preservative, which also constitutes a potentially important precursor of urinary HA excretion[30].

To conclude, a higher fruit and FV intake during growth may beneficially affect adult insulin metabolism and restrict IGF-1 bioavailability as indicated by higher levels of IGFBP-2. As suggested by our biomarker analyses, these associations might be partly attributable to the high polyphenol contents of FV. Our study does not support a major relevance of fruit or FV for adult IGF-1 or IGFBP-3 concentrations, but methodological considerations hamper a definite conclusion on these biological plausible associations.

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The authors' contributions are as follows: D. K., T. R. and A. E. B. conceived the project. D. K. carried out the statistical analyses and drafted the manuscript. T. R. and A. E. B.

contributed to the study design, the manuscript drafting and data interpretation. K. J. P. contributed to the flavonoid assignment procedure and data analyses. K. B. contributed to the statistical analyses and data interpretation. Measurements of IGF-1 and IGFBP were carried out in the laboratory of S. A. W. All authors critically revised the manuscript for important intellectual content.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114515004742

References

1. Liu RH (2013) Health-promoting components of fruits and vegetables in the diet. *Adv Nutr* **4**, 384S–392S.
2. Boeing H, Bechthold A, Bub A, *et al.* (2012) Critical review: vegetables and fruit in the prevention of chronic diseases. *Eur J Nutr* **51**, 637–663.
3. Potischman N, Weiss HA, Swanson CA, *et al.* (1998) Diet during adolescence and risk of breast cancer among young women. *J Natl Cancer Inst* **90**, 226–233.
4. Maynard M, Gunnell D, Emmett P, *et al.* (2003) Fruit, vegetables, and antioxidants in childhood and risk of adult cancer: the Boyd Orr cohort. *J Epidemiol Community Health* **57**, 218–225.
5. Key TJ (2011) Fruit and vegetables and cancer risk. *Br J Cancer* **104**, 6–11.
6. Aune D, Chan DS, Vieira AR, *et al.* (2012) Fruits, vegetables and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Breast Cancer Res Treat* **134**, 479–493.
7. Noratto G, Porter W, Byrne D, *et al.* (2009) Identifying peach and plum polyphenols with chemopreventive potential against estrogen-independent breast cancer cells. *J Agric Food Chem* **57**, 5219–5226.
8. Aiyer HS, Warri AM, Woode DR, *et al.* (2012) Influence of berry polyphenols on receptor signaling and cell-death pathways: implications for breast cancer prevention. *J Agric Food Chem* **60**, 5693–5708.
9. Juul A (2003) Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Horm IGF Res* **13**, 113–170.
10. Jogie-Brahim S, Feldman D & Oh Y (2009) Unraveling insulin-like growth factor binding protein-3 actions in human disease. *Endocr Rev* **30**, 417–437.
11. Ben-Shlomo Y, Holly J, McCarthy A, *et al.* (2005) Prenatal and postnatal milk supplementation and adult insulin-like growth factor I: long-term follow-up of a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev* **14**, 1336–1339.
12. Joslowski G, Remer T, Assmann KE, *et al.* (2013) Animal protein intakes during early life and adolescence differ in their relation to the growth hormone-insulin-like-growth-factor axis in young adulthood. *J Nutr* **143**, 1147–1154.
13. Koyama S, Cobb LJ, Mehta HH, *et al.* (2010) Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis. *Growth Horm IGF Res* **20**, 55–62.
14. Vijayababu MR, Arunkumar A, Kanagaraj P, *et al.* (2006) Effects of quercetin on insulin-like growth factors (IGFs) and their binding protein-3 (IGFBP-3) secretion and induction of apoptosis in human prostate cancer cells. *J Carcinog* **5**, 10.
15. Akhtar S, Meeran SM, Katiyar N, *et al.* (2009) Grape seed proanthocyanidins inhibit the growth of human non-small cell lung cancer xenografts by targeting insulin-like growth factor binding protein-3, tumor cell proliferation, and angiogenic factors. *Clin Cancer Res* **15**, 821–831.
16. Harper CE, Cook LM, Patel BB, *et al.* (2009) Genistein and resveratrol, alone and in combination, suppress prostate cancer in SV-40 tag rats. *Prostate* **69**, 1668–1682.
17. Arafat AM, Weickert MO, Frystyk J, *et al.* (2009) The role of insulin-like growth factor (IGF) binding protein-2 in the insulin-mediated decrease in IGF-I bioactivity. *J Clin Endocrinol Metab* **94**, 5093–5101.
18. Babu PV, Liu D & Gilbert ER (2013) Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *J Nutr Biochem* **24**, 1777–1789.
19. Gallagher EJ & LeRoith D (2010) The proliferating role of insulin and insulin-like growth factors in cancer. *Trends Endocrinol Metab* **21**, 610–618.
20. Kroke A, Manz F, Kersting M, *et al.* (2004) The DONALD Study. History, current status and future perspectives. *Eur J Nutr* **43**, 45–54.
21. Sichert-Hellert W, Kersting M & Schoch G (1998) Underreporting of energy intake in 1 to 18 year old German children and adolescents. *Z Ernahrungswiss* **37**, 242–251.
22. Remer T, Neubert A & Maser-Gluth C (2002) Anthropometry-based reference values for 24-h urinary creatinine excretion during growth and their use in endocrine and nutritional research. *Am J Clin Nutr* **75**, 561–569.
23. Sichert-Hellert W, Kersting M, Chada C, *et al.* (2007) German food composition database for dietary evaluations in children and adolescents. *J Food Compost Anal* **20**, 63–70.
24. US Department of Agriculture (2014) USDA database for the flavonoid content of selected foods, release 3.1. http://www.ars.usda.gov/Services/docs.htm?docid=24953 (accessed September 2014).
25. US Department of Agriculture (2004) USDA database for the proanthocyanidin content of selected foods. http://www.ars.usda.gov/Services/docs.htm?docid=24953 (accessed September 2014).
26. US Department of Agriculture (2008) USDA database for the isoflavone content of selected foods, release 2.0. http://www.ars.usda.gov/Services/docs.htm?docid=24953 (accessed September 2014)
27. Bhagwat S, Haytowitz DB, Wasswa-Kintu SI, *et al.* (2013) USDA develops a database for flavonoids to assess dietary intakes. *Procedia Food Sci* **2**, 81–86.
28. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventos RM, *et al.* (2010) Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). *J Am Diet Assoc* **110**, 390–398.
29. Frank T, Netzel G, Kammerer DR, *et al.* (2012) Consumption of *Hibiscus sabdariffa* L. aqueous extract and its impact on systemic antioxidant potential in healthy subjects. *J Sci Food Agric* **92**, 2207–2218.
30. Krupp D, Doberstein N, Shi L, *et al.* (2012) Hippuric acid in 24-hour urine collections is a potential biomarker for fruit and vegetable consumption in healthy children and adolescents. *J Nutr* **142**, 1314–1320.
31. Tomokuni K & Ogata M (1972) Direct colorimetric determination of hippuric acid in urine. *Clin Chem* **18**, 349–351.
32. Rechner AR, Kuhnle G, Bremner P, *et al.* (2002) The metabolic fate of dietary polyphenols in humans. *Free Radic Biol Med* **33**, 220–235.
33. Bring A, Penczynski K, Krupp D, *et al.* (2014) P43: Relative Validierung der Hippursäureausscheidung im 24-Stunden-Sammelurin als Biomarker für die Flavonoidzufuhr aus Obst

und Gemüse bei Jugendlichen (Relative validity of hippuric acid excretion in 24-h urine samples as a biomarker for flavonoid intake from fruits and vegetables among adolescents). In *Abstractband der 9. Epidemiologie als Schlüssel für Prävention und bessere Versorgung*, pp. 182–183 [D Rothenbacher, editor]. Ulm: Jahrestagung der Deutschen Gesellschaft für Epidemiologie.
34. Blum WF, Ranke MB & Bierich JR (1986) Isolation and partial characterization of six somatomedin-like peptides from human plasma Cohn fraction IV. *Acta Endocrinol (Copenh)* **111**, 271–284.
35. DuBois D & DuBois EF (1916) A formula to estimate the approximate surface area if height and weight are known. *Arch Intern Med* **17**, 863–871.
36. Neuhauser H, Schienkiewitz A, Schaffrath Rosario A, *et al.* (2011) *Referenzperzentile für anthropometrische Maßzahlen und Blutdruck aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS) 2003–2006 (Reference Percentiles for Body Measurements and Blood Pressure from German National Health Interview and Examination Survey for Children and Adolescents (KiGGS) 2003–2006)*. Berlin: Robert Koch-Institut.
37. Deurenberg P, Pieters JJ & Hautvast JG (1990) The assessment of the body fat percentage by skinfold thickness measurements in childhood and young adolescence. *Br J Nutr* **63**, 293–303.
38. Slaughter MH, Lohman TG, Boileau RA, *et al.* (1988) Skinfold equations for estimation of body fatness in children and youth. *Hum Biol* **60**, 709–723.
39. Hu FB, Stampfer MJ, Rimm E, *et al.* (1999) Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol* **149**, 531–540.
40. Bihuniak JD, Simpson CA, Sullivan RR, *et al.* (2013) Dietary protein-induced increases in urinary calcium are accompanied by similar increases in urinary nitrogen and urinary urea: a controlled clinical trial. *J Acad Nutr Diet* 113, 447–451.
41. McGreevy KM, Hoel BD, Lipsitz SR, *et al.* (2007) Impact of nutrients on insulin-like growth factor-I, insulin-like growth factor binding protein-3 and their ratio in African American and white males. *Public Health Nutr* **10**, 97–105.
42. Du H, van der AD, van Bakel MM, *et al.* (2008) Glycemic index and glycemic load in relation to food and nutrient intake and metabolic risk factors in a Dutch population. *Am J Clin Nutr* **87**, 655–661.
43. Allen NE, Appleby PN, Davey GK, *et al.* (2002) The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev* **11**, 1441–1448.
44. Curtis PJ, Sampson M, Potter J, *et al.* (2012) Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-year CVD risk in medicated postmenopausal women with type 2 diabetes: a 1-year, double-blind, randomized, controlled trial. *Diabetes Care* **35**, 226–232.
45. Linseisen J & Rohrmann S (2008) Biomarkers of dietary intake of flavonoids and phenolic acids for studying diet-cancer relationship in humans. *Eur J Nutr* **47**, Suppl. 2, 60–68.
46. Cassidy A, O'Reilly EJ, Kay C, *et al.* (2011) Habitual intake of flavonoid subclasses and incident hypertension in adults. *Am J Clin Nutr* **93**, 338–347.
47. Mink PJ, Scrafford CG, Barraj LM, *et al.* (2007) Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am J Clin Nutr* **85**, 895–909.
48. Kaaks R, Toniolo P, Akhmedkhanov A, *et al.* (2000) Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. *J Natl Cancer Inst* **92**, 1592–1600.
49. Krajcik RA, Borofsky ND, Massardo S, *et al.* (2002) Insulin-like growth factor I (IGF-I), IGF-binding proteins, and breast cancer. *Cancer Epidemiol Biomarkers Prev* **11**, 1566–1573.
50. Hoeflich A, Reisinger R, Lahm H, *et al.* (2001) Insulin-like growth factor-binding protein 2 in tumorigenesis: protector or promoter? *Cancer Res* **61**, 8601–8610.
51. Suh YA, Kim JH, Sung MA, *et al.* (2013) A novel antitumor activity of deguelin targeting the insulin-like growth factor (IGF) receptor pathway via up-regulation of IGF-binding protein-3 expression in breast cancer. *Cancer Lett* **332**, 102–109.
52. Gunnell D, Oliver SE, Peters TJ, *et al.* (2003) Are diet-prostate cancer associations mediated by the IGF axis? A cross-sectional analysis of diet, IGF-I and IGFBP-3 in healthy middle-aged men. *Br J Cancer* **88**, 1682–1686.
53. Suzuki K, Ito Y, Hashimoto S, *et al.* (2009) Association of serum retinol and carotenoids with insulin-like growth factors and insulin-like growth factor binding protein-3 among control subjects of a nested case-control study in the Japan Collaborative Cohort Study. *Asian Pac J Cancer Prev* **10**, Suppl., 29–35.
54. Rogers IS, Gunnell D, Emmett PM, *et al.* (2005) Cross-sectional associations of diet and insulin-like growth factor levels in 7- to 8-year-old children. *Cancer Epidemiol Biomarkers Prev* **14**, 204–212.
55. Keku TO, Sandler RS, Simmons JG, *et al.* (2008) Local IGFBP-3 mRNA expression, apoptosis and risk of colorectal adenomas. *BMC Cancer* **8**, 143.
56. Muller C, Wallaschofski H, Brabant G, *et al.* (2014) The association between IGF-I/IGFBP-3 and subclinical endpoints-epidemiology faces the limits. *J Clin Endocrinol Metab* **99**, 2804–2812.
57. Rinaldi S, Kaaks R, Zeleniuch-Jacquotte A, *et al.* (2005) Insulin-like growth factor-I, IGF binding protein-3, and breast cancer in young women: a comparison of risk estimates using different peptide assays. *Cancer Epidemiol Biomarkers Prev* **14**, 48–52.
58. Zamora-Ros R, Forouhi NG, Sharp SJ, *et al.* (2014) Dietary intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations. *J Nutr* **144**, 335–343.
59. Behrendt I & Krawinkel M (2012) [Children should eat more fruit and vegetables. Results of PRO GREENS]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* **55**, 254–259.