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 cancer1,2. 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 life3,4,5,6. 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 assessment5,6. 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 vivo7,8,9,10.

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 markers9,10. Specifically, whereas higher IGF-1 levels seem to be associated with an increased cancer risk10, the antiproliferative and pro-apoptotic actions of its major binding protein IGFBP-3 suggest that higher IGFBP-3 levels might be related to lower risk for cancer11.

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. The GH–IGF axis is also susceptible to nutri-
tional influences, 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, thereby possibly explain-
ing 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 and animal studies 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. Higher flavonoid consumption from FV may improve insulin sensitivity, thereby decreasing insulin levels, which could in turn contribute to decreased cancer risk. 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. 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 BMI was below age- and sex-specific cut-off values. 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 Digitas 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 LEITAB (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(26).

Laboratory measurements

Annual 24-h urine collections are usually performed at the 3rd visit 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 method and creatinine excretion is determined by the kinetic Jaffé method(31). 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(26).

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/m², 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–S5). 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.
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. As the partial attenuation of FV intake on IGFBP-2 was independently higher in women consuming a vegan diet compared with those eating meat or following a vegetarian diet, we 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.

(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).

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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)

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<td>Model B</td>
<td>143</td>
<td>123, 166</td>
<td>131</td>
<td>114, 152</td>
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<td>IGF-1 (μg/l)</td>
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<td></td>
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<td></td>
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<tr>
<td>Model A</td>
<td>333</td>
<td>308, 357</td>
<td>302</td>
<td>277, 326</td>
</tr>
<tr>
<td>Model B</td>
<td>337</td>
<td>312, 363</td>
<td>304</td>
<td>277, 331</td>
</tr>
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</table>

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[43,46]. Although several prospective studies calculating flavonoid intakes from the USDA databases observed meaningful associations of these dietary compounds with...
hypothesis of a role of IGFBP-2 in promoting 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(12,16,51). 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 detailed. More studies are needed to further elucidate the role of flavonoids in the modulation of IGF pathways.
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)

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<th>T3</th>
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<tr>
<td>FV intake (g/d)</td>
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<tr>
<td>Median</td>
<td>273</td>
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<td>628</td>
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<tr>
<td>IQR</td>
<td>216–336</td>
<td>379–474</td>
<td>566–735</td>
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<tr>
<td>IGFBP-3 (mg/l)</td>
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<td></td>
<td></td>
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<tr>
<td>Model A†</td>
<td>3.9 3.6 4.2</td>
<td>3.9 3.6 4.2</td>
<td>3.9 3.6 4.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Model B‡</td>
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<td>3.9 3.6 4.2</td>
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<tr>
<td>IGFBP-2 (μg/l)‡</td>
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<td>142 124, 162</td>
<td>142 124, 162</td>
<td>0.05</td>
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<td>147 129, 167</td>
<td>147 129, 167</td>
<td>0.045</td>
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<tr>
<td>IGF-1 (μg/l)§</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>276 254, 298</td>
<td>276 254, 298</td>
<td>276 254, 298</td>
<td>0.09</td>
</tr>
<tr>
<td>Model B</td>
<td>273 251, 295</td>
<td>273 251, 295</td>
<td>273 251, 295</td>
<td>0.2</td>
</tr>
</tbody>
</table>

| Fruit intake (g/d)  |               |               |               |                        |
| Median              | 66            | 122           | 194           |                        |
| IQR                 | 41–79         | 103–146       | 171–229       |                        |
| FLAVFV (mg/d)       |               |               |               |                        |
| Median              | 73            | 126           | 206           |                        |
| IQR                 | 57–88         | 110–147       | 179–232       |                        |

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 A additionally adjusted for intakes of SFA and protein, for birth weight and maternal overweight (BMI ≥ 25 kg/m², yes/no).
§ Model B additionally adjusted for intake of SFA, high maternal educational status and maternal overweight (BMI ≥ 25 kg/m², yes/no).
¶ Model B for IGFBP-3: model A additionally adjusted for intake of SFA, protein, for birth weight and maternal overweight (BMI ≥ 25 kg/m², yes/no).
\(\dagger\) Model B for IGFBP-2: model A additionally adjusted for intake of SFA, high maternal educational status and maternal overweight (BMI ≥ 25 kg/m², yes/no).
\(\ddagger\) Model B for IGFBP-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\(^{(10)}\). 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 part depend on the method of measurement\(^{(56,57)}\). In our study, the same in-house RIA were used for both
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. 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 contributed to dilution of possible influences of fruit, FV and FlavFV on these outcomes.

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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


