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Dietary intake, body composition and biomarkers in children and adolescents

Thesis

submitted for a Doctoral degree in Human Biology at the Faculty of Medicine,

Ludwig-Maximilians-University, Munich, Germany

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from

Port Moresby, Papua New Guinea

2018

With approval of the Medical Faculty of the
Ludwig-Maximilians-University of Munich

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Date of oral examination:	10.09.2018

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Abbreviations

BIA	bioelectrical impedance analysis
BLS	Bundeslebensmittelschlüssel (German Nutrient Database)
CRP	C-reactive protein
FAME	fatty acid methyl esters
FFQ	food frequency questionnaire
FFMI	fat-free mass index
FMI	fat mass index
GINIplus	*G*erman *I*nfant *N*utritional *I*ntervention *plus* environmental and genetic influences on allergy development
HDL	high-density lipoprotein
hs-CRP	high-sensitivity C-reactive protein
IL-6	interleukin-6
LDL	low-density lipoprotein
LISAplus	Influence of *L*ifestyle-Related Factors on the *I*mmune *S*ystem and the Development of *A*llergies in Childhood *plus* the influence of traffic emissions and genetics
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
TAG	triglycerides
TOTAL:HDL	ratio of total cholesterol to HDL

Summary

Growing evidence suggests that non-communicable diseases begin early in life with the development of intermediate risk factors. These include a number of metabolic dysfunctions, which can appear and remain in subclinical form, years before disease onset. The current prevalence of overweight and obesity in children and adolescents is alarmingly high, and other associated conditions such as dyslipidaemia and low-grade inflammation have also been detected at young ages.

Foods and nutrients are reported to be of relevance in the development of intermediate risk factors. It is hypothesised that associations between diet and disease risk factors can be observed during childhood and persist throughout adolescence. Early, informed dietary modification is therefore considered a key strategy for primary prevention of non-communicable diseases. Nevertheless, for many dietary components, evidence is often lacking in children and adolescents in order to reach definitive conclusions. A deeper understanding of the associations between dietary intake and intermediate risk factors is therefore needed in this population.

Among the many foods and nutrients suggested to play an important role in health, high meat intake has been associated with weight gain, while dietary fatty acids are proposed to influence circulating lipid levels and to modify inflammatory processes. The present thesis uses data from the GINIplus and LISAplus birth cohort studies to evaluate these relationships during the critical period of adolescence, and includes four publications.

In the first publication the prospective role of meat intake was assessed in relation to body composition at age 15 years. Associations with different meat types as well as their respective protein contents were assessed. Significant associations were observed in males only, with higher poultry intakes being associated with fat mass, and total and red meat being associated with lean mass. Protein from total and red meat was also associated with higher lean mass.

In the second publication, associations between dietary fatty acids and changes in blood lipids during adolescence were investigated. Higher intakes of saturated fatty acids were associated with reduced triglyceride levels. The importance of interpreting such associations in the context of other nutrients was highlighted by results from substitution analyses. Here, the theoretical isocaloric replacement of saturated fatty acids with carbohydrates was associated with detrimental changes in the blood lipid profile of females.

The third publication explored the role of fatty acids in low-grade inflammation in 10-year-old children. Fatty acid composition was analysed in serum glycerophospholipids and assessed in relation to levels of common inflammatory markers (hs-CRP and IL-6). Linoleic acid and total polyunsaturated fatty acids were associated with reduced inflammation. Palmitic acid, total saturated fatty acids, arachidonic acid, highly-unsaturated fatty acids, and the ratio of arachidonc to linoleic acid were associated with increased inflammation.

The final publication describes changes in dietary intake from childhood to adolescence. Average intake changes were assessed, as well as individual tracking levels (the stability

of food intake behaviours over time, with respect to the rest of the population). Additionally, possible determinants of dietary changes were evaluated. Mostly fair tracking levels were observed, despite a general trend in the population to reduce starchy vegetable, margarine and dairy intakes. Family income and parental education predominantly influenced individual dietary changes.

Together these results support the hypothesis that certain components of dietary intake are associated with intermediate risk factors for noncommunicable diseases as early as childhood and adolescence. Clinical trials should follow in order to confirm causal relationships. Primary prevention strategies targeting children could then benefit from these findings to achieve optimal impact.

Zusammenfassung

Die Annahme, dass sich nichtübertragbare Krankheiten bereits früh mit der Entstehung von intermediären Risikofaktoren entwickeln, wurde vermehrt bestätigt. Diese Risikofaktoren umfassen eine Reihe metabolischer Dysfunktionen, die sich bereits Jahre vor dem Auftreten der eigentlichen Erkrankung in subklinischer Form manifestieren. Die derzeitige Prävalenz von Übergewicht und Adipositas bei Kindern und Jugendlichen ist besorgniserregend hoch und intermediäre Risikofaktoren wie Dyslipidämie und chronisch erhöhte Entzündungswerte sind schon in jungem Alter nachweisbar.

Es wurde berichtet, dass Lebensmittel und Nährstoffe bei der Entstehung intermediärer Risikofaktoren eine Rolle spielen. Dabei wird angenommen, dass sich der Zusammenhang zwischen Ernährung und Risikofaktoren für die Entstehung von Krankheiten bereits in der Kindheit entwickelt und auch in der Jugend weiter bestehen bleibt. Eine frühe Anpassung der Ernährung kann daher eine Schlüsselstrategie bei der primären Prävention von nichtübertragbaren Krankheiten sein. Dennoch fehlt bisher für viele Lebensmittel der nötige Nachweis dieses Zusammenhangs bei Kindern und Jugendlichen – eine Voraussetzung um entsprechende Schlussfolgerungen ziehen zu können. Ein besseres Verständnis des Zusammenhangs von Ernährung mit intermediären Risikofaktoren in dieser Altersgruppe ist daher notwendig.

Viele Lebensmittel und Nährstoffe scheinen eine gesundheitlich relevante Rolle zu haben. Unter anderem wird ein Zusammenhang von Fleischverzehr und Gewichtszunahme vermutet. Ebenso wird spekuliert, dass die Fettsäurenzusammensetzung der Nahrung die Blutfettwerte sowie Inflammationsprozesse beeinflusst. Die vorliegende Arbeit untersucht diese Zusammenhänge im Zeitfenster von der Kindheit bis zum Jugendalter und umfasst vier Publikationen, die auf Daten der GINIplus und LISAplus Geburtskohorten basieren.

Die erste Publikation beschreibt den prospektiven Zusammenhang des Konsums verschiedener Fleischsorten und deren Proteingehalt mit der Körperzusammensetzung im Alter von 15 Jahren. Signifikante Zusammenhänge wurden nur für Jungen beobachtet, wobei ein höherer Verzehr von Geflügel mit höherer Fettmasse und ein höherer Verzehr von rotem Fleisch mit einer höheren fettfreien Masse einhergingen.

In der zweiten Publikation wurde der Zusammenhang von Fettsäurenverzehr mit Veränderungen der Blutlipide während der Pubertät betrachtet. Ein höherer Verzehr von gesättigten Fettsäuren war dabei mit niedrigeren Triglyzeridwerten assoziiert. Für die Interpretation ist es von großer Relevanz die übrigen Nährstoffe zu berücksichtigen, weshalb eine Analyse der Nährstoffsubstitution verwendet wurde. Dabei führte der theoretische isokalorische Austausch von gesättigten Fettsäuren mit Kohlenhydraten zu ungünstigen Veränderungen des Blutlipidprofils bei Mädchen.

In der dritten Publikation wurde der Einfluss von Fettsäuren auf Entzündungswerte bei 10-jährigen Kindern analysiert. Die Fettsäurenzusammensetzung wurde in Glycerophospholipiden im Serum gemessen und deren Zusammenhang mit Inflammationsparametern (hs-CRP und IL-6) ausgewertet. Ein höherer Anteil an Linolsäure und mehrfach ungesättigten Fettsäuren führte zu niedrigeren Entzündungswerten, während höhere Konzentrationen von Palmitinsäure, gesättigten Fettsäuren, Arachidonsäure, hoch un-

gesättigten Fettsäuren und das Verhältnis von Arachidon- zu Linolsäure mit höheren Entzündungsmarkern assoziiert waren.

Die letzte Publikation beschreibt die Veränderung der Ernährung von der Kindheit bis zum Jugendalter. Sowohl die mittlere Veränderung in der Verzehrsmenge bestimmter Lebensmittel, als auch die individuellen Veränderungen (definiert als die Beibehaltung der relativen Verzehrsmenge über die Zeit, im Vergleich zur restlichen Population) wurden untersucht. Zusätzlich wurden mögliche Determinanten dieser Ernährungsveränderung bestimmt. Auf individuellem Niveau wurde nur eine mäßige Veränderung beobachtet, wobei es in der Gesamtpopulation einen generellen Trend hin zu niedrigeren Verzehrsmengen von stärkehaltigem Gemüse, Margarine und Milchprodukten gab. Das Familieneinkommen und das elterliche Bildungsniveau hatten dabei den größten Einfluss auf die individuellen Veränderungen der Verzehrsmenge.

Diese Ergebnisse stützen die Hypothese, dass bestimmte Lebensmittel und Nährstoffe mit intermediären Risikofaktoren nichtübertragbarer Erkrankungen bereits in der Kindheit und Jugend zusammenhängen. Klinische Studien sollten folgen um die Kausalität des Zusammenhangs nachzuweisen. Bestehende primäre Präventionsmaßnahmen für diese Altersgruppe könnten dann diese Ergebnisse berücksichtigen, um ihre Wirksamkeit zu optimieren.

1 Introduction

1.1 Children and adolescents in primary prevention

Noncommunicable diseases are a leading cause of mortality world-wide, and constitute a major public health challenge[1,2]. In 2015 around 31% of global deaths were reported to have resulted from cardiovascular diseases alone[1]. This is a disproportionately high rate, considering that these diseases are very often caused by unhealthy lifestyles, and are thus largely preventable[3,4]. Although morbidity typically presents in older age groups, risk factors tend to appear sooner, and include metabolic disruptions, such as the accumulation of excess body fat, dyslipideamia, or chronic systemic inflammation, among others[5,6]. Frequently, an intermediate phenotype develops, characterised by the aggregation of a number of these traits, years before disease onset[7].

As stated in the constitution of the World Health Organisation, "health is not merely the absence of disease"[8]. This principle is of particular relevance in the context of children and adolescents who, despite showing no symptoms of disease, can present signs of developing intermediate risk factors. Indeed, the current prevalence of overweight and obesity in children and adolescents is alarmingly high[9]. Disturbed blood lipid profiles and low-grade inflammation have also been detected in these age groups[10,11]. Importantly, a growing body of evidence indicates that risk factors developed during childhood tend to track into adulthood[12–15]. Optimizing health in children and adolescents is therefore a key component of action strategies aiming for the primary prevention of noncommunicable diseases[4].

1.2 Body composition and biomarkers

The assessment of intermediate risk factors relies on the measurement of established biological markers. Different parameters can be measured which are not necessarily causal in the path of disease progression, but are known to be associated with disease outcome and are often used for the detection of high-risk individuals. In younger populations these parameters might reflect very early phases of risk development[16]. The following paragraphs describe measurements of body composition and biomarkers considered to be of value for early risk prediction in children and adolescents.

Body composition

Excess fat mass accumulation underlies overweight and obesity, and is associated with systemic inflammation, insulin resistance and abnormal lipid metabolism, all important risk factors for future development of cardiovascular diseases[17,18]. On the other hand, increasing lean body mass through skeletal muscle gain is considered beneficial for metabolic health[19,20]. The measurement of body composition allows an estimation of both these body compartments, providing important information on nutritional status. Such information is often unavailable and unfortunately not appreciable from other commonly-used measurements, such as total body weight or body mass index. Since fat mass and lean mass together comprise total body weight, individuals with similar weights can present largely varying profiles of body composition.

Blood lipids

Disturbed lipoprotein metabolism is considered an underlying cause of atherosclerosis, which can lead to cardiovascular diseases[21]. Adverse blood lipid profiles have been found to be associated with vascular lesions in children and young adults[22,23]. Improving overall serum lipid concentrations among children and adolescents has hence represented an important target for primary prevention for quite some time[24]. An atherogenic blood lipid profile is characterised by high levels of circulating triglycerides (TAG) and low-density lipoprotein (LDL), and low levels of high-density lipoprotein (HDL)[25]. In particular, high TAG levels have often been associated with insulin resistance, a state which can adversely modify a range of biochemical responses leading to further metabolic dysfunction[26]. Additionally, a high ratio of total cholesterol to HDL (TOTAL:HDL) has been reported to be a strong predictor of cardiovascular disease risk[27].

Inflammatory markers

Chronic low-grade inflammation, characterised by elevated levels of circulating inflammatory markers, has been identified as an important intermediate risk factor[28,29]. Amongst the known proinflammatory markers associated with cardiovascular risk, the cytokine interleukin-6 (IL-6)[30], and the acute-phase reactant C-reactive protein (CRP)[31], have been observed to be associated with arterial changes in children[32,33], indicating a possible role of low-grade inflammation in the early phases of atherosclerosis. IL-6 is suggested, among other functions, to play a causal role in chronic inflammation[34,35]. CRP is synthesised primarily in response to IL-6[36] and is considered a highly valuable non-specific inflammatory marker[37]. Raised concentrations of these markers could be indicative of early systemic inflammation associated with the intermediate phenotype in young populations.

1.3 The role of dietary intake

An unhealthy diet is recognized to be among the main modifiable lifestyle factors associated with noncommunicable disease risk[38,39]. Its impact is usually reflected through the development of the intermediate phenotype, where different dietary components have been found to be of relevance. Despite considerable advances in the field of nutrition and health, the roles of many foods and nutrients continue to be discussed[40]. Especially in children and adolescents, evidence is often lacking to bring about definitive conclusions[41].

Meat intake and body composition

Diets composed of high amounts of meat have been reported to be associated with weight gain in a number of observational studies[42]. Excess fat mass accumulation is implied, however, measures of body composition are typically not reported. Numerous clinical trials have observed body fat loss and weight maintenance with higher meat and protein intakes[43]. Given that protein is the primary macronutrient component of meat, and plays a role in the development and maintenance of lean tissue[44], the assessment of body composition is key in understanding the true role of meat intake. Additionally, different types of meat can vary in their macronutrient and energy composition, as

well as in how they are processed. Indeed, assessing separate meat types in relation to body weight has shown this to be of relevance[45]. The association of meat intake with body composition has not been assessed in long-term epidemiological studies during adolescence, so it is yet unclear whether meat (or meat protein) intake might induce changes in fat mass or in lean mass, or both, in this age-group.

Dietary fatty acids and blood lipids

Fatty acids are often referred to under three main umbrella terms, based on their molecular structure: saturated fatty acids (SFA), containing no double bonds, monounsaturated fatty acids (MUFA), containing one double bond, and polyunsaturated fatty acids (PUFA), which contain two or more double bonds. Additionally, two PUFA families exist, n-6 PUFA and n-3 PUFA[46]. Dietary fatty acids have been reported to alter circulating blood lipids in different ways. In particular, high SFA intakes have often been suggested to promote dyslipidaemia[47], and reduced consumption has been advised[48]. However, emerging studies question whether a truly independent association between SFA and the blood lipid profile exists[49,50]. Findings in adults highlight the importance of considering the nutrient context, by indicating the differing effects on blood lipids when SFA is replaced with different nutrients, e.g with PUFA (mostly beneficial) or with carbohydrates (mostly detrimental)[51]. Longitudinal studies addressing this concept in children and adolescents are lacking. Indeed, some evidence exists linking dietary fatty acids to blood lipids, but results are few and inconsistent[52–54]. Further, without information on substituting nutrients, associations cannot be considered independent of other correlated nutrients.

Serum fatty acids and low-grade inflammation

Specific fatty acids are known to influence the inflammatory process[46]. It has been shown that long-chain PUFA are transformed into lipid mediators, which play an active role in the metabolic mechanisms of inflammation[55]. For example, long chain n-3 PUFA exhibit anti-inflammatory properties, in part by limiting levels of arachidonic acid, a precursor of pro-inflammatory mediators[56]. While some fatty acids, such as linoleic and α-linoleic acid, can be obtained only through dietary intake, others are mainly derived from endogenous metabolism. The measurement of fatty acids in serum glycerophospholipids is suggested to reflect both[57]. The ratio of product-to-precursor fatty acids can also be calculated as a surrogate to estimate activity of the enzymes involved in the endogenous conversion of fatty acids[58]. Assessing the relationship between serum fatty acids and markers of inflammation has allowed the pin-pointing of different fatty acids involved in the inflammatory process. Emerging studies in children also suggest a potential relevance of different fatty acids in the early progression of low-grade inflammation, but such studies are scarce and findings inconsistent.

To assume that the associations of dietary intake with intermediate risk factors in children and adolescents would be similar to those observed in adults, might be an oversimplification. The period of transition from childhood to adolescence is a time of rapid growth, as well as changes in body composition[59]. Dietary components observed to influence health outcomes in adults may hence behave differently during this life phase. Specific individual and environmental determinants could also influence dietary behaviour in this population group.

2 Specific Aims and Results

2.1 Specific Aims

This thesis aimed to investigate the associations of different components of dietary intake with body composition and biomarkers in children and adolescents. The main objectives are described below:

- to assess the prospective associations of different meat types and their respective protein contents during childhood, with measures of body composition during adolescence.
- to evaluate the associations of dietary fatty acids with changes in serum lipids during adolescence, considering the theoretical isocaloric replacements of saturated fatty acids with other fatty acids or carbohydrates.
- to explore possible existing associations between different fatty acids, as well as fatty acid groups and ratios assumed to play relevant roles in inflammatory processes, with markers of low-grade inflammation in 10-year-old children.
- to describe changes in dietary intake during puberty, including their association with individual or environmental determinants.

This thesis describes the work of four studies, published in Nutrition Journal, Nutrients, European Journal of Clinical Nutrition and BMC Public Health. I wrote the manuscript and am hence first author of all included publications. I contributed significantly towards developing the research questions and design of all studies, and carried out the statistical analyses and interpretation of results. The manuscripts were revised by co-authors, and their comments and suggestions were included in the final versions. The large number of co-authors is explained by the fact that data was derived from two multicentre cohort studies and results are based on multidisciplinary research.

2.2 Study Population and Methods

2.2.1 Study Population

The data used in all studies were obtained from children enrolled in the LISAplus (chapter 6) or in the GINIplus (chapter 7) study, or from both studies combined (chapters 4 and 5). These studies are ongoing, population-based, prospective birth cohort studies, for which full-term neonates were recruited in obstetric clinics of different regions in Germany. In both studies, the 15-year follow-up has been completed, and GINIplus is currently in the 20-year follow-up stage. In brief, for the German Infant study on the Influence of Nutrition Intervention plus environmental and genetic influences on allergy development (GINIplus), a total of 5991 infants were recruited in two cities, Munich and Wesel, between September 1995 and June 1998. Those with at least one allergic parent and/or sibling were allocated to the study intervention arm (randomized to one of three hydrolysed formulae or to conventional cow's milk). Recruited participants without have a family history of allergy or whose parents withheld consent to the intervention, were assigned to the study observation arm. For the Influence of

Life-style factors on the Immune System and the development of Allergies in childhood plus the influence of traffic emissions and genetics (LISAplus) study, 3097 participants were recruited in Munich, Wesel, Leipzig and Bad Honnef, between the years 1997 and 1999, of which 3 removed consent (3094 remained). Comprehensive descriptions of the GINIplus and LISAplus studies have been published elsewhere[60,61]. To address the aims of the current thesis, the study population was limited to children participating in the 10- and/or 15-year follow-up assessments.

2.2.2 Exposure assessment

In the first, second, and fourth manuscripts (chapters 4, 5, and 7), dietary intake was assessed by means of a self-administered food frequency questionnaire (FFQ). For the third manuscript (chapter 6), blood samples were collected during the physical examination of the 10-year follow-up, and analysis of fatty acids in serum glycerophospholipids was carried out.

Food frequency questionnaire

The FFQ was designed to assess habitual food and nutrient intake in 10-year-old children, with particular focus on estimating energy, fatty acid and antioxidant intakes. Participants were asked to report for a list of 80 food items, how often these were consumed over the past 12 months, as well as estimated quantities. Frequency and quantity estimates were combined for the calculation of average intakes in grams per day. Various questions regarding preferences of fat and energy content, diets and preparation practices, among others, were also included in the FFQ. Daily energy and nutrient intake for each food item were computed by linking estimated daily intakes and information from additional questions to the German Nutrient Data Base (BLS) version II.3.1[62]. A detailed description of the FFQ design and validation, as well as of the calculation of estimated intakes, has been published by Stiegler et al.[63].

Analysis of serum glycerophospholipids

Fatty acids in serum glycerophospholipids have been shown to reflect dietary fatty acids consumed over the past weeks to months[64], which makes them plausible markers of habitual fatty acid intake. A high-throughput method was applied, details of which are published elsewhere[65]. Briefly, 100 ml of internal standard and 0.6 ml methanol were added to 100 ml of serum, obtained from blood withdrawn during the physical examination. After centrifugation the supernatant was transferred to new vials and sodium methoxide solution was added for synthesis of fatty acid methyl esters (FAME). FAME were extracted and dried under nitrogen flow at room temperature. The residue was analysed by gas chromatography and proportions relative to total fatty acids were calculated.

2.2.3 Outcome assessment

In children who attended the follow-up physical examinations, body composition measurements and withdrawal of blood samples were carried out. Blood samples were used for the analysis of serum lipids and markers of inflammation.

Body composition

Measures of fat mass and fat free mass were obtained by means of phase sensitive bioelectrical impedance analysis (BIA) at the 15-year follow-up examination. Fat mass index (FMI) and fat-free mass index (FFMI) were calculated by dividing fat mass and fat-free mass (kg), respectively, by height squared (kg/m^2), which was measured barefoot during the same visit. These parameters provide discrete indices of relative fat mass and fat free mass, normalised for body size, hence allowing an independent evaluation of both fat and lean components of body weight[66].

Blood lipids

Concentrations of total cholesterol, LDL, HDL, and TAG were measured in serum using homogenous enzymatic colorimetric methods on a Modular Analytics System from Roche Diagnostics GmbH Mannheim according to the manufactures instructions. External controls were used in accordance with the guidelines of the German Society of Clinical Chemistry and Laboratory Medicine. The ratio TOTAL:HDL was calculated by dividing total cholesterol by HDL.

Inflammatory markers

Serum concentrations of high-sensitivity CRP (hs-CRP) were measured using the Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitive assay; and concentrations of IL-6 by flow cytometry using a cytometric bead array (BD CBA Human Soluble Flex Set system; Becton Dickinson, Heidelberg, Germany), according to manufacturer instructions.

2.2.4 Analyses

First manuscript: Meat intake and body composition (chapter 4)

The associations of intakes of different meat types, and their respective protein contents, at age 10 years, with FMI and FFMI at age 15 years, were evaluated among 1610 children from the GINIplus and LISAplus studies. Information on meat and protein intake was obtained from the FFQ, including total meat, processed meat, red meat and poultry. Prospective associations with measures of body composition were assessed by linear regression, stratified by sex. Sensitivity analyses included additional adjustment for potentially correlated nutrients (essential amino acids, SFA, MUFA and PUFA), and further stratification for initial weight status. Secondary analyses was carried out to test associations with changes in blood lipid parameters.

Second manuscript: Dietary fatty acids and blood lipids (chapter 5)

The second manuscript assessed the prospective associations of four major groups of dietary fatty acids with changes in blood lipid concentrations during adolescence. Theoretical isocaloric nutrient substitutions were also considered. Among participants of the GINIplus and LISAplus studies, data on intakes of fatty acids and carbohydrates were obtained from the 10-year follow-up FFQ. Lipid concentrations were measured in serum of blood samples collected at ages 10 and 15 years. In 1398 children, the associations of SFA, MUFA, *n*–6 PUFA and *n*–3 PUFA with changes in LDL, HDL, TAG and TOTAL:HDL, were evaluated by linear regression. Further, substitution models

assessed the isocaloric replacement of SFA for other fatty acids or carbohydrates. Analyses were performed in the total population and separately for females and males.

Third manuscript: Serum fatty acids and low-grade inflammation (chapter 6)

The role of fatty acid composition in the inflammatory process in children was investigated in this exploratory study. The analyses included 958 children who participated in the 10-year follow-up blood-withdrawal of the LISAplus study. The association of 20 different fatty acid exposures (including individual fatty acids as well as relevant fatty acid groups and ratios) with hs-CRP and IL-6 were assessed in the total population and stratified by sex. As concentrations of hs-CRP and IL-6 were often below the detection limit, both markers were highly skewed. Therefore, the variables were categorised into 3 levels, and analyses were carried out by multinomial logistic regression.

Fourth manuscript: Changes in dietary intake (chapter 7)

The final manuscript involved describing changes in dietary intakes from age 10 to 15 years, in children participating in the GINIplus study. Intakes of 17 food groups, macronutrients and antioxidant vitamins were described in terms of stability or change over time, at a population level and at an individual level. Average intake changes were assessed, as well as individual tracking levels (the maintenance of food intake behaviour over time, relative to the rest of the population). Determinants of individual changes (increase or decrease vs tracking) in intakes of different dietary components were investigated by assessing their associations with education level, parental education, family income, body mass index, pubertal onset and screen-time sedentary behaviour. Information on determinants was obtained from questionnaires completed at the 10-year follow-up. A total of 1232 children were included, with complete information for all relevant variables.

2.3 Results

First manuscript: Meat intake and body composition (chapter 4)

The analyses of meat and meat protein intakes in relation to body composition indicated sex-specific associations. A direct association was observed in males between poultry intake and FMI, and between total and red meat intakes with FFMI. Protein intakes from total and red meat were also associated with increased FFMI in males. Sensitivity analyses including the adjustment for essential amino acids rendered the association between red meat and FFMI in males no longer significant, indicating a possible relevant role of amino acids present in meat protein. Of note, red meat intake was also associated with higher TAG levels in males, as revealed by secondary analyses.

Second manuscript: Dietary fatty acids and blood lipids (chapter 5)

Investigating the association between intakes of major fatty acid groups with changes in blood lipids during adolescence indicated an inverse association between SFA intake and TAG concentrations. Nevertheless, it is important to highlight that the context of other nutrients is not considered in the single nutrient analysis approach, which means other correlated nutrients could be indirectly driving the observed association. No

associations were observed between MUFA, *n*–6 PUFA or *n*–3 PUFA intakes with any of the assessed blood lipid parameters. Substitution models evaluating the theoretical isocaloric replacement of SFA with other nutrients, indicated elevated LDL, TAG and TOTAL:HDL levels in females, when consuming carbohydrates at the expense of SFA.

Third manuscript: Serum fatty acids and low-grade inflammation (chapter 6)

A number of associations were observed between fatty acids measured in serum glycerophospholipids with markers of low-grade inflammation in 10-year-old children. Arachidonic acid, *n*–6 highly-unsaturated fatty acids, and the ratio of arachidonic to linoleic acid were associated with increased inflammation, as indicated by elevated levels of hs-CRP. Low-grade inflammation was also observed in association with palmitic acid and total SFA, as indicated by increased levels of IL-6. On the other hand, linoleic acid and total PUFA were associated with lower IL-6 levels.

Fourth manuscript: Changes in dietary intake (chapter 7)

Changes in dietary intake from childhood to adolescence were assessed at a population level and at an individual level. Average intakes of water, starchy vegetables, margarine and dairy were reduced over time in both sexes. Additionally, females reduced intakes of meat and retinol, and increased their intakes of vegetables, grains, oils and tea. Males reduced fruit and carbohydrate intakes and increased their intakes of meat, caloric drinks, protein, total fat, PUFA, vitamin C and α-tocopherol. Despite these general changes, individual intakes of the various food groups presented fair tracking (maintenance relative to the rest of the population) from age 10 to 15 years. Changes in individual intakes were mainly associated with parental education and family income.

2.4 Strengths and Limitations

The strengths and limitations of the different analyses are explained in detail within each manuscript. Some aspects are common to all the performed studies, while others are specific to the data used.

A main strength within all four studies was the availability of large, homogeneous, study populations from one or both of the GINIplus and LISAplus birth cohorts. Additionally, the repeated measurements within these two cohorts provided the opportunity to perform longitudinal analyses in three of the four manuscripts (chapter 6 was a cross-sectional study). Although causality cannot be implied due to the observational nature of these studies, the temporal aspect of the applied longitudinal analyses grants better grounds for a causal interpretation of the observed associations.

The assessment of dietary intake was carried out by means of an FFQ (chapters 4, 5, 7), designed to estimate dietary intake in school-aged children. Nevertheless, self-reported dietary assessment is prone to possible reporting bias, leading to under- or over-estimation of specific foods or nutrients. Additionally, the food-item list, although comprehensive, may not include all food items consumed. This was particularly of note in the assessment of meat and protein intake in relation to body composition (chapter 4), since a number of typically vegetarian food sources were not covered in the FFQ. Despite this, additional sensitivity analyses did not significantly alter our findings. In the study of fatty acid intakes relative to blood lipids (chapter 5), this aspect was

less likely to represent a limitation, as a main focus of the FFQ was the assessment of dietary fatty acid intakes. Finally, in the description of changes in dietary intake (chapter 7), inter-reporter differences cannot be excluded, since at age 10 years a parent or guardian helped the participant complete the FFQ, whereas at age 15 this was not required. Nevertheless, a combined effort in FFQ completion at age 10 years was considered necessary in order to enhance response accuracy, since experience has shown that children younger than 12 years usually struggle to recall intakes and to understand the concept of portion sizes[67]. On the other hand, studies have indicated that the parental estimation of the dietary intake of their children is moderately valid[68].

In terms of study design, results from analyses including a large number of exposures, and/or multiple tests performed, are more prone to type-1 error. In order to avoid the appearance of chance findings in such cases, we applied Bonferonni correction for multiple testing where appropriate. Furthermore, in nutritional epidemiology a main drawback is the correlation between nutrients, which cannot always be accounted for without encountering problems of multicollinearity. Where possible (chapters 4 and 5), we attempted to tackle this problem by residualising highly correlated nutrients, hence allowing their simultaneous inclusion in the analyses. This was of particular value in the substitution model approach (chapter 5), where consideration of the nutrient context was highly relevant, in order to avoid misleading conclusions arising from strong correlations naturally occurring within the diet.

Finally, a common limitation in cohort studies is non-random loss to follow-up, whereby children of lower social classes tend to be under-represented. In all studies, adjustment for parental education as a surrogate for social status was performed; nevertheless, results might not be entirely representative of the study area.

3 Conclusion and Outlook

The aim of this thesis was to investigate the roles of different dietary components in the development of intermediate risk factors of cardiovascular diseases in children and adolescents. Dietary changes and their determinants were also described.

A number of associations were observed which are broadly in line with existing findings in adults. However, sex-specific determinants also seemed relevant, possibly owing to differences in dietary behaviours, sex hormones or pubertal stage. Considering the sex-specific changes which take place during pubertal development at both behavioural and physiological levels, it is conceivable that metabolic responses to dietary intake would also differ. During pubertal maturation, males undergo a significant increase in lean tissue, and protein utilization is more efficient[59]. Hence, it is possible that the male metabolic response to meat intake during adolescence differs from that in females or adults (chapter 4). Potentially adverse effects of carbohydrates were specific to females (chapter 5), which could be attributed to dietary behaviours, amongst which the type of carbohydrates consumed is likely to be of relevance[69,70]. It has also been reported that physiological insulin resistance can arise with pubertal maturation[71], and more females than males had reached puberty among the studied population. Further, oestrogen may influence fatty acid metabolism, and could have affected observed sex-specific inflammatory responses to fatty acids (chapter 6).

The analyses of the role of different quality nutrients would perhaps allow a better understanding of metabolic aspects involved in the observed associations. However, analyses based on foods or dietary patterns are also warranted to aid understanding of diet as a whole, an important aspect when communicating public health messages. In terms of physiological differences, sex-stratified analyses presented in this thesis allow only for speculations. Future studies might consider longitudinal analyses including interactions with different stages of pubertal development. Given the evidence for a role of adipose tissue in chronic inflammation and the disruption of lipid metabolism[72], body fat as a potential effect modifier might also be considered in further analyses, making use of measures of body composition. The role of metabolomics is becoming highly relevant in nutrition science and could help improve our understanding of nutrient metabolites at a cellular level, including their interactions with enzymes, proteins or their influence on gene expression.

The studies presented in this thesis address the gap in the literature regarding habitual intakes of common foods and nutrients and their relation to biomarkers in children and adolescents. This age-group represents an increasingly important target-population for early disease prevention. Whether the associations observed can be considered of clinical relevance might be questioned. However, given that the participants of the studies were not from a high risk population, mostly presenting normal levels for the assessed parameters, the associations observed might indicate a possible early relevance of these dietary components in the longer-term development of cardiovascular diseases. Clinical trials should follow in order to confirm causal relationships. Finally, fair tracking of dietary intake observed from childhood to adolescence (chapter 7) support the rationale for targeting children early for primary prevention. Sex-specific sub-populations, such as children with lower socio-economic status, or lower education levels, might be identified for added impact.

References

[1] World Health Organization, "Global health estimates 2015: Deaths by cause, age, sex, by country and by region, 2000-2015." Geneva, 2016.

[2] World Health Organization, "Global health risks: mortality and burden of disease attributable to selected major risks," Geneva, 2009.

[3] M. Ezzati and E. Riboli, "Behavioral and dietary risk factors for noncommunicable diseases," *New England Journal of Medicine*, vol. 369, no. 10, pp. 954–964, 2013, pMID: 24004122.

[4] World Health Organization, "Global action plan for the prevention and control of NCDs 2013-2020," Geneva, 2013.

[5] S. Klein, L. E. Burke, G. A. Bray, S. Blair, D. B. Allison, X. Pi-Sunyer *et al.*, "Clinical implications of obesity with specific focus on cardiovascular disease," *Circulation*, vol. 110, no. 18, pp. 2952–2967, 2004.

[6] G. Hotamisligil, "Inflammation and metabolic disorders," *Nature*, vol. 444, no. 7121, pp. 860–867, 2006.

[7] G. Hulsegge, A. M. W. Spijkerman, Y. T. van der Schouw, S. J. L. Bakker, R. T. Gansevoort, H. A. Smit *et al.*, "Trajectories of metabolic risk factors and biochemical markers prior to the onset of cardiovascular disease – The Doetinchem Cohort Study," *PLoS ONE*, vol. 11, no. 5, pp. 1–15, 05 2016.

[8] World Health Organization, "Preamble to the Constitution of WHO as adopted by the International Health Conference, New York, 19 June - 22 July 1946; signed on 22 July 1946 by the representatives of 61 States (official records of WHO, no. 2, p. 100) and entered into force on 7 April 1948." 1948.

[9] M. Bibiloni, A. Pons, and J. Tur, "Prevalence of overweight and obesity in adolescents: A systematic review," *ISRN Obesity*, vol. 2013, p. 14, 2013.

[10] R. Weiss, J. Dziura, T. S. Burgert, W. V. Tamborlane, S. E. Taksali, C. W. Yeckel *et al.*, "Obesity and the metabolic syndrome in children and adolescents," *New England Journal of Medicine*, vol. 350, no. 23, pp. 2362–2374, 2004.

[11] M. J. Järvisalo, A. Harmoinen, M. Hakanen, U. Paakkunainen, J. Viikari, J. Hartiala *et al.*, "Elevated serum C-reactive protein levels and early arterial changes in healthy children," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 8, pp. 1323–1328, 2002.

[12] J. A. Morrison, L. A. Friedman, and C. Gray-McGuire, "Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: The Princeton Lipid Research Clinics Follow-up Study," *Pediatrics*, vol. 120, no. 2, pp. 340–345, 2007.

[13] M. Simmonds, A. Llewellyn, C. G. Owen, and N. Woolacott, "Predicting adult obesity from childhood obesity: a systematic review and meta-analysis," *Obesity Reviews*, vol. 17, no. 2, pp. 95–107, 2016, oBR-05-15-2260.R1.

[14] T. A. Nicklas, S. P. von Duvillard, and G. S. Berenson, "Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: The Bogalusa Heart Study," *Int J Sports Med*, vol. 23, no. S1, pp. 39–43, 2002.

[15] J. Juhola, C. G. Magnussen, J. S. Viikari, M. Kähönen, N. Hutri-Kähönen, A. Jula *et al.*, "Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: The Cardiovascular Risk in Young Finns Study," *The Journal of Pediatrics*, vol. 159, no. 4, pp. 584 – 590, 2011.

[16] J. Canas, S. Sweeten, and P. Balagopal, "Biomarkers for cardiovascular risk in children," *Current Opinion in Cardiology*, vol. 28, no. 2, pp. 103–114, 2013.

[17] J.-P. Després, "Body fat distribution and risk of cardiovascular disease," *Circulation*, vol. 126, no. 10, pp. 1301–1313, 2012.

[18] A. H. Berg and P. E. Scherer, "Adipose tissue, inflammation, and cardiovascular disease," *Circulation Research*, vol. 96, no. 9, pp. 939–949, 2005.

[19] B. Pedersen and M. Febbraio, "Muscles, exercise and obesity: skeletal muscle as a secretory organ," *Nat Rev Endocrinol*, vol. 8, no. 8, pp. 457–465, 2012.

[20] R. R. Wolfe, "The underappreciated role of muscle in health and disease," *The American Journal of Clinical Nutrition*, vol. 84, no. 3, pp. 475–482, 2006.

[21] R. Ross, "The pathogenesis of atherosclerosis: a perspective for the 1990s," *Nature*, vol. 362, pp. 801–809, 1993.

[22] G. S. Berenson, S. R. Srinivasan, W. Bao, W. P. Newman, R. E. Tracy, and W. A. Wattigney, "Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults," *New England Journal of Medicine*, vol. 338, no. 23, pp. 1650–1656, 1998, pMID: 9614255.

[23] W. P. I. Newman, D. S. Freedman, A. W. Voors, P. D. Gard, S. R. Srinivasan, J. L. Cresanta *et al.*, "Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis," *New England Journal of Medicine*, vol. 314, no. 3, pp. 138–144, 1986, pMID: 3455748.

[24] World Health Organization Expert Committee on Prevention in Childhood and Youth of Adult Cardiovascular Diseases, "Prevention in childhood and youth of adult cardiovascular diseases: time for action / report of a WHO Expert Committee," Geneva, 1990.

[25] L. A. Solberg and J. P. Strong, "Risk factors and atherosclerotic lesions. A review of autopsy studies." *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 3, no. 3, pp. 187–198, 1983.

[26] S. M. Grundy, "Hypertriglyceridemia, insulin resistance, and the metabolic syndrome," *The American Journal of Cardiology*, vol. 83, no. 9, Supplement 2, pp. 25 – 29, 1999.

[27] J. Millán, X. Pintó, A. Muñoz, M. Zúñiga, J. Rubiés-Prat, L. F. Pallardo *et al.*, "Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention," *Vascular health and risk management*, vol. 5, pp. 1033–1042, 2009.

[28] T. A. Pearson, G. A. Mensah, R. W. Alexander, J. L. Anderson, R. O. Cannon, M. Criqui *et al.*, "Markers of inflammation and cardiovascular disease," *Circulation*, vol. 107, no. 3, pp. 499–511, 2003.

[29] J. Danesh, R. Collins, P. Appleby, and R. Peto, "Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: Meta-analyses of prospective studies," *JAMA*, vol. 279, no. 18, pp. 1477–1482, 1998.

[30] P. M. Ridker, N. Rifai, M. J. Stampfer, and C. H. Hennekens, "Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men," *Circulation*, vol. 101, no. 15, pp. 1767–1772, 2000.

[31] W. Koenig, M. Sund, M. Fröhlich, H.-G. Fischer, H. Löwel, A. Döring *et al.*, "C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men," *Circulation*, vol. 99, no. 2, pp. 237–242, 1999.

[32] M. J. Järvisalo, A. Harmoinen, M. Hakanen, U. Paakkunainen, J. Viikari, J. Hartiala *et al.*, "Elevated serum C-reactive protein levels and early arterial changes in healthy children," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 8, pp. 1323–1328, 2002.

[33] S. Kapiotis, G. Holzer, G. Schaller, M. Haumer, H. Widhalm, D. Weghuber *et al.*, "A proinflammatory state is detectable in obese children and is accompanied by functional and morphological vascular changes," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 11, pp. 2541–2546, 2006.

[34] J. Hartman and W. H. Frishman, "Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy," *Cardiology in review*, vol. 22, no. 3, pp. 147–151, 2014.

[35] The Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, "The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis," *The Lancet*, vol. 379, no. 9822, pp. 1214 – 1224, 2012.

[36] P. Heinrich, J. Castell, and T. Andus, "Interleukin-6 and the acute phase response," *Biochemical Journal*, vol. 265, no. 3, pp. 621–636, 1990, cited By 1832.

[37] M. B. Pepys and G. M. Hirschfield, "C-reactive protein: a critical update," *The Journal of Clinical Investigation*, vol. 111, no. 12, pp. 1805–1812, 6 2003.

[38] WHO Regional Office for Europe, "Vienna declaration on nutrition and noncommunicable diseases in the context of health 2020," Copenhagen, 2013.

[39] M. Ezzati and E. Riboli, "Behavioral and dietary risk factors for noncommunicable diseases," *New England Journal of Medicine*, vol. 369, no. 10, pp. 954–964, 2013, pMID: 24004122.

[40] D. Katz and S. Meller, "Can we say what diet is best for health?" *Annual Review of Public Health*, vol. 35, pp. 83–103, 2014.

[41] A. N. Funtikova, E. Navarro, R. A. Bawaked, M. Fíto, and H. Schröder, "Impact of diet on cardiometabolic health in children and adolescents," *Nutrition Journal*, vol. 14, no. 1, p. 118, 2015.

[42] M. H. Rouhani, A. Salehi-Abargouei, P. J. Surkan, and L. Azadbakht, "Is there a relationship between red or processed meat intake and obesity? A systematic review and meta-analysis of observational studies," *Obesity Reviews*, vol. 15, no. 9, pp. 740–748, 2014.

[43] P. Clifton, D. Condo, and J. Keogh, "Long term weight maintenance after advice to consume low carbohydrate, higher protein diets – a systematic review and meta analysis," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 24, no. 3, pp. 224 – 235, 2014.

[44] J. W. Krieger, H. S. Sitren, M. J. Daniels, and B. Langkamp-Henken, "Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression," *The American Journal of Clinical Nutrition*, vol. 83, no. 2, pp. 260–274, 2006.

[45] A.-C. Vergnaud, T. Norat, D. Romaguera, T. Mouw, A. M. May, N. Travier *et al.*, "Meat consumption and prospective weight change in participants of the EPIC-PANACEA study," *The American Journal of Clinical Nutrition*, vol. 92, no. 2, pp. 398–407, 2010.

[46] P. C. Calder, "Polyunsaturated fatty acids and inflammation," *Biochemical Society Transactions*, vol. 33, no. 2, pp. 423–427, 2005.

[47] A. Keys, J. T. Anderson, and F. Grande, "Serum cholesterol response to changes in the diet," *Metabolism*, vol. 14, no. 7, pp. 776 – 787, 1965.

[48] World Health Organization, "Diet, nutrition and the prevention of chronic diseases," *World Health Organization Technical Report Series*, vol. 916, pp. 1–149, Generva, Switzerland, 2003.

[49] P. W. Siri-Tarino, Q. Sun, F. B. Hu, and R. M. Krauss, "Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease," *The American Journal of Clinical Nutrition*, vol. 91, no. 3, pp. 535–546, 2010.

[50] R. Chowdhury, S. Warnakula, S. Kunutsor, F. Crowe, H. A. Ward, L. Johnson *et al.*, "Association of dietary, circulating, and supplement fatty acids with coronary risk: A systematic review and meta-analysis," *Annals of Internal Medicine*, vol. 160, no. 6, pp. 398–406, 2014.

[51] FAO, "Fats and fatty acids in human nutrition; report of an expert consultation," *FAO Food and Nutrition Paper*, vol. 91, pp. 1–166, Food and Agriculture Organization: Rome, Italy, 2010.

[52] S. Bel-Serrat, T. Mouratidou, I. Huybrechts, I. Labayen, M. Cuenca-García, G. Palacios *et al.*, "Associations between macronutrient intake and serum lipid profile depend on body fat in european adolescents: the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study," *British Journal of Nutrition*, vol. 112, no. 12, pp. 2049–2059, 2014.

[53] C.-Y. Ku, B. A. Gower, T. R. Nagy, and M. I. Goran, "Relationships between dietary fat, body fat, and serum lipid profile in prepubertal children," *Obesity Research*, vol. 6, no. 6, pp. 400–407, 1998.

[54] H. Niinikoski, K. Pahkala, M. Ala-Korpela, J. Viikari, T. Rönnemaa, H. Lagström *et al.*, "Effect of repeated dietary counseling on serum lipoproteins from infancy to adulthood," *Pediatrics*, vol. 129, no. 3, pp. e704–e713, 2012.

[55] P. C. Calder, "Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale," *Biochimie*, vol. 91, no. 6, pp. 791 – 795, 2009.

[56] P. C. Calder, "Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?" *British Journal of Clinical Pharmacology*, vol. 75, no. 3, pp. 645–662, 2013.

[57] M. Nakamura and T. Nara, "Structure, function, and dietary regulation of Δ^6, Δ^5, and Δ^9 desaturases," *Annual Review of Nutrition*, vol. 24, pp. 345–376, 2004.

[58] B. Vessby, I.-B. Gustafsson, S. Tengblad, M. Boberg, and A. Andersson, "Desaturation and elongation of fatty acids and insulin action," *Annals of the New York Academy of Sciences*, vol. 967, no. 1, pp. 183–195, 2002.

[59] A. D. Rogol, J. N. Roemmich, and P. A. Clark, "Growth at puberty," *Journal of Adolescent Health*, vol. 31, no. 6, Supplement, pp. 192 – 200, 2002, health Futures of Youth II: Pathways to Adolescent Health.

[60] A. von Berg, U. Krämer, E. Link, C. Bollrath, J. Heinrich, I. Brockow *et al.*, "Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years," *Clin Exp Allergy*, 2010.

[61] J. Heinrich, G. Bolte, B. Hölscher, J. Douwes, I. Lehmann, B. Fahlbusch *et al.*, "Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates," *Eur. Respir. J.*, vol. 20, no. 3, pp. 617–623, 2002.

[62] B. M. Hartmann, S. Bell, A. Vásquez-Caicedo, A. Götz, J. Erhardt, and C. Brombach, *Der Bundeslebensmittelschlüssel. German Nutrient Database.*, Federal Research Centre for Nutrition and Food (BfEL): Karlsruhe, Germany, 2005.

[63] P. Stiegler, S. Sausenthaler, A. E. Buyken, P. Rzehak, D. Czech, J. Linseisen *et al.*, "A new FFQ designed to measure the intake of fatty acids and antioxidants in children," *Public Health Nutr.*, vol. 13, no. 1, pp. 38–46, 2010.

[64] E. Riboli, H. Ronnholm, and R. Saracci, "Biological markers of diet," *Cancer Surv.*, vol. 6, no. 4, pp. 685–718, 1987.

[65] C. Glaser, H. Demmelmair, and B. Koletzko, "High-throughput analysis of fatty acid composition of plasma glycerophospholipids," *J. Lipid Res.*, vol. 51, no. 1, pp. 216–221, 2010.

[66] J. C. K. Wells, "Toward body composition reference data for infants, children, and adolescents," *Advances in Nutrition: An International Review Journal*, vol. 5, no. 3, pp. 320S–329S, 2014.

[67] M. B. E. Livingstone, P. J. Robson, and J. M. W. Wallace, "Issues in dietary intake assessment of children and adolescents," *British Journal of Nutrition*, vol. 92, no. S2, pp. S213–S222, 2004.

[68] W. Willett, *Nutritional Epidemiology*. Oxford, USA: Oxford University Press, 1998.

[69] L. A. Te Morenga, A. J. Howatson, R. M. Jones, and J. Mann, "Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids," *The American Journal of Clinical Nutrition*, vol. 100, no. 1, pp. 65–79, 2014.

[70] P. W. Siri-Tarino, S. Chiu, N. Bergeron, and R. M. Krauss, "Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment," *Annual review of nutrition*, vol. 35, pp. 517–543, 2015.

[71] S. A. Amiel, R. S. Sherwin, D. C. Simonson, A. A. Lauritano, and W. V. Tamborlane, "Impaired insulin action in puberty," *New England Journal of Medicine*, vol. 315, no. 4, pp. 215–219, 1986, pMID: 3523245.

[72] H. E. Bays, P. P. Toth, P. M. Kris-Etherton, N. Abate, L. J. Aronne, W. V. Brown *et al.*, "Obesity, adiposity, and dyslipidemia: A consensus statement from the national lipid association," *Journal of Clinical Lipidology*, vol. 7, no. 4, pp. 304 – 383, 2013.

4 Paper 1: Meat Consumption and Measures of Body Composition during Adolescence

(Harris et al. Nutrition Journal, 2016)

Original title:	Prospective associations of meat consumption during childhood with measures of body composition during adolescence: results from the GINIplus and LISAplus birth cohorts
Authors:	C Harris, A Buyken, A von Berg, D Berdel, I Lehmann, B Hoffmann, S Koletzko, B Koletzko, J Heinrich, M Standl
Journal:	Nutrition Journal
Volume:	15
Pages:	101
Year:	2016
DOI:	10.1186/s12937-016-0222-5

Harris et al. Nutrition Journal (2016) 15:101
DOI 10.1186/s12937-016-0222-5

Nutrition Journal

RESEARCH Open Access

Prospective associations of meat consumption during childhood with measures of body composition during adolescence: results from the GINIplus and LISAplus birth cohorts

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Abstract

Background: Higher meat and protein intakes have been associated with increased body weight in adults, but studies evaluating body composition are scarce. Furthermore, our knowledge in adolescents is limited. This study aimed to investigate the prospective associations of intakes of different meat types, and their respective protein contents during childhood, with body composition during adolescence.

Methods: Dietary (using food frequency questionnaires) and body composition (measured by bioelectrical impedance) data were collected from the 10- and 15-year follow-up assessments respectively, of the GINIplus and LISAplus birth cohort studies. Sex-stratified prospective associations of meat and meat protein intakes (total, processed, red meat and poultry) with fat mass index (FMI) and fat free mass index (FFMI), were assessed by linear regression models ($N = 1610$).

Results: Among males, higher poultry intakes at age 10 years were associated with a higher FMI at age 15 years [$\beta = 0.278$ (SE = 0.139), $p = 0.046$]; while higher intakes of total and red meat were prospectively associated with higher FFMI [0.386 (0.143), $p = 0.007$, and 0.333 (0.145), $p = 0.022$, respectively]. Additionally in males, protein was associated with FFMI for total and red meat [0.285 (0.145) and 0.356 (0.144), respectively].

Conclusions: Prospective associations of meat consumption with subsequent body composition in adolescents may differ by sex and meat source.

Keywords: Meat intake, Body composition, Adolescence, Protein, Longitudinal study, Fat mass, Fat free mass

Background

Concerns regarding excessive meat intake include increased risks of all-cause mortality [1], cancer [2], CVD [3] and diabetes mellitus [4]. Observational studies have also associated high meat intakes with increased risk of weight gain and obesity [5]. Red and processed meats in particular, have been associated with increased weight gain. However, meat types are very diverse, and differ substantially from each other in terms of macronutrient and energy composition as well as processing. A number of observational studies have reported animal protein, the main macronutrient component of meat, to be directly associated with weight gain [6]. On the other hand, animal protein is known to increase satiety and thermogenesis [7], and intervention studies have reported beneficial effects of high protein diets on fat loss and weight maintenance [8]. Amino acids obtained from meat protein have been proposed to exert an anabolic effect on muscle

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mass, and may be important in the development and maintenance of lean tissue [9]. It is hence possible that the associations reported between meat intake and weight gain in observational studies could be due to gains in lean mass rather than fat mass. Indeed, positive prospective associations between animal protein intake and lean body mass from puberty to young adulthood have been reported in females [10].

A better understanding of the role of different meat types and their respective protein contents is needed in order to shed light on the underlying factors driving associations between meat intake and weight gain. Furthermore, the evaluation of body composition can determine whether weight gains associated with meat intake are a result of accumulating fat mass, fat-free mass, or both. Hence, in order to appreciate the true role of meat intake in adiposity, accurate body composition data are necessary.

In a large proportion of German adolescents, meat intakes exceed recommended amounts [11], and the prevalence of overweight and obesity is high and rising further [12]. Considering that overweight in adolescence is known to track into adulthood [13], the identification of meat as a contributor towards increased fat mass in adolescence could have important implications for the early prevention of overweight and associated comorbidities. There is a need for longitudinal studies on the association between meat intake and body composition during adolescence, a critical life stage during which fast weight-gain occurs [14]. The aims of the present study were thus to investigate prospective associations of the consumption of different sources of meat and meat-protein during childhood, with fat mass and fat-free mass during adolescence.

Methods

Subjects

The present study used data from the 10- and 15-year follow-up assessments of the ongoing GINIplus (*G*erman *I*nfant *N*utritional *I*ntervention *plus* environmental and genetic influences on allergy development) and LISAplus (Influence of *L*ifestyle-Related Factors on the *I*mmune *S*ystem and the Development of *A*llergies in Childhood *plus* the Influence of Traffic Emissions and Genetics) birth cohort studies. Healthy full-term new-borns were recruited from obstetric clinics within four German cities between 1995 and 1999. Information was collected using identical questionnaires and at physical examinations. The study designs, recruitment and exclusion criteria have been described previously [15, 16]. For both studies, approval by the local ethics committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participant's families were obtained.

Exposure variables

Dietary intake data was obtained from the 10-year follow-up assessment, using a self-administered food frequency questionnaire (FFQ), designed and validated to assess food and nutrient intake over the past year in school-aged children [17]. In brief, subjects were asked to report estimated frequency and portion size of intakes of 80 food items. A quality control procedure was applied based on recommendations by Willett et al. for data cleaning in nutritional epidemiology [18].

Four meat types were defined: *processed meat* (salami, liver sausage, cold meat, bratwurst and wiener- or pork-sausage), *red meat* (pork, beef, veal), *poultry* (any poultry meat) and *other meats* (offal and ready meals with meat). The protein content (g/day) of each of the different meat types was calculated based on the German Food Code and Nutrient Database (BLS) version II.3.1 [19], and converted to kcal/d (g/d multiplied by 4). The daily intakes (mg/d) of essential amino acids (EAA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were also obtained from the FFQ by use of the same database. Total meat intake (the sum of all meat types) and each individual meat type, as well as their respective protein contents, were included as exposures in the statistical analyses. The food-group "other meats" was rarely consumed and was not individually analysed.

Outcome variables

Measures of fat mass and fat free mass were obtained during the 15-year physical examination by means of phase sensitive bioelectrical impedance (BIA). Fat mass index (FMI) and fat-free mass index (FFMI) were calculated by dividing fat mass and fat-free mass (kg), respectively, by height squared (kg/m^2) measured without shoes at the same examination. Blood samples were also obtained from willing participants during the 10- and 15-year follow-up physical examinations. The concentrations (mmol/L) of total cholesterol, LDL, HDL, and triglycerides (TAG) were measured in serum using homogenous enzymatic colorimetric methods on a Modular Analytics System from Roche Diagnostics GmbH Mannheim according to the manufactures instructions. External controls were used in accordance with the guidelines of the German Society of Clinical Chemistry and Laboratory Medicine. The ratio of total to HDL cholesterol (TOTAL:HDL) was calculated by dividing total cholesterol by HDL.

Adjustment variables

Statistical models were adjusted for study (GINI observation arm; GINI intervention arm; LISA), recruitment region (Munich; Wesel; Bad Honnef; Leipzig), parental education level (highest level achieved by mother or father: $\leq 10^{th}$grade = low/medium; $>10^{th}$grade = high),

exact age at BIA measurement (years), sedentary behaviour at age 15 years (≤2 h screen time/day = low; > 2 h screen time/day = high), pubertal onset (any presence of acne or spots, pubic or axillary hair, breast development, menstruation, penis or testicle enlargement at age 10 years: yes; no), and weight category at age 10 years (BMI z-score ≤ 1 = normal weight; BMI z-score > 1 = overweight). BMI z-scores used to categorize body weight were calculated according to the 2007 BMI-for-age WHO growth reference for school-aged children and adolescents [20]. Due to non-random loss-to follow-up, children with low parental education were underrepresented in our study population (Additional file 1: Table S1), therefore low (<10thgrade) and medium (10thgrade) parental education were combined into low/medium.

Statistical analysis

Subjects providing complete data for outcome, exposure and adjustment variables, were included ($N = 1736$). Participants were excluded if they reported an illness affecting diet at 10 or 15 years (e.g. diabetes, anorexia, coeliac disease, cancer) or medical dietary indications, such as gluten-free or lactose-free diets, at age 15 years ($n = 82$). Clear outliers in outcomes ($n = 2$) and exposures ($n = 42$) were visually identified using descriptive plots and excluded from the analyses (Additional file 2: Figure S1). Meat and meat protein intake variables were adjusted for daily caloric intake using the nutrient residual model. For this we computed sex-specific residuals from a regression model where meat and protein variables (kcal/day) were regressed on energy intake (kcal/day) at age 10 years. As these residuals are uncorrelated with total energy intake the variation due to the nutrient composition of the diet, rather than the combination with total amount of food, can be evaluated. Due to non-linearity, residuals were categorized into sex-specific tertiles (T1 = low, T2 = medium and T3 = high intake).

Main subject characteristics for the total study population, and stratified by energy-adjusted meat intake tertiles, were described by medians (25th percentile; 75th percentile) or counts (%). Differences between meat intake tertiles were tested using Kruskal-Wallis test for continuous variables and χ^2-test for categorical variables. All statistical analyses were performed and presented stratified by sex. Prospective associations of consumption of meat (total meat, processed, red meat, poultry) and meat protein (total meat protein, processed meat protein, red meat protein, poultry protein) at age 10 years with FMI and FFMI at age 15 years, were assessed by linear regression models. First, minimally adjusted models (MIN) were fit, adjusting for study, recruitment region, parental education level, pubertal onset, age at BIA measurement and sedentary behaviour. As significant associations between meat intake and BMI at age 10 years have been previously reported [21], main models (MAIN) were fit separately, further adjusting for weight category at age 10 years. We performed additional analyses where we further adjusted the main model for EAA, SFA, MUFA or PUFA, respectively. These variables were included in the model as energy-adjusted residuals (computed as described above for meat and protein residuals). We also tested for possible interactions by including an interaction term between the meat or protein exposures and weight category, following which stratified analyses (normal weight; overweight) were performed. Finally, we repeated our main analyses using blood lipid parameters as secondary outcomes in a subgroup of the study population who provided measurements at ages 10 and 15 years ($n = 1309$). Linear regression models were used to assess the prospective associations of consumption of meat and meat protein at age 10 years with changes in blood lipids (ΔLDL, ΔHDL, ΔTAG and ΔTOTAL:HDL) from age 10 to 15 years. Models were adjusted as in the previously described main model, with further adjustment for the respective blood lipid measurement at age 10 years.

Results are presented as β-coefficients (β), along with their standard errors (SE) with reference to the lowest intake tertile (T1). Meat intake residual coefficients have an isocaloric substitution interpretation. A two-sided α-level of 5% was considered significant. For the stratified analyses, we corrected for multiple testing using Bonferroni correction, yielding a corrected two-sided alpha level of 0.025 (0.05/2 = 0.025). Since the meat group "poultry" was composed by only one food item (poultry meat), each poultry tertile includes the same subjects as its respective poultry protein tertile; therefore, the calculated regression coefficients for poultry are identical for both meat and meat protein intakes and are hence only reported when referring to meat intakes. All analyses were conducted using R (www.r-project.org), version 3.2.2 [22].

Results

Study population

Data from 1610 participants (797 females and 813 males) were included in the analyses (Figure S1). Descriptive characteristics are displayed in Table 1. At age 10 years, 16.7% females and 22.5% males were overweight according to WHO cut-off criteria (10.3 and 10.8%, respectively, according to IOTF cut-offs [23]). Children in the highest meat intake tertile were significantly more likely to be overweight at age 10 years. Most children in the study population were from Munich and from families with high parental education.

Regression analyses

Primary outcomes (FMI and FFMI)

Results of the minimally adjusted (MIN) and main (MAIN) linear regression models are presented in

Table 1 Basic characteristics of study population by tertiles of total meat intake at age 10 years

	Females					Males				
	Total meat ($n = 797$)	Total meat tertiles				Total meat ($n = 813$)	Total meat tertiles			
		T1 ($n = 266$)	T2 ($n = 266$)	T3 ($n = 265$)	p-val[a]		T1 ($n = 271$)	T2 ($n = 271$)	T3 ($n = 271$)	p-val[a]
10 years										
BMI (kg/m^2)	16.7 (15.5; 18.3)	16.4 (15.5; 17.9)	16.9 (15.5; 18.3)	16.9 (15.5; 18.6)	**0.037**	16.7 (15.6; 18.4)	16.5 (15.4; 18.3)	16.7 (15.5; 18.1)	16.9 (15.9; 18.9)	**0.030**
Overweight, n (%)[b]	133 (16.7)	32 (12)	44 (16.5)	57 (21.5)	**0.014**	183 (22.5)	54 (19.9)	54 (19.9)	75 (27.7)	**0.045**
Age (years)	10.7 (10.5; 11.2)	10.7 (10.5; 11.2)	10.8 (10.5; 11.2)	10.7 (10.4; 11.1)	0.162	10.7 (10.4; 11.1)	10.7 (10.4; 11.1)	10.7 (10.4; 11)	10.7 (10.4; 11.1)	0.923
Sedentary behaviour [high][c], n (%)	65 (8.2)	15 (5.7)	23 (8.7)	27 (10.3)	0.149	103 (12.8)	37 (13.7)	31 (11.6)	35 (13.1)	0.759
Pubertal onset [Yes][d], n (%)	366 (45.9)	117 (44)	120 (45.1)	129 (48.7)	0.526	81 (10)	30 (11.1)	25 (9.2)	26 (9.6)	0.750
15 years										
BMI (kg/m^2)	20.3 (18.8; 22.1)	20.1 (18.6; 21.6)	20.4 (19.1; 22.3)	20.4 (18.8; 22.5)	0.066	19.9 (18.5; 21.9)	19.6 (18.2; 21.5)	19.8 (18.3; 21.6)	20.4 (18.9; 22.6)	0.001
Overweight, n (%)[b]	105 (13.2)	22 (8.3)	38 (14.3)	45 (17)	**0.010**	151 (18.6)	46 (17)	43 (15.9)	62 (22.9)	0.078
Fat mass index (kg/m^2)	5.5 (4.6; 6.6)	5.2 (4.5; 6.2)	5.6 (4.6; 6.7)	5.8 (4.7; 6.9)	0.008	3.6 (2.8; 4.7)	3.5 (2.8; 4.5)	3.5 (2.7; 4.5)	3.8 (2.8; 5.1)	0.028
Fat free mass index (kg/m^2)	14.9 (13.8; 15.8)	14.8 (13.8; 15.5)	14.9 (13.8; 16)	14.9 (13.8; 15.9)	0.411	16.3 (15.3; 17.6)	16 (15.2; 17.3)	16.3 (15.2; 17.4)	16.6 (15.6; 18)	0.002
Age (years)	15.2 (15; 15.3)	15.2 (15; 15.3)	15.2 (15.1; 15.3)	15.1 (15; 15.3)	0.336	15.1 (15; 15.3)	15.2 (15; 15.3)	15.1 (15; 15.3)	15.1 (15; 15.3)	0.704
Sedentary behaviour [high][c], n (%)	386 (48.4)	116 (43.6)	134 (50.4)	136 (51.3)	0.152	522 (64.2)	172 (63.5)	166 (61.3)	184 (67.9)	0.260
Basis characteristics										
Study										
GINI control, n (%)	282 (35.4)	92 (34.6)	103 (38.7)	87 (32.8)	0.226	258 (31.7)	82 (30.3)	91 (33.6)	85 (31.4)	0.862
GINI intervention, n (%)	254 (31.9)	86 (32.3)	89 (33.5)	79 (29.8)		238 (29.3)	85 (31.4)	74 (27.3)	79 (29.2)	
LISA, n (%)	261 (32.7)	88 (33.1)	74 (27.8)	99 (37.4)		317 (39)	104 (38.4)	106 (39.1)	107 (39.5)	
Region										
Munich, n (%)	417 (52.3)	154 (57.9)	137 (51.5)	126 (47.5)	0.094	416 (51.2)	138 (50.9)	142 (52.4)	136 (50.2)	0.960
Leipzig, n (%)	69 (8.7)	22 (8.3)	18 (6.8)	29 (10.9)		79 (9.7)	24 (8.9)	29 (10.7)	26 (9.6)	
Bad Honef, n (%)	34 (4.3)	14 (5.3)	10 (3.8)	10 (3.8)		40 (4.9)	15 (5.5)	11 (4.1)	14 (5.2)	
Wesel, n (%)	277 (34.8)	76 (28.6)	101 (38)	100 (37.7)		278 (34.2)	94 (34.7)	89 (32.8)	95 (35.1)	
Parental educ. [High], n (%)[e]	578 (72.5)	205 (77.1)	193 (72.6)	180 (67.9)	0.062	552 (67.9)	192 (70.8)	182 (67.2)	178 (65.7)	0.415

Values are medians for continuous variables (25th percentile; 75th percentile) and n (%) for categorical variables. [a]Differences between tertiles were tested by Kruskal-Walis test for continuous variables and X^2-test for categorical variables; [b]BMI z-score > 1; [c]Hours spent on screen activities > 2; [d]Presence of any sign of pubertal onset; [e]Highest level achieved by mother or father > 10y. Significant p-values marked in bold

Table 2. In females, the MIN models showed that high (T3) total meat and poultry intakes at age 10 years were related to higher FMI at 15 years (p-value for linear trend = 0.006 and 0.019, respectively). These associations were no longer significant in the MAIN models. Similar results were observed for protein intakes in females. In males, the MIN models indicated that high (T3) poultry intakes at age 10 years were associated with higher FMI at age 15 years, and high (T3) red and processed meat intakes were related to higher FFMI; while high (T3) total meat intakes were related to both higher FMI and higher FFMI. Following further adjustment for BMI category in the MAIN model, high (T3) poultry intake at age 10 years remained significantly associated with higher FMI at age 15 years [0.278 (0.139)] (p-value for linear trend = 0.047), while high (T3) total and red meat intakes at age 10 years were significantly associated with higher FFMI at age 15 years [0.386 (0.143) and 0.333 (0.145), respectively] (p-value for linear trend = 0.007 and 0.022, respectively). Similar associations were observed with the respective protein intakes of all meat types [0.285 (0.145) for high total meat protein with higher FFMI, and 0.356 (0.144) for high red meat protein with higher FFMI].

Results from the further adjusted models (adjusted for EAA, SFA, MUFA or PUFA) are presented in Additional file 3: Tables S3a for females and S3b for males. In females, additional adjustment for MUFA or PUFA resulted in significant positive associations between high (T3) total meat and meat protein intakes with FMI. When adjusting for PUFA, high (T3) poultry intakes were also significantly associated with FMI. In males, when adjusting for EAA, SFA, MUFA or PUFA, the association between high poultry intake and FMI no longer reached statistical significance (except with adjustment for MUFA, where it was weakened but remained borderline significant). The associations between red meat, total meat protein and red meat protein with FFMI in males were no longer significant following adjustment for EAA, while the association of total meat with FFMI was weakened. On the other hand, when adjusting for SFA, an additional positive association was observed between high (T3) processed meat and FFMI. When adjusting for MUFA or PUFA, the association between total meat protein intakes with FFMI was no longer significant.

Stratified analyses (normal weight/ overweight)

Stratified analyses results are presented in Fig. 1 (exact values in Additional file 4: Tables S2a for females and S2b for males). In females, high (T3) intakes of poultry in children with normal weight at age 10 years were related to higher FMI at age 15 years [0.314 (0.125)]. In males high (T3) total meat intakes in normal weight children at age 10 years was related to higher FFMI at age 15 years [0.350 (0.150)].

Secondary outcomes (ΔLDL, ΔHDL, ΔTAG and ΔTOTAL:HDL)

Blood samples at both age 10 and 15 years were available in a subsample of 1309 participants (636 females and 673 males). In males, high (T3) red meat and red meat protein intakes were associated with increasing TAG concentrations [0.131 (0.060), p-value = 0.030; and 0.130 (0.060), p-value = 0.031, respectively]. No significant associations were observed for any of the meat or meat protein types with the other blood lipid parameters (data not shown).

Discussion

The present study aimed at assessing the associations of meat intake at the age of 10 years with later body composition during adolescence, and to determine the role of protein in such associations. Our findings suggest that a higher poultry intake during childhood in males may lead to an accumulation of body fat during adolescence. This finding is in line with the notion that higher meat intakes promote increased weight gain, proposed in a number of observational studies [5]. Amongst these, Vergnaud et al. [24] have highlighted poultry as a possible determinant of gains in weight and waist circumference in adults. Contrary to other observational studies [5], our results suggest a beneficial association between the consumption of red meats and later lean body mass in adolescent males.

Two major differences between our and many other existing observational studies should be noted. Firstly, studies on the association of meat intake with overweight typically describe changes in body weight or BMI. These measures cannot indicate possible variation in body composition. Hence, gains in BMI or body weight are not analogous to gains in body fat, and without supporting information cannot be interpreted as such. Secondly, most studies reporting associations of different meat types with overweight have been carried out in adults. Our study population consisted of children assessed over a five-year follow-up period during adolescence, from ages 10 to 15 years. We have previously reported cross-sectional associations between higher total meat intakes and increased BMI in 10-year old children from the GINIplus and LISAplus birth cohort studies [21]. Additionally, Bradlee et al. [25] reported that adolescent boys (aged 12–16) with smaller waist circumferences tended to eat less meat. Nevertheless, in view of the present study findings, it could be suggested that associations between red meat and weight gain in adolescents may reflect increased lean mass rather than fat mass in males. Additional analyses indicated that associations between total meat and FFMI were stronger in

Table 2 Prospective association of tertiles of meat and meat protein intakes with FMI and FFMI

	FMI							FFMI						
	T2 vs T1			T3 vs T1				T2 vs T1			T3 vs T1			
	β	SE	*p*-val	β	SE	*p*-val	p-trend	β	SE	*p*-val	β	SE	*p*-val	p-trend
Females														
Total meat														
MIN	0.255	0.149	0.088	0.411	0.150	**0.006**	**0.006**	0.108	0.130	0.405	0.106	0.130	0.418	0.418
MAIN	0.177	0.130	0.174	0.224	0.131	0.088	0.088	0.053	0.119	0.658	−0.028	0.120	0.818	0.818
Processed														
MIN	0.077	0.149	0.604	0.265	0.150	0.077	0.077	0.209	0.129	0.105	0.164	0.130	0.208	0.206
MAIN	0.005	0.130	0.967	0.194	0.130	0.137	0.138	0.159	0.118	0.180	0.113	0.119	0.340	0.338
Red meat														
MIN	−0.178	0.150	0.236	0.095	0.152	0.531	0.544	0.080	0.130	0.539	0.038	0.132	0.776	0.771
MAIN	−0.116	0.131	0.374	−0.063	0.133	0.636	0.627	0.124	0.119	0.297	−0.076	0.121	0.533	0.550
Poultry														
MIN	−0.104	0.149	0.487	0.355	0.150	**0.018**	**0.019**	−0.122	0.130	0.349	0.115	0.131	0.378	0.380
MAIN	−0.146	0.130	0.260	0.254	0.131	0.052	0.053	−0.152	0.119	0.202	0.044	0.120	0.714	0.716
Total meat protein														
MIN	0.067	0.150	0.653	0.419	0.152	**0.006**	**0.006**	0.094	0.130	0.471	0.088	0.132	0.503	0.502
MAIN	−0.013	0.131	0.922	0.246	0.133	0.064	0.065	0.037	0.119	0.758	−0.035	0.121	0.772	0.773
Processed (Protein)														
MIN	0.105	0.149	0.482	0.226	0.150	0.131	0.131	0.011	0.129	0.933	0.233	0.130	0.073	0.074
MAIN	0.077	0.130	0.552	0.077	0.131	0.558	0.555	−0.009	0.118	0.943	0.128	0.119	0.283	0.287
Red meat (Protein)														
MIN	−0.113	0.150	0.453	0.100	0.153	0.512	0.522	0.079	0.130	0.543	0.014	0.132	0.915	0.909
MAIN	−0.088	0.131	0.503	−0.052	0.133	0.696	0.690	0.097	0.119	0.415	−0.094	0.121	0.438	0.450
Poultry (protein)[a]														
MIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MAIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Males														
Total meat														
MIN	−0.084	0.162	0.604	0.395	0.162	**0.015**	**0.015**	0.032	0.162	0.846	0.552	0.162	**0.001**	**0.001**
MAIN	−0.096	0.138	0.489	0.213	0.139	0.124	0.125	0.021	0.143	0.884	0.386	0.143	**0.007**	**0.007**
Processed														
MIN	0.076	0.164	0.641	0.232	0.163	0.155	0.155	0.049	0.164	0.763	0.398	0.163	**0.015**	**0.015**
MAIN	0.096	0.139	0.493	0.095	0.139	0.495	0.495	0.067	0.144	0.641	0.273	0.144	0.057	0.057
Red meat														
MIN	−0.029	0.163	0.858	0.166	0.164	0.312	0.316	0.182	0.163	0.264	0.433	0.165	**0.009**	**0.009**
MAIN	−0.031	0.139	0.826	0.057	0.140	0.686	0.690	0.181	0.143	0.207	0.333	0.145	**0.022**	**0.022**
Poultry														
MIN	−0.018	0.162	0.914	0.418	0.163	**0.010**	**0.011**	−0.082	0.164	0.617	0.159	0.164	0.333	0.336
MAIN	−0.041	0.139	0.766	0.278	0.139	**0.046**	**0.047**	−0.104	0.144	0.471	0.028	0.144	0.844	0.849
Total meat protein														
MIN	−0.045	0.163	0.783	0.412	0.164	**0.012**	**0.012**	0.080	0.164	0.626	0.479	0.164	**0.004**	**0.004**
MAIN	−0.007	0.139	0.960	0.202	0.140	0.151	0.153	0.115	0.144	0.426	0.285	0.145	**0.050**	**0.050**
Processed (Protein)														

Table 2 Prospective association of tertiles of meat and meat protein intakes with FMI and FFMI *(Continued)*

MIN	−0.015	0.163	0.929	0.312	0.163	0.055	0.055	−0.092	0.163	0.575	0.376	0.163	**0.021**	**0.021**
MAIN	0.043	0.139	0.758	0.131	0.139	0.346	0.346	−0.039	0.144	0.786	0.211	0.144	0.143	0.144
Red meat (Protein)														
MIN	−0.107	0.163	0.509	0.162	0.164	0.323	0.328	0.072	0.163	0.660	0.442	0.164	**0.007**	**0.007**
MAIN	−0.045	0.139	0.747	0.069	0.140	0.623	0.627	0.129	0.143	0.367	0.356	0.144	**0.014**	**0.014**
Poultry (protein)[a]														
MIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MAIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–

MIN: adjusted for study, region, age at BIA measurement, parental education, pubertal onset10; MAIN: MIN model further adjusted for overweight at 10y; *p*-val: *p*-value from linear regression; p-trend: *p*-value indicating linear trend. Significant *p*-values marked in bold

[a]Estimates for poultry protein not presented, as categories for protein were identical to those for poultry meat, and hence estimates are also identical

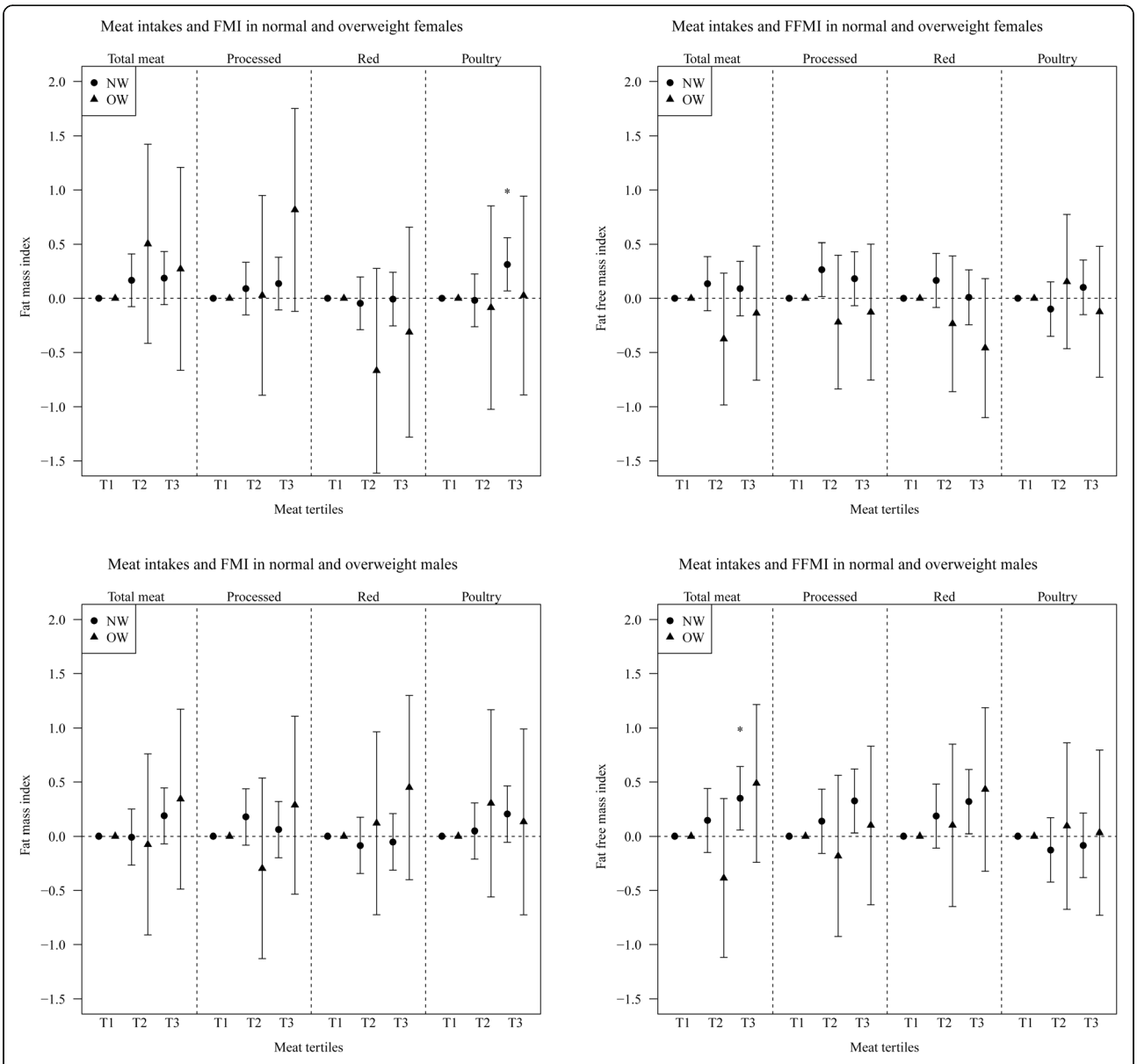

Fig. 1 Prospective association of tertiles of meat intakes (T2 and T3 vs T1) with FMI (left) and FFMI (right), stratified by normal weight (NW) and overweight (OW) subjects in females (top) and males (bottom). *P value < 0.025 (significant after correction for multiple testing)

lean than in overweight subjects. This suggests that the development of lean tissue triggered by higher meat intakes occurs more readily in leaner males, although it is possible that due to the smaller number of overweight individuals, there was not sufficient power for associations in this group to reach statistical significance.

Our data indicated that the subsequent increase in lean mass observed following higher red meat intakes could be attributed to the high protein content of this meat type. These findings are not unexpected, considering that red meat protein is a rich source of essential amino acids, known to be important for the development and preservation of lean tissue [9]. Indeed, when further adjusting our analyses for dietary EAA, the associations between red meat and red meat protein with FFMI in males were no longer significant. This was not the case when adjusting for other nutrients, suggesting that EAA might be a potentially responsible component in the association of red meat with lean body mass. Our findings are consistent with intervention studies which propose that higher protein intakes contribute towards increasing lean tissue, and can be beneficial for weight loss and maintenance [26]. Most observational studies however, fail to reproduce these findings longitudinally. Some studies even propose a detrimental effect of protein, in particular animal protein, on weight gain [27]. However, measurements of body composition are also scarce in these studies. A Danish study did report that the energy intake from protein was positively related to total fat mass in 36-year-old men and women [28]. On the other hand, a prospective relation between animal protein intake during puberty and FFMI in young adulthood was reported among females (and also in males when adjusting for FMI) [10]. Furthermore, in another study, higher protein was prospectively associated with higher FFMI in overweight and lower FMI in lean girls aged 8–10 years [29]. It is however of note that despite the greater lean mass observed in males in the present study, our secondary analyses also revealed an association of red meat and red meat protein with increasing TAG levels in males. This finding supports prospective studies which have reported a link between red meat and CVD [3, 30]. Attempts to explain positive associations between meat intake and blood lipids often refer to the high SFA content of meat as the responsible component [31]. Studies have also indicated that the consumption of lean meat (low in SFA) could have beneficial effects on cardio-metabolic risk markers [32]. Nevertheless, in our analyses, adjustment for SFA did not alter the observed association between red meat and TAG (data not shown). Further research is warranted in order to evaluate the specific role of lean meat on blood lipids in adolescence. Until this area is better understood, it is unclear whether all red meats represent a healthy dietary protein source for adolescents attempting to promote lean body mass development. Furthermore, we cannot exclude that other dietary components consumed in the dietary pattern along with red meat, could have contributed to its observed association with lean body mass. Unfortunately, we were not able to look at the separate role of protein intake in the association of poultry with FMI, which would have been interesting considering it promotes changes in body composition which oppose those of red meats. Adjusting for EAA, SFA or PUFA weakened the association of poultry with FMI in males, whilst a positive association was observed in females with adjustment for PUFA. These results reflect a complex, sex-specific role of this meat subtype in fat mass accumulation, which, from the present analyses cannot be attributed to any specific nutrient.

We highlight that the present study was carried out during adolescence, a period where growth occurs at its most rapid rate since infancy, and where significant weight gain and important changes in body composition take place [14]. Furthermore our findings were limited to males, who, under the influence of testosterone, at this stage undergo a significant increase in lean body tissue [14]. This process could be enhanced by higher protein intakes; however it has been suggested that increasing protein consumption is not entirely necessary to maintain nitrogen retention, due to an increased efficiency of protein utilization at this life-stage [33]. It is hence plausible that the metabolic response to meat intake in adolescents is different to that occurring in adults [14]. Considering the evidence for increased risk of disease associated with red and processed meat in particular [2], these findings should be interpreted with caution, keeping in mind that similar findings are not necessarily expected to be observed in adults.

A major strength of the present study is that it is based on data from two large population-based birth cohorts. The large sample size allows for robust prospective analyses, lacking thus far in observational studies concerning meat consumption and body composition. Our data allows us to evaluate specific associations of meat consumption with fat and lean body mass. Although we additionally assessed associations with changes in blood lipids, we were unfortunately not able to assess blood biomarkers of obesity such as adipokines, which would have provided further insight into the biological effects of meat intake in parallel to those reflected by our anthropometric measurements. Additionally, some other limitations were also encountered. Although study sampling was primarily population-based, non-random loss-to-follow-up, often occurring in cohort studies, meant children of lower social classes were underrepresented in the present analyses, and hence findings cannot be considered representative of

the study area. Furthermore, the 10-year FFQ was completed by parents alongside their children, as young children might have difficulties recalling intakes or understanding portion sizes. Nevertheless, the questionnaire produced plausible values in terms of energy intake and any misreporting was most likely detected through extensive quality control. The improved quality of the data was obtained at the expense of reducing the sample size, although the study sample remained large with no substantial loss of power. The FFQ lacks questions regarding typically vegetarian protein sources such as tofu or pulses. Therefore, the relative caloric contribution of meat intake – as used in this study – could be overestimated among children whose diets are high in vegetable protein. When excluding children following a meat-free diet at age 10 years, our results remained consistent (data not shown).

Conclusions

In conclusion, prospective associations of meat consumption with subsequent body composition in adolescents may differ by sex and meat source. We found that in males high poultry intake is prospectively associated with increased fat mass, while red meat in males is related to higher fat free mass. Protein from red meat likely plays a major role in its association with lean mass. These findings provide important insight into the underlying changes in body composition occurring with meat and meat protein intakes during the period of pubertal development.

Additional files

Additional file 1: Table S1. Comparison of lost-to-follow-up and not-lost-to-follow-up study participants. (PDF 173 kb)

Additional file 2: Figure S1. Study participants. (PDF 84 kb)

Additional file 3: Table S3a. Prospective association of tertiles of meat and meat protein intakes with FMI and FFMI in females ($n = 636$) adjusted for EAA, SFA, MUFA or PUFA. **Table S3b.** Prospective association of tertiles of meat and meat protein intakes with FMI and FFMI in males ($n = 673$) adjusted for EAA, SFA, MUFA or PUFA. (PDF 272 kb)

Additional file 4: Table S2a. Prospective association of tertiles of meat and meat protein intakes (T2 and T3 vs T1) with FMI and FFMI, stratified by BMI at 10y (normal weight/overweight), in females. **Table S2b.** Prospective association of tertiles of meat and meat protein intakes (T2 and T3 vs T1) with FMI and FFMI, stratified by BMI at 10y (normal weight/overweight), in males. (PDF 248 kb)

Abbreviations

FFMI: Fat free mass index; FFQ: Food frequency questionnaire; FMI: Fat mass index

Acknowledgements

The authors thank all the families for their participation in the GINIplus and LISAplus studies. Furthermore, we thank all members of the GINIplus and LISAplus Study Groups for their excellent work. The GINIplus Study group consists of the following: Institute of Epidemiology I, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg (Heinrich J, Brüske I, Schulz H, Flexeder C, Zeller C, Standl M, Schnappinger M, Sußmann M, Thiering E, Tiesler C); Department of Pediatrics, Marien-Hospital, Wesel (Berdel D, von Berg A); Ludwig-Maximilians-University of Munich, Dr von Hauner Children's Hospital (Koletzko S); Child and Adolescent Medicine, University Hospital rechts der Isar of the Technical University Munich (Bauer CP, Hoffmann U); IUF- Environmental Health Research Institute, Düsseldorf (Hoffmann B, Link E, Klümper C).
The LISAplus Study group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Schnappinger M, Brüske I, Sußmann M, Lohr W, Schulz H, Zeller C, Standl M); Department of Pediatrics, Municipal Hospital "St. Georg", Leipzig (Borte M, Gnodtke E); Marien Hospital Wesel, Department of Pediatrics, Wesel (von Berg A, Berdel D, Stiers G, Maas B); Pediatric Practice, Bad Honnef (Schaaf B); Helmholtz Centre of Environmental Research – UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (Lehmann I, Bauer M, Röder S, Schilde M, Nowak M, Herberth G , Müller J, Hain A); Technical University Munich, Department of Pediatrics, Munich (Hoffmann U, Paschke M, Marra S); Clinical Research Group Molecular Dermatology, Department of Dermatology and Allergy, Technische Universität München (TUM), Munich (Ollert M).

Funding

The GINIplus study was mainly supported for the first 3 years of the Federal Ministry for Education, Science, Research and Technology (interventional arm) and Helmholtz Zentrum Munich (former GSF) (observational arm). The 4 year, 6 year, 10 year and 15 year follow-up examinations of the GINIplus study were covered from the respective budgets of the 5 study centres (Helmholtz Zentrum Munich (former GSF), Research Institute at Marien-Hospital Wesel, LMU Munich, TU Munich and from 6 years onwards also from IUF - Leibniz Research-Institute for Environmental Medicine at the University of Düsseldorf) and a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). The LISAplus study was mainly supported by grants from the Federal Ministry for Education, Science, Research and Technology and in addition from Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research - UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Pediatric Practice, Bad Honnef for the first 2 years. The 4 year, 6 year, 10 year and 15 year follow-up examinations of the LISAplus study were covered from the respective budgets of the involved partners (Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research - UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Pediatric Practice, Bad Honnef, IUF – Leibniz-Research Institute for Environmental Medicine at the University of Düsseldorf) and in addition by a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). Further, the 15 year follow-up examination of the GINIplus and LISAplus studies was supported by the Commission of the European Communities, the 7th Framework Program: MeDALL project. The 15 year follow-up examination of the GINIplus study was additionally supported l by the Mead Johnson and Nestlé companies. The work of BK is financially supported in part by the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG – no.322605).

Availability of data and materials

The datasets generated during and/or analysed during the current study are not publicly available due to the ongoing nature of the GINIplus and LISAplus studies.

Authors' contributions

CH and MS were involved in the conception and design of the study; AvB, DB, IL, BH, SK, and JH in the data acquisition; CH and MS in the statistical analyses; CH, MS, BK and AB in the interpretation; CH drafted the manuscript; all authors revised it critically for important intellectual content, and approved the final version to be published.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate
For both studies, approval by the local ethics committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participant's families were obtained.

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Received: 25 July 2016 Accepted: 29 November 2016
Published online: 05 December 2016

References

1. Sinha R, Cross AJ, Graubard BI, Leitzmann MF, Schatzkin A. Meat intake and mortality: a prospective study of over half a million people. Arch Intern Med. 2009;169(6):562–71.
2. Bouvard V, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, Guha N, Mattock H, Straif K. Carcinogenicity of consumption of red and processed meat. Lancet Oncol. 2015;16(16):1599–1600.
3. Abete I, Romaguera D, Vieira AR, Lopez de Munain A, Norat T. Association between total, processed, red and white meat consumption and all-cause, CVD and IHD mortality: a meta-analysis of cohort studies. Br J Nutr. 2014; 112(5):762–75.
4. Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. Circulation. 2010;121(21):2271–83.
5. Rouhani MH, Salehi-Abargouei A, Surkan PJ, Azadbakht L. Is there a relationship between red or processed meat intake and obesity? A systematic review and meta-analysis of observational studies. Obes Rev. 2014;15(9):740–8.
6. Halkjaer J, Olsen A, Overvad K, Jakobsen MU, Boeing H, Buijsse B, Palli D, Tognon G, Du H, van der AD, et al. Intake of total, animal and plant protein and subsequent changes in weight or waist circumference in European men and women: the Diogenes project. Int J Obes (2005). 2011;35(8):1104–13.
7. Paddon-Jones D, Westman E, Mattes RD, Wolfe RR, Astrup A, Westerterp-Plantenga M. Protein, weight management, and satiety. Am J Clin Nutr. 2008;87(5):1558s–61.
8. Clifton PM, Condo D, Keogh JB. Long term weight maintenance after advice to consume low carbohydrate, higher protein diets–a systematic review and meta analysis. Nutr Metab Cardiovasc Dis. 2014;24(3):224–35.
9. Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. Am J Clin Nutr. 2006;83(2):260–74.
10. Assmann KE, Joslowski G, Buyken AE, Cheng G, Remer T, Kroke A, Gunther AL. Prospective association of protein intake during puberty with body composition in young adulthood. Obesity (Silver Spring, Md). 2013;21(12): E782–9.
11. Mensink GBM, Richter A, Vohmann C, Stahl A, Six J, Kohler S, Fischer J, Heseker H. EsKiMo. The nutrition module of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS). Ernährung - Wissenschaft und Praxis. 2007;1(5):225–9.
12. Bibiloni Mdel M, Pons A, Tur JA. Prevalence of overweight and obesity in adolescents: a systematic review. ISRN Obes. 2013;2013:392747.
13. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev. 2016;17(2):95–107.
14. Rogol AD, Roemmich JN, Clark PA. Growth at puberty. J Adolesc Health. 2002;31(6 Suppl):192–200.
15. Berg A, Kramer U, Link E, Bollrath C, Heinrich J, Brockow I, Koletzko S, Grubl A, Filipiak-Pittroff B, Wichmann HE, et al. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. Clin Exp Allergy. 2010;40(4):627–36.
16. Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I, Fahlbusch B, Bischof W, Weiss M, Borte M, Wichmann HE. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. Eur Respir J. 2002;20(3):617–23.
17. Stiegler P, Sausenthaler S, Buyken AE, Rzehak P, Czech D, Linseisen J, Kroke A, Gedrich K, Robertson C, Heinrich J. A new FFQ designed to measure the intake of fatty acids and antioxidants in children. Public Health Nutr. 2010; 13(1):38–46.
18. Willett W. Nutritional epidemiology. USA: Oxford University Press; 1998.
19. Hartmann BM, Bell S, Va'squez-Caiquedo AL. Der Bundeslebensmittelschlüssel. German Nutrient DataBase. Karlsruhe: Federal Research Centre for Nutrition and Food (BfEL); 2005.
20. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ. 2007;85(9):660–7.
21. Pei Z, Flexeder C, Fuertes E, Standl M, Buyken A, Berdel D, von Berg A, Lehmann I, Schaaf B, Heinrich J. Food intake and overweight in school-aged children in Germany: results of the GINIplus and LISAplus studies. Ann Nutr Metab. 2014;64(1):60–70.
22. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2015.
23. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ (Clin Res ed). 2000;320(7244):1240.
24. Vergnaud AC, Norat T, Romaguera D, Mouw T, May AM, Travier N, Luan J, Wareham N, Slimani N, Rinaldi S, et al. Meat consumption and prospective weight change in participants of the EPIC-PANACEA study. Am J Clin Nutr. 2010;92(2):398–407.
25. Bradlee ML, Singer MR, Qureshi MM, Moore LL. Food group intake and central obesity among children and adolescents in the Third National Health and Nutrition Examination Survey (NHANES III). Public Health Nutr. 2010;13(6):797–805.
26. Clifton P. Effects of a high protein diet on body weight and comorbidities associated with obesity. Br J Nutr. 2012;108 Suppl 2:S122–9.
27. Bujnowski D, Xun P, Daviglus ML, Van Horn L, He K, Stamler J. Longitudinal association between animal and vegetable protein intake and obesity among men in the United States: the Chicago Western Electric Study. J Am Diet Assoc. 2011;111(8):1150–5. e1151.
28. Koppes LL, Boon N, Nooyens AC, van Mechelen W, Saris WH. Macronutrient distribution over a period of 23 years in relation to energy intake and body fatness. Br J Nutr. 2009;101(1):108–15.
29. van Vught AJ, Heitmann BL, Nieuwenhuizen AG, Veldhorst MA, Brummer RJ, Westerterp-Plantenga MS. Association between dietary protein and change in body composition among children (EYHS). Clin Nutr (Edinburgh, Scotland). 2009;28(6):684–8.
30. Snowdon DA, Phillips RL, Fraser GE. Meat consumption and fatal ischemic heart disease. Prev Med. 1984;13(5):490–500.
31. Li D, Sinclair A, Mann N, Turner A, Ball M, Kelly F, Abedin L, Wilson A. The association of diet and thrombotic risk factors in healthy male vegetarians and meat-eaters. Eur J Clin Nutr. 1999;53(8):612–9.
32. Li D, Siriamornpun S, Wahlqvist ML, Mann NJ, Sinclair AJ. Lean meat and heart health. Asia Pac J Clin Nutr. 2005;14(2):113–9.
33. Soliman A, De Sanctis V, Elalaily R. Nutrition and pubertal development. Indian J Endocrinol Metab. 2014;18 Suppl 1:S39–47.

Paper 1: Supplementary Material

Table S1 Comparison of lost-to-follow-up and not-lost-to-follow-up study participants

	Females			Males		
	Lost to Follow-up	Not lost to Follow-up	p-value	Lost to Follow-up	Not lost to Follow-up	p-value
N	729	797		755	813	
10 years						
Age (years)	10.8 (0.5)	10.8 (0.5)	0.426	10.9 (0.5)	10.8 (0.5)	**0.000**
BMI (kg/m2)	17.2 (2.5)	17.1 (2.3)	0.627	17.2 (2.6)	17.2 (2.3)	0.935
Total caloric intake (kcal/d)	18.1 (4.9)	18.1 (4.9)	0.836	21.1 (6)	20.9 (5.7)	0.464
Overweight (%)	19.4	16.7	0.196	22.8	22.5	0.902
Sedentary behaviour[a] [high] (%)	9.4	8.2	0.466	13.8	12.8	0.600
Pubertal onset [yes](%)	47.5	45.9	0.535	12.6	10	0.107
15 years						
Age (years)	15.5 (0.3)	15.4 (0.3)	0.156	15.5 (0.3)	15.4 (0.3)	0.050
BMI (kg/m2)	20.5 (2.9)	20.7 (2.9)	0.195	20.7 (3.3)	20.6 (3.2)	0.382
Total caloric intake (kcal/d)	17.5 (5.7)	18 (5.6)	0.142	23.6 (6.9)	23.7 (6.6)	0.803
Overweight (%)	12	13.2	0.564	19.5	18.6	0.681
Sedentary behaviour[a] [high] (%)	46.8	48.4	0.583	59.5	64.2	0.069
Basic characteristics						
Study						
GINI observation	38.7	35.4	0.087	36.8	31.7	0.070
GINI intervention	26.7	31.9		28.9	29.3	
LISA	34.6	32.7		34.3	39	
Region						
Munich	49.1	52.3	**0.046**	51.3	51.2	**0.019**
Leipzig	5.9	8.7		6.2	9.7	
Bad Honef	5.1	4.3		3.6	4.9	
Wesel	39.9	34.8		38.9	34.2	
Parental education[b] (%)						
Low	6.3	3.9	**0.029**	8.4	4.3	**0.005**
Medium	26.4	23.6		26.6	27.8	
High	67.2	72.5		65.1	67.9	

Lost-to-follow-up: Dietary data at age 10 years but no available body composition data at age 15 years;
Not-lost-to-follow-up: Dietary data at age 10 years and available body composition data at age 15 years (current study sample);
Categorical variables presented as percentages, tested by Fisher's exact test (variables with 2 levels), or by Pearson's Chi2 test (variables with > 2 levels);
Continuous variables presented as mean (standard deviation), tested by t-test; [a]Hours spent on screen behaviours (≤ 2 hours = low; >2 hours = high); [b]Highest level achieved by mother or father (<10 years = low; 10 years = medium; >10 years = high); Significant p-values marked in bold.

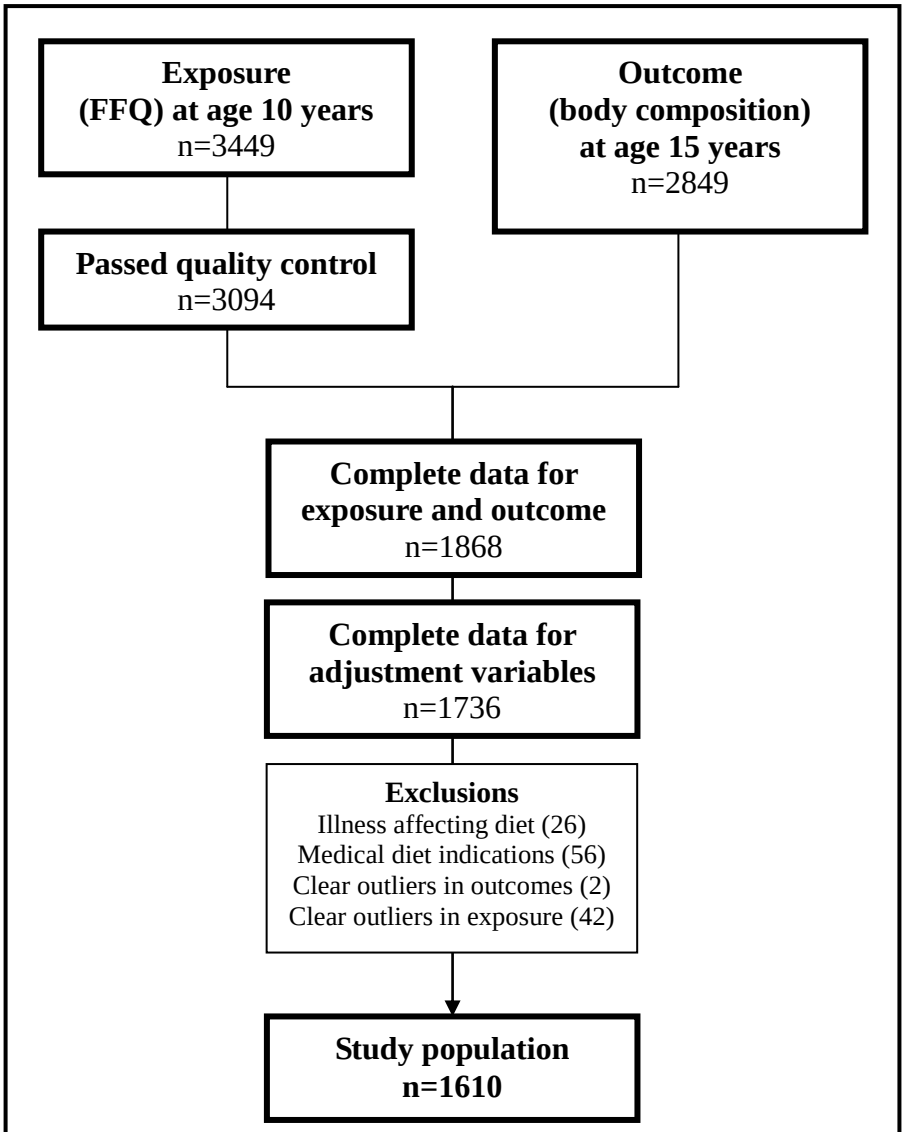

Figure S1 Study participants

Exposure: total meat, processed meat, red meat, poultry and respective protein variables obtained from FFQ; Outcome: fat mas index and fat free mass index; Adjustment variables: study, region, parental education, pubertal onset, age at body composition measurement, sedentary behaviour. Illness affecting diet: e.g. diabetes, anorexia, coeliac disease, cancer; Medical dietary indications: e.g. gluten-free, lactose-free diets.

Table S2a Prospective association of tertiles of meat and meat protein intakes (T2 and T3 vs T1) with FMI and FFMI, stratified by BMI at 10y (normal weight/overweight), in females

	FMI									FFMI								
	T2 vsT1				T3 vs T1					T2 vs T1				T3 vs T1				
	β	SE	p-val	p-int	β	SE	p-val	p-int	p-trend	β	SE	p-val	p-int	β	SE	p-val	p-int	p-trend
TOTAL MEAT																		
Normal weight	0.167	0.124	0.180	0.365	0.187	0.125	0.135	0.487	0.135	0.136	0.128	0.289	0.563	0.091	0.129	0.482	0.392	0.481
Overweight	0.503	0.469	0.285		0.271	0.478	0.571		0.547	-0.375	0.310	0.229		-0.137	0.316	0.665		0.634
PROCESSED																		
Normal weight	0.090	0.124	0.466	0.525	0.136	0.124	0.273	0.135	0.273	0.265	0.127	0.037	**0.097**	0.181	0.127	0.155	0.236	0.152
Overweight	0.028	0.470	0.953		0.816	0.478	0.090		0.092	-0.220	0.316	0.487		-0.127	0.321	0.692		0.686
RED MEAT																		
Normal weight	-0.046	0.125	0.712	0.378	-0.007	0.126	0.957	0.908	0.955	0.165	0.128	0.195	0.187	0.010	0.130	0.941	0.558	0.935
Overweight	-0.668	0.482	0.168		-0.312	0.494	0.528		0.523	-0.234	0.319	0.465		-0.459	0.327	0.164		0.162
POULTRY																		
Normal weight	-0.019	0.125	0.881	**0.016**	0.314	0.125	**0.012**	0.481	**0.012**	-0.100	0.129	0.439	0.132	0.102	0.129	0.429	0.525	0.426
Overweight	-0.085	0.478	0.859		0.026	0.468	0.955		0.960	0.154	0.316	0.628		-0.124	0.309	0.689		0.702
TOTAL MEAT PROTEIN																		
Normal weight	-0.034	0.125	0.788	0.718	0.154	0.127	0.224	0.209	0.224	0.042	0.129	0.743	0.172	-0.006	0.131	0.961	0.529	0.961
Overweight	0.196	0.488	0.689		-0.109	0.477	0.820		0.826	-0.335	0.319	0.296		-0.576	0.312	0.067		0.066
PROCESSED (PROTEIN)																		
Normal weight	0.111	0.124	0.372	0.268	0.071	0.124	0.570	0.390	0.566	0.117	0.127	0.357	**0.016**	0.230	0.128	0.071	0.111	0.071
Overweight	-0.316	0.473	0.505		0.304	0.485	0.532		0.570	-0.291	0.314	0.356		-0.311	0.322	0.335		0.321
RED MEAT (PROTEIN)																		
Normal weight	-0.010	0.124	0.937	0.337	-0.036	0.126	0.778	0.662	0.778	0.208	0.127	0.103	0.198	-0.037	0.129	0.775	0.601	0.789
Overweight	-0.668	0.482	0.168		-0.312	0.494	0.528		0.523	-0.234	0.319	0.465		-0.459	0.327	0.164		0.162
POULTRY (PROTEIN)[a]																		
Normal weight	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overweight	-	-	-		-	-	-			-	-	-		-	-	-		

Presented as beta coefficients (β) and standard errors (SE). Normal weight: BMI z-score ≤ 1; Overweight: BMI z-score > 1. P-val: p-value for the stratified model coefficients. Significant p-values marked in bold (<0.025 after adjustment for multiple testing). P-int: P-value for the interaction term coefficients of the interaction model ($p<0.1$ is marked as statistically significant). p-val: p-value from linear regression; p-trend: p-value indicating linear trend. Significant p-values marked in bold.

[a]Estimates for poultry protein not presented as categories for protein were identical to those for poultry meat, and hence estimates are also identical.

Table S2b Prospective association of tertiles of meat and meat protein intakes (T2 and T3 vs T1) with FMI and FFMI, stratified by BMI at 10y (normal weight/overweight), in males

	FMI									FFMI								
	T2 vsT1				T3 vs T1					T2 vs T1				T3 vs T1				
	β	SE	p-val	p-int	β	SE	p-val	p-int	p-trend	β	SE	p-val	p-int	β	SE	p-val	p-int	p-trend
TOTAL MEAT																		
Normal weight	-0.008	0.132	0.951	0.365	0.188	0.131	0.153	0.979	0.153	0.146	0.150	0.333	**0.027**	0.350	0.150	**0.020**	0.823	**0.020**
Overweight	-0.076	0.426	0.859		0.343	0.424	0.419		0.416	-0.386	0.374	0.303		0.488	0.372	0.191		0.191
PROCESSED																		
Normal weight	0.178	0.132	0.178	0.394	0.061	0.132	0.645	0.437	0.652	0.138	0.151	0.360	0.222	0.325	0.151	0.031	0.480	0.031
Overweight	-0.296	0.426	0.487		0.287	0.419	0.494		0.488	-0.182	0.379	0.633		0.100	0.374	0.790		0.785
RED MEAT																		
Normal weight	-0.085	0.132	0.523	0.505	-0.053	0.133	0.692	0.114	0.690	0.186	0.151	0.217	0.764	0.319	0.151	0.035	0.744	0.035
Overweight	0.120	0.431	0.782		0.450	0.434	0.301		0.298	0.101	0.382	0.793		0.433	0.385	0.263		0.259
POULTRY																		
Normal weight	0.048	0.132	0.716	0.980	0.205	0.132	0.122	0.593	0.123	-0.127	0.151	0.403	0.905	-0.084	0.152	0.581	0.239	0.578
Overweight	0.304	0.441	0.491		0.133	0.438	0.762		0.776	0.094	0.392	0.811		0.033	0.389	0.933		0.938
TOTAL MEAT PROTEIN																		
Normal weight	0.002	0.132	0.988	0.350	0.189	0.133	0.156	0.623	0.156	0.104	0.151	0.491	0.580	0.305	0.152	0.044	0.584	0.044
Overweight	-0.327	0.437	0.455		0.097	0.437	0.825		0.816	-0.170	0.389	0.662		0.093	0.389	0.811		0.805
PROCESSED (PROTEIN)																		
Normal weight	0.131	0.132	0.324	0.167	0.037	0.132	0.782	0.602	0.788	0.057	0.151	0.704	**0.092**	0.272	0.151	0.072	0.500	0.072
Overweight	-0.278	0.429	0.518		0.312	0.423	0.462		0.457	-0.315	0.382	0.411		-0.070	0.377	0.854		0.859
RED MEAT (PROTEIN)																		
Normal weight	-0.083	0.132	0.528	0.520	-0.031	0.133	0.813	**0.078**	0.811	0.166	0.151	0.272	0.690	0.304	0.151	0.045	0.689	0.045
Overweight	0.196	0.427	0.647		0.488	0.431	0.259		0.257	0.169	0.379	0.656		0.467	0.382	0.224		0.222
POULTRY (PROTEIN)[a]																		
Normal weight	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overweight	-	-	-		-	-	-			-	-	-		-	-	-		

Presented as beta coefficients (β) and standard errors (SE). Normal weight: BMI z-score ≤ 1; Overweight: BMI z-score > 1. P-val: p-value for the stratified model coefficients. Significant p-values marked in bold (<0.025 after adjustment for multiple testing). P-int: P-value for the interaction term coefficients of the interaction model (p<0.1 is marked as statistically significant). p-val: p-value from linear regression; p-trend: p-value indicating linear trend. Significant p-values marked in bold.

[a]Estimates for poultry protein not presented as categories for protein were identical to those for poultry meat, and hence estimates are also identical.

Table S3a Prospective association of tertiles of meat and meat protein intakes with FMI and FFMI in females (n=636) adjusted for EAA, SFA, MUFA or PUFA

	FMI						FFMI					
	T2 vsT1			T3 vsT1			T2 vsT1			T3 vsT1		
	β	SE	p-val	β	SE	p-val	β	SE	p-val	β	SE	p-val
EAA												
TOTAL MEAT	0.146	0.130	0.263	0.096	0.141	0.499	0.028	0.119	0.816	-0.131	0.129	0.310
PROCESSED	-0.009	0.129	0.944	0.134	0.132	0.309	0.150	0.118	0.206	0.076	0.121	0.527
RED MEAT	-0.167	0.131	0.203	-0.186	0.138	0.179	0.092	0.120	0.443	-0.154	0.126	0.224
POULTRY	-0.207	0.132	0.117	0.134	0.141	0.340	-0.197	0.121	0.105	-0.045	0.129	0.730
TOTAL MEAT PROTEIN	-0.069	0.133	0.604	0.101	0.149	0.499	-0.017	0.122	0.891	-0.174	0.136	0.202
PROCESSED (PROTEIN)	0.059	0.129	0.650	0.003	0.133	0.984	-0.018	0.118	0.877	0.089	0.122	0.465
RED MEAT (PROTEIN)	-0.139	0.131	0.290	-0.173	0.138	0.211	0.064	0.120	0.594	-0.173	0.126	0.171
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-
SFA												
TOTAL MEAT	0.172	0.130	0.186	0.251	0.132	0.058	0.050	0.119	0.674	-0.013	0.121	0.913
PROCESSED	0.023	0.130	0.862	0.240	0.133	0.071	0.169	0.119	0.154	0.141	0.121	0.243
RED MEAT	-0.115	0.131	0.379	-0.065	0.133	0.627	0.125	0.119	0.294	-0.077	0.121	0.527
POULTRY	-0.151	0.130	0.244	0.235	0.132	0.075	-0.155	0.119	0.192	0.030	0.121	0.807
TOTAL MEAT PROTEIN	-0.022	0.131	0.868	0.249	0.133	0.061	0.032	0.120	0.792	-0.033	0.121	0.784
PROCESSED (PROTEIN)	0.089	0.130	0.493	0.108	0.132	0.415	0.000	0.118	0.999	0.150	0.120	0.214
RED MEAT (PROTEIN)	-0.084	0.131	0.519	-0.054	0.133	0.684	0.099	0.119	0.404	-0.096	0.121	0.431
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-
MUFA												
TOTAL MEAT	0.198	0.132	0.134	0.295	0.148	**0.047**	0.057	0.120	0.639	-0.014	0.135	0.916
PROCESSED	0.036	0.132	0.786	0.278	0.150	0.063	0.180	0.121	0.136	0.172	0.137	0.207
RED MEAT	-0.115	0.131	0.378	-0.061	0.134	0.648	0.126	0.119	0.292	-0.072	0.122	0.553
POULTRY	-0.145	0.130	0.267	0.256	0.131	0.051	-0.149	0.119	0.211	0.046	0.120	0.701
TOTAL MEAT PROTEIN	0.001	0.132	0.996	0.284	0.140	**0.042**	0.040	0.120	0.737	-0.025	0.128	0.847
PROCESSED (PROTEIN)	0.089	0.132	0.500	0.110	0.147	0.454	0.014	0.120	0.909	0.190	0.134	0.156
RED MEAT (PROTEIN)	-0.087	0.131	0.509	-0.050	0.134	0.709	0.099	0.119	0.407	-0.091	0.122	0.457
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-
PUFA												
TOTAL MEAT	0.220	0.133	0.098	0.310	0.141	**0.029**	0.053	0.121	0.664	-0.028	0.129	0.831
PROCESSED	0.042	0.132	0.750	0.261	0.138	0.059	0.169	0.121	0.161	0.132	0.126	0.295
RED MEAT	-0.112	0.131	0.392	-0.055	0.133	0.682	0.124	0.119	0.297	-0.075	0.122	0.536
POULTRY	-0.128	0.131	0.327	0.290	0.134	**0.030**	-0.150	0.120	0.211	0.048	0.122	0.695
TOTAL MEAT PROTEIN	0.017	0.132	0.899	0.312	0.139	**0.025**	0.037	0.121	0.762	-0.036	0.128	0.780
PROCESSED (PROTEIN)	0.106	0.132	0.422	0.126	0.138	0.361	0.001	0.121	0.993	0.144	0.125	0.251
RED MEAT (PROTEIN)	-0.083	0.131	0.524	-0.043	0.133	0.745	0.097	0.119	0.415	-0.094	0.122	0.441
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-

EAA = essential amino acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted EAA)
SFA = saturated fatty acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted SFA)
MUFA = essential amino acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted MUFA)
PUFA = essential amino acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted PUFA)
Presented as beta coefficients (β) and standard errors (SE). Significant p-values marked in bold. [a]Estimates for poultry protein not presented as categories for protein were identical to those for poultry meat, and hence estimates are also identical.

Table S3b Prospective association of tertiles of meat and meat protein intakes with FMI and FFMI in males (n=673) adjusted for EAA, SFA, MUFA or PUFA

	FMI						FFMI					
	T2 vsT1			T3 vsT1			T2 vsT1			T3 vsT1		
	β	SE	p-val	β	SE	p-val	β	SE	p-val	β	SE	p-val
EAA												
TOTAL MEAT	-0.118	0.145	0.415	0.179	0.154	0.245	-0.019	0.149	0.899	0.324	0.158	**0.041**
PROCESSED	0.079	0.141	0.573	0.069	0.142	0.628	0.038	0.145	0.795	0.226	0.147	0.123
RED MEAT	-0.052	0.141	0.711	0.006	0.151	0.967	0.154	0.145	0.290	0.269	0.155	0.084
POULTRY	-0.050	0.141	0.724	0.260	0.149	0.082	-0.160	0.146	0.276	-0.082	0.155	0.596
TOTAL MEAT PROTEIN	-0.023	0.146	0.874	0.172	0.161	0.287	0.069	0.150	0.649	0.199	0.166	0.233
PROCESSED (PROTEIN)	0.033	0.140	0.815	0.106	0.143	0.457	-0.059	0.144	0.682	0.161	0.147	0.274
RED MEAT (PROTEIN)	-0.064	0.140	0.648	0.021	0.150	0.890	0.105	0.145	0.467	0.297	0.155	0.056
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-
SFA												
TOTAL MEAT	-0.084	0.138	0.543	0.249	0.141	0.077	0.034	0.143	0.812	0.427	0.145	**0.003**
PROCESSED	0.122	0.141	0.387	0.144	0.144	0.317	0.100	0.145	0.492	0.335	0.149	**0.024**
RED MEAT	-0.029	0.139	0.837	0.062	0.140	0.661	0.183	0.143	0.201	0.338	0.145	**0.020**
POULTRY	-0.037	0.139	0.791	0.270	0.139	0.053	-0.098	0.144	0.494	0.020	0.144	0.891
TOTAL MEAT PROTEIN	0.003	0.139	0.982	0.221	0.141	0.119	0.125	0.144	0.385	0.305	0.146	**0.037**
PROCESSED (PROTEIN)	0.069	0.141	0.624	0.179	0.144	0.213	-0.011	0.145	0.941	0.263	0.148	0.077
RED MEAT (PROTEIN)	-0.040	0.139	0.770	0.075	0.140	0.590	0.134	0.143	0.350	0.363	0.144	**0.012**
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-
MUFA												
TOTAL MEAT	-0.093	0.141	0.512	0.222	0.159	0.163	0.049	0.146	0.736	0.462	0.164	**0.005**
PROCESSED	0.076	0.145	0.602	0.052	0.165	0.752	0.090	0.150	0.548	0.322	0.170	0.058
RED MEAT	-0.042	0.140	0.763	0.043	0.141	0.760	0.176	0.144	0.221	0.327	0.146	**0.025**
POULTRY	-0.048	0.139	0.727	0.273	0.139	**0.050**	-0.110	0.144	0.447	0.025	0.144	0.864
TOTAL MEAT PROTEIN	-0.013	0.142	0.926	0.189	0.152	0.216	0.121	0.147	0.412	0.298	0.157	0.059
PROCESSED (PROTEIN)	0.030	0.145	0.835	0.104	0.161	0.520	-0.030	0.149	0.839	0.229	0.167	0.170
RED MEAT (PROTEIN)	-0.057	0.140	0.684	0.055	0.141	0.698	0.125	0.144	0.387	0.351	0.146	**0.016**
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-
PUFA												
TOTAL MEAT	-0.111	0.139	0.427	0.174	0.145	0.228	0.014	0.144	0.925	0.367	0.149	**0.014**
PROCESSED	0.066	0.141	0.640	0.042	0.145	0.775	0.049	0.146	0.736	0.241	0.150	0.109
RED MEAT	-0.047	0.139	0.736	0.044	0.140	0.755	0.168	0.144	0.243	0.322	0.145	**0.026**
POULTRY	-0.057	0.139	0.682	0.255	0.140	0.070	-0.121	0.144	0.401	0.003	0.146	0.983
TOTAL MEAT PROTEIN	-0.026	0.140	0.854	0.161	0.146	0.269	0.101	0.145	0.488	0.255	0.151	0.091
PROCESSED (PROTEIN)	0.015	0.141	0.915	0.087	0.144	0.546	-0.062	0.146	0.672	0.175	0.149	0.241
RED MEAT (PROTEIN)	-0.063	0.139	0.649	0.055	0.140	0.694	0.114	0.144	0.427	0.345	0.144	**0.017**
POULTRY (PROTEIN)[a]	-	-	-	-	-	-	-	-	-	-	-	-

EAA = essential amino acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted EAA)
SFA = saturated fatty acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted SFA)
MUFA = essential amino acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted MUFA)
PUFA = essential amino acids (MAIN models for the different meat exposures additionally adjusted for energy-adjusted PUFA)
Presented as beta coefficients (β) and standard errors (SE). Significant p-values marked in bold. [a]Estimates for poultry protein not presented as categories for protein were identical to those for poultry meat, and hence estimates are also identical.

5 Paper 2: Dietary Fatty Acids and Changes in Blood Lipids during Adolescence

(Harris et al. Nutrients, 2017)

Original title:	Dietary fatty acids and changes in blood lipids during adolescence: the role of substituting nutrient intakes
Authors:	C Harris, A Buyken, S Koletzko, A von Berg, D Berdel, T Schikowski, B Koletzko, J Heinrich, M Standl
Journal:	Nutrients
Volume:	9
Pages:	127
Year:	2017
DOI:	10.3390/nu9020127

nutrients

MDPI

Article

Dietary Fatty Acids and Changes in Blood Lipids during Adolescence: The Role of Substituting Nutrient Intakes

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Received: 2 December 2016; Accepted: 6 February 2017; Published: 11 February 2017

Abstract: The relevance of dietary fatty acids (FA) for blood lipids should be assessed in the context of substituting nutrients. Such evidence is lacking for adolescents. This study describes prospective associations of dietary FA with changes in serum lipids during adolescence, and considers the theoretical isocaloric replacements of saturated FA (SFA) with other FA or carbohydrates (CHO). Children from the GINIplus and LISAplus birth cohorts, with data on FA intakes (at age 10 years) and serum lipids (at age 10 and 15 years), were included (n = 1398). Associations of SFA, monounsaturated FA (MUFA), n-3 polyunsaturated FA (n-3 PUFA) and n-6 PUFA, with changes in low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TAG), and total cholesterol to HDL ratio (TOTAL:HDL), were assessed by linear regression. Substitution models assessed isocaloric replacements of SFA with MUFA, n-3 PUFA, n-6 PUFA or CHO. Higher SFA intakes were associated with decreasing TAG. No associations were observed for fatty acid intakes with LDL, HDL or TOTAL:HDL. In females, replacing SFA with CHO was associated with increasing LDL, TAG and TOTAL:HDL. Our findings confirm observations in adults, although sex-specific determinants seem relevant in our adolescent population. Overlooking the nutrient context when limiting SFA intakes might have detrimental consequences appreciable as early as adolescence.

Keywords: fatty acids; lipids; isocaloric substitution; diet; carbohydrates; adolescence; epidemiology

1. Introduction

Since the first appearance of evidence suggesting a detrimental role of saturated fatty acids (SFA) in the development of coronary heart disease [1,2], the advice to reduce SFA consumption has become a major component of health-promoting strategies [3]. Nevertheless, inconsistent findings among emerging studies have led scientists to question the independent association of SFA with the development of cardiovascular disease (CVD) [4–6]. It has become clear that evidence supporting

a reduction of SFA intake must be interpreted in the context of the specific nutrients consumed in its place [7,8]. In 2008, the FAO and the WHO stated convincing evidence for an improved lipoprotein profile in adults when replacing SFA with polyunsaturated fatty acids (PUFA) and, to a lesser extent, with monounsaturated fatty acids (MUFA). On the other hand, replacing SFA with carbohydrates (CHO) was reported to reduce low-density lipoprotein (LDL) but also high-density lipoprotein (HDL) levels [9].

It is currently well established that CVD risk factors progress from childhood and adolescence into adulthood [10]. Results from numerous longitudinal cohort studies have indicated strong tracking of serum lipids from childhood to adulthood [11–13]. Considering the implications this can have for later disease development, improving our understanding of the role of dietary fatty acid intakes in children is of major importance for the early implementation of dietary advice. The period concerning pubertal development is of interest due to the rapid growth and development as well as behavioral changes occurring at this stage [14,15]. However, despite the growing evidence in adults [16–18], the amount of reliable and comparable data on dietary fatty acid intakes in children and adolescents is scarce [19]. Studies observing the associations of total [20–22] and saturated fat [23,24] with blood lipid concentrations have reported mixed results. In particular, longitudinal studies on the theoretical implication of different replacements of SFA on lipid profiles in children and adolescents are lacking. A 2002 study using repeated measures at ages 8 and 11 years, suggested associations with serum lipids similar to those observed in adults when replacing SFA with MUFA or PUFA [25]. Further studies are required to learn whether such associations persist during the period of pubertal development.

The current study therefore aims to describe the prospective associations of fatty acid intakes during childhood with changes in serum lipid concentrations during adolescence. Furthermore, we are interested in observing how associations with SFA may depend on the choice of substituting nutrient. We therefore consider changes in blood lipids following the theoretical reduction of SFA in the context of different isocaloric replacements with other fatty acids or with carbohydrates.

2. Materials and Methods

2.1. Participants

The present study used data from the 10- and 15-year follow-up assessments of the ongoing GINIplus (German Infant Nutritional Intervention plus environmental and genetic influences on allergy development) and LISAplus (Influence of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood plus the Influence of Traffic Emissions and Genetics) birth cohort studies. Healthy full-term newborns were recruited from obstetric clinics in four German cities. Information was collected using identical questionnaires and at physical examinations. The study designs, recruitment and exclusion criteria have been described previously [26,27]. For both studies, approval by the local ethics committees (Bavarian General Medical Council, University of Leipzig, Medical Council of North-Rhine-Westphalia) and written consent from participants' families were obtained.

2.2. Dietary Intake

Dietary intake data were collected at the 10-year follow-up assessment, using a self-administered food frequency questionnaire (FFQ) designed to assess food and nutrient intake over the past year in school-aged children, and validated to estimate energy, fatty acid and antioxidant intake [28]. In brief, subjects were asked to report estimated frequency and portion size of intakes of 80 food items. A quality control procedure was applied based on recommendations by Willett et al. for data cleaning in nutritional epidemiology [29,30]. Total daily energy intake and the intakes of SFA, MUFA, *n*-6 and *n*-3 PUFA, protein, carbohydrate and alcohol were calculated (in kcal/day) based on the German Food Code and Nutrient Database (BLS) version II.3.1 [31]. Each nutrient was expressed as its percentage contribution towards total daily energy intake (%EI), calculated as the ratio of energy from each nutrient to total daily energy intake, multiplied by 100.

2.3. Blood Lipids

Blood samples were obtained during the 10- and 15-year follow-up physical examinations. The concentrations (mmol/L) of total cholesterol, LDL, HDL, and triglycerides (TAG) were measured in serum using homogenous enzymatic colorimetric methods on a Modular Analytics System from Roche Diagnostics GmbH Mannheim according to the manufactures instructions. External controls were used in accordance with the guidelines of the German Society of Clinical Chemistry and Laboratory Medicine. The ratio of total to HDL cholesterol (TOTAL:HDL) was calculated by dividing total cholesterol by HDL.

2.4. Statistical Analyses

Participants with complete data on FA intakes at age 10 years, serum lipids at age 10 and 15 years, and all adjustment variables were included in the study (Figure 1). To test for differences due to attrition bias, we compared characteristics of participants lost to follow-up (data only available for exposure and outcome at 10 years) to those included in the present study analyses, who adhered at follow-up (data available for exposure at 10 years and outcome at 10 years and 15 years). Categorical variables, presented as percentages, were tested by Fisher's exact test (binary variables) or Pearson's Chi-squared test (variables with more than 2 levels). Continuous variables, presented as means (standard deviation), were tested by Student's *t*-test.

Statistical analyses were carried out in the total population and stratified by sex. Subject characteristics at ages 10 and 15 years were described by medians (25th percentile; 75th percentile) or counts (%). Differences from 10 to 15 years were tested using paired Wilcoxon signed rank test for continuous variables and McNemar's χ^2-test for categorical variables. Differences in characteristics between males and females at each assessment were tested using Wilcoxon signed rank test for continuous variables and χ^2-test for categorical variables (Fisher's exact test for binary variables). Changes (Δ) in lipid concentrations and in TOTAL:HDL ratio were calculated by subtracting each measurement at the 10-year follow-up from its respective measurement at the 15-year follow-up.

Using linear regression, two modelling approaches were applied. First, single nutrient models were fit to observe the changes in blood lipids when increasing habitual intakes of a single nutrient at a constant energy intake. Intakes of different fatty acids assessed at age 10 years were considered as the exposures of interest. Separate regression models were run for each exposure (SFA, MUFA, *n*-3 PUFA or *n*-6 PUFA) with the different blood lipid parameters (ΔLDL, ΔHDL, ΔTAG, ΔTOTAL:HDL). Through this prospective approach, we aim to avoid any misleading findings emerging from the possible bidirectional relationship between fatty acid intakes and blood lipids assessed at a single time-point only. Second, substitution models were fit to observe the effect of replacing SFA with other fatty acids (MUFA, *n*-6 PUFA and *n*-3 PUFA) or with CHO, on the different blood lipid parameters (ΔLDL, ΔHDL, ΔTAG, ΔTOTAL:HDL). These models included the exposure nutrient of interest as well as all other energy-bearing nutrients except SFA (the nutrient being "replaced"). In this way, energy intakes of protein, carbohydrate, alcohol and other fats are held constant; and by additionally including total energy intake in the model it is possible to interpret the resulting coefficients for each nutrient as its theoretical substitution for an equal amount of energy (%EI) from saturated fat, being the only energy-bearing nutrient not accounted for in the model. All models were adjusted for potential covariates in two steps. First, we adjusted for basic covariates (M_{BASIC}): study (GINI observation arm; GINI intervention arm; LISA), recruitment region (Munich; Wesel; Bad Honnef; Leipzig), sex (male; female)—not in sex-stratified models—exact age at 10-year blood sampling (years), fasting status at blood sampling (not fasted (46%); fasted at one assessment (45%); fasted at both assessments (9%)), BMI (kg/m^2) at age 10 years, screen-time (daily hours spent on activities in front of a screen: $\leq$2 h = low; >2 h = high) at age 10 years, total energy intake (kcal/day) at age 10 years, and lipid concentration (mmol/L) at age 10 years. In a second step, models were further adjusted for other potential confounders (M_{ADJ}): parental education level (highest level achieved by mother or father: $\leq$10th grade = low/medium; >10th grade = high) and pubertal onset at age

10 years (oestrogen $\geq$ 18.5 pmol/L or testosterone $\geq$ 0.1 nmol/L = yes; oestrogen < 18.5 pmol/L or testosterone < 0.1 mmol/L = no). Given the high intercorrelation typically present amongst dietary components [32], we calculated correlation coefficients between pairs of nutrient variables, using Pearson's product-moment correlation coefficient. A high negative correlation was observed between MUFA and CHO. By linearly regressing MUFA onto CHO and vice-versa, we computed residuals ($MUFA_{RESID}$ and CHO_{RESID}), which were uncorrelated with each other [33]. In order to avoid multicollinearity, these were included in the models as a stand-in for the original variable only when acting as a covariate (i.e., when assessing the effect of replacing SFA with CHO, CHO was included in its original form as the main predictor variable, and $MUFA_{RESID}$ was included in place of MUFA, along with all other covariates, and vice versa). Results from the linear regression analyses are presented as regression coefficients (β) per interquartile range (IQR) increase in the relevant exposure variable, along with their 95% confidence interval (95% CI). A two-sided α-level of 5% was considered significant for the total population analyses. For the sex-stratified analysis we corrected for multiple testing using Bonferroni correction: the α-level was divided by 2 (2.5%) as the dataset was analyzed by sub-groups of two levels (male/female). Statistical analyses were conducted using R (www.r-project.org), version 3.3.0 [34].

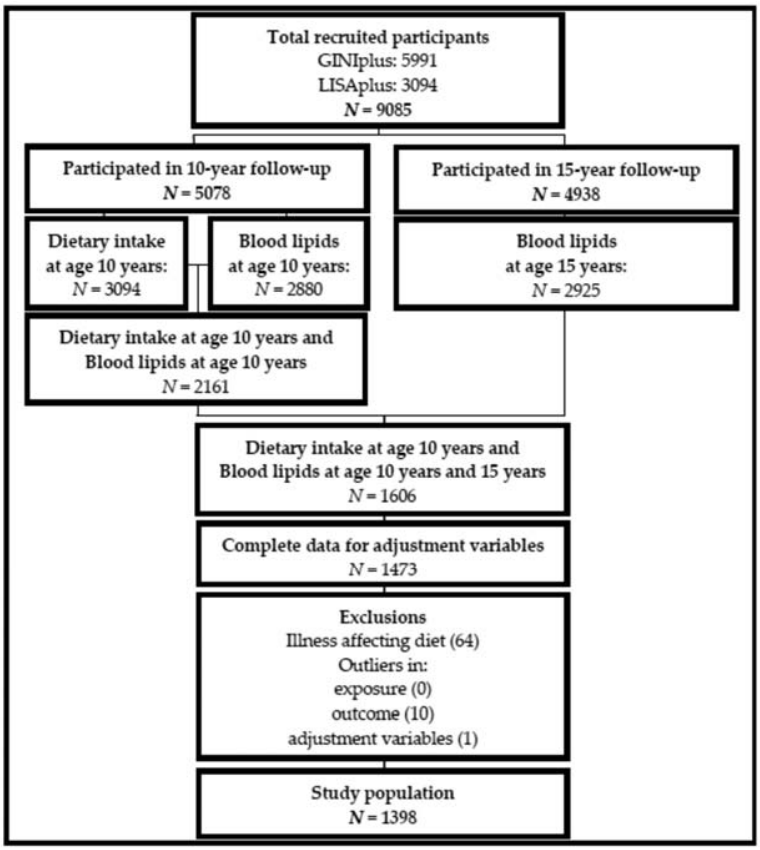

Figure 1. Study participants Dietary intake: intakes of fatty acids (saturated, monounsaturated, *n*-3 polyunsaturated, *n*-6 polyunsaturated), carbohydrate, protein, and alcohol obtained from FFQ; Blood lipids: low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides; adjustment variables: study, region, age, fasting status, BMI, screen-time, total energy intake, lipid concentration at age 10 years, parental education and pubertal onset; Illness affecting diet: e.g., diabetes, anorexia, coeliac disease, cancer, or medical dietary indications (e.g., gluten-free, lactose-free diets).

3. Results

3.1. Study Population

The present analyses comprised of 1398 participants (681 females and 717 males). The derivation of the study population is presented in Figure 1. Subjects providing complete dietary intake data at age 10 years, measures of serum lipids at both 10 and 15 years, as well as information on all adjustment variables, were included (n = 1473). Differences in descriptive characteristics between participants included in the analyses and participants lost to follow-up are presented in the Table S1). Participants were excluded if they reported an illness affecting diet (e.g., diabetes, anorexia, coeliac disease, cancer) or medical dietary indications, such as gluten-free or lactose-free diets (n = 64). Clear outliers in blood lipid concentrations (n = 10), or adjustment variables (n = 1) were visually identified using descriptive plots and excluded from the analyses. Basic characteristics of the study population at age 10 and 15 years are described in Table 1. Both sexes had higher levels of LDL and HDL and lower levels of TAG and TOTAL:HDL at age 15 years compared to 10 years. Significant differences over time were observed for BMI, fasting status at blood sampling, screen-time and total daily energy intake, with higher values at age 15 years in both sexes (except energy intake, which decreased in females). Males reported higher screen-time and daily energy intake than females at both time-points, as well as higher fat and protein intakes at age 15. On the other hand, females at age 15 years reported higher carbohydrate intakes. Overall, most participants were from Munich (57.7%) with a high parental education (71.3%). Notably, more females than males had reached pubertal onset at the age of 10 years (74.4% females vs. 24.1% males).

3.2. Single Nutrient Models

The prospective associations of dietary fatty acid intakes (in %EI) at age 10 years with changes in serum lipid concentrations from age 10 to age 15 years are described in Table 2. Values are presented for basic (M_{BASIC}) and fully adjusted models (M_{ADJ}). The resulting β-coefficients indicate the changes in blood lipid concentrations (mmol/L) per IQR increase in the %EI of a given fatty acid, while maintaining total energy intake constant. A significant inverse association was observed between the intake of SFA (IQR increase in %EI) at age 10 years and the change in TAG concentrations from age 10 to age 15 years (M_{ADJ}: β = −0.038 (95% CI = −0.075; −0.001), p-value = 0.042). A similar association was observed in females only, which was borderline statistically significant when corrected for multiple testing (M_{ADJ}: −0.053 (−0.100; −0.007), p-value = 0.025). No associations were observed for any of the fatty acid exposures with the other assessed blood lipid parameters.

3.3. Substitution Models

Table 3 shows the prospective associations of different dietary fatty acid intakes and CHO at age 10 years with changes in serum lipid concentrations from age 10 to age 15 years, when considering their theoretical substitution for SFA. Values are presented for basic (M_{BASIC}) and fully adjusted models (M_{ADJ}). Coefficients (β) obtained from these models represent an isocaloric substitution, i.e., the change in blood lipid concentrations when theoretically replacing the intake of SFA with another (specific) fatty acid or CHO, while maintaining total energy intake constant. A direct association was observed in the basic model for the substitution of CHO (IQR increase in %EI) for SFA with ΔLDL (M_{BASIC}: 0.063 (0.000; 0.127), p-value = 0.05). Sex-stratified analyses indicated significant associations in females only, after correction for multiple testing: direct associations were observed for the substitution of CHO (IQR increase in %EI) for SFA with ΔLDL (M_{ADJ}: 0.125 (0.021; 0.229), p-value = 0.019), ΔTAG (M_{ADJ}: 0.098 (0.020; 0.176), p-value = 0.014), and ΔTOTAL:HDL (M_{ADJ}: 0.115 (0.015; 0.215), p-value = 0.024).

Table 1. Basic characteristics of the study population.

Variables	Total (*N* = 1398)			Females (*N* = 681)			Males (*N* = 717)		
	10 Years	15 Years	*p*-Value [a]	10 Years	15 Years	*p*-Value [a]	10 Years	15 Years	*p*-Value [a]
Blood lipids									
LDL (mmol/L)	2.1 (1.7; 2.5)	2.3 (1.9; 2.7)	<0.01	2.1 (1.8; 2.5) †	2.4 (2.0; 2.9) †	<0.01	2.0 (1.7; 2.5)	2.2 (1.8; 2.6)	<0.01
HDL (mmol/L)	1.2 (1.1; 1.4)	1.5 (1.2; 1.7)	<0.01	1.2 (1.1; 1.4)	1.6 (1.4; 1.8) †	<0.01	1.3 (1.1; 1.5) §	1.4 (1.2; 1.6)	<0.01
TAG (mmol/L)	1.2 (0.9; 1.6)	1.0 (0.8; 1.4)	<0.01	1.2 (0.9; 1.6)	1.0 (0.8; 1.3)	<0.01	1.1 (0.8; 1.6)	1.0 (0.7; 1.4)	<0.01
TOTAL:HDL	3.8 (3.2; 4.5)	2.9 (2.5; 3.4)	<0.01	3.9 (3.4; 4.6) †	2.9 (2.5; 3.4)	<0.01	3.6 (3.2; 4.4)	3.0 (2.5; 3.5) §	<0.01
Fatty acids									
SFA (%EI)	12.6 (10.9; 14.7)	12.7 (10.8; 14.7)	0.621	12.5 (10.7; 14.7)	12.6 (10.6; 14.6)	0.190	12.8 (11.1; 14.8)	12.9 (10.9; 14.9)	0.512
MUFA (%EI)	10.7 (9.3; 12.3)	10.8 (9.4; 12.3)	0.133	10.7 (9.2; 12.1)	10.5 (9.1; 12.2)	0.608	10.7 (9.5; 12.4)	11.2 (9.6; 12.6) §	<0.01
n-3 PUFA (%EI)	0.54 (0.49; 0.62)	0.56 (0.49; 0.65)	<0.01	0.55 (0.49; 0.63)	0.57 (0.49; 0.64)	0.009	0.54 (0.48; 0.62)	0.56 (0.48; 0.65)	<0.01
n-6 PUFA (%EI)	3.7 (3.2; 4.3)	3.9 (3.3; 4.6)	<0.01	3.7 (3.2; 4.3)	3.9 (3.3; 4.7)	0.002	3.7 (3.2; 4.3)	3.9 (3.3; 4.6)	<0.01
Covariates									
Age (years)	10.2 (10.1; 10.3)	15.1 (15.0; 15.3)	<0.01	10.2 (10.1; 10.3)	15.1 (15; 15.3)	<0.01	10.2 (10.1; 10.3)	15.1 (15; 15.3)	<0.01
BMI (kg/m^2)	16.7 (15.6; 18.4)	20.2 (18.7; 22.2)	<0.01	16.8 (15.6; 18.5)	20.4 (18.9; 22.3)	<0.01	16.7 (15.7; 18.4)	20 (18.6; 22.1)	<0.01
Fasting (yes)	237 (17.0)	649 (46.4)	<0.01 [b]	121 (17.8)	309 (45.2)	<0.01 [b]	116 (16.2)	341 (47.6)	<0.01 [b]
Screen-time (high)	134 (9.6)	763 (55.3)	<0.01 [b]	48 (7.0)	322 (47.8)	<0.01 [b]	86 (12.0) §	442 (62.5) §	<0.01 [b]
Energy intake (kcal)	1933 (1591; 2292)	2011 (1584; 2532)	<0.01	1798 (1486; 2124)	1734 (1360; 2115)	0.016	2061 (1705; 2447) §	2361 (1884; 2866) §	<0.01
Fat (%EI)	30.1 (26.7; 34.2)	30.5 (27.1; 34.8)	0.128	29.9 (26.1; 33.9)	30.0 (26.5; 34.2)	0.982	30.2 (27.3; 34.4)	30.9 (27.6; 35.3) §	0.029
Carbohydrate (%EI)	54.1 (49.6; 58.0)	53.2 (48.6; 57.7)	0.004	54.3 (49.7; 58.4)	54.1 (49.1; 58.4) †	0.696	53.7 (49.6; 57.5)	52.4 (47.7; 56.7)	<0.01
Protein (%EI)	14.5 (12.9; 16.0)	14.8 (13.1; 16.6)	<0.01	14.4 (12.8; 16.1)	14.5 (12.7; 16.3)	0.452	14.5 (13.1; 16.0)	15.1 (13.4; 16.8) §	<0.01
Study									
GINI observation	452 (32.3)			221 (32.5)			231 (32.2)		
GINI intervention	437 (31.3)			224 (32.9)			213 (29.7)		
LISA	509 (36.4)			236 (34.7)			273 (38.1)		
Region									
Munich	807 (57.7)			389 (57.1)			418 (58.3)		
Leipzig	123 (8.8)			60 (8.8)			63 (8.8)		
Bad Honnef	65 (4.6)			29 (4.3)			36 (5.0)		
Wesel	403 (28.8)			203 (29.8)			200 (27.9)		
Parental education (High)	997 (71.3)			497 (73.0)			500 (69.7)		
Pubertal onset (Yes)	680 (48.6)			507 (74.4) †			173 (24.1)		

Values are medians (25th percentile; 75th percentile) or counts (%); LDL = low-density lipoprotein; HDL = high-density lipoprotein; TAG = triglycerides; SFA = saturated fatty acids; TOTAL:HDL = total cholesterol to HDL ratio; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; [a] tested by paired Wilcoxon signed rank test; [b] tested by McNemar's chi-squared test; † value is significantly greater in females than in males at the respective time-point (*p*-value < 0.05, tested by Wilcoxon signed rank test or Fisher's exact test); § value is significantly greater in males than in females at the respective time-point (*p*-value < 0.05, tested by Wilcoxon signed rank test or Fisher's exact test).

Table 2. Single nutrient model: prospective associations of dietary fatty acid intakes at age 10 years (per IQR increase in %EI) with changes in blood lipid concentrations (mmol/L) from age 10 to 15 years.

Fatty Acids		ΔLDL			ΔHDL			ΔTAG			ΔTOTAL:HDL		
		β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value
TOTAL													
SFA	M_{BASIC}	−0.038	−0.077; 0.001	0.057	0.005	−0.017; 0.027	0.653	**−0.038**	**−0.075; −0.001**	**0.042**	−0.030	−0.075; 0.015	0.196
	M_{ADJ}	−0.036	−0.075; 0.003	0.068	0.005	−0.017; 0.027	0.638	**−0.038**	**−0.075; −0.001**	**0.042**	−0.029	−0.074; 0.016	0.209
MUFA	M_{BASIC}	−0.012	−0.048; 0.024	0.519	0.011	−0.010; 0.031	0.306	−0.012	−0.046; 0.023	0.507	−0.017	−0.059; 0.025	0.428
	M_{ADJ}	−0.011	−0.048; 0.025	0.534	0.011	−0.009; 0.031	0.297	−0.012	−0.046; 0.023	0.503	−0.017	−0.059; 0.025	0.430
***n*-3 PUFA**	M_{BASIC}	−0.027	−0.058; 0.003	0.075	0.005	−0.012; 0.022	0.590	0.001	−0.028; 0.030	0.953	−0.017	−0.052; 0.018	0.342
	M_{ADJ}	−0.027	−0.057; 0.003	0.082	0.005	−0.012; 0.022	0.540	0.000	−0.028; 0.029	0.981	−0.017	−0.053; 0.018	0.332
***n*-6 PUFA**	M_{BASIC}	−0.003	−0.034; 0.028	0.866	0.015	−0.002; 0.032	0.089	0.009	−0.020; 0.039	0.525	−0.012	−0.048; 0.024	0.504
	M_{ADJ}	−0.003	−0.034; 0.028	0.849	0.016	−0.002; 0.033	0.074	0.009	−0.021; 0.038	0.559	−0.013	−0.049; 0.023	0.465
FEMALES													
SFA	M_{BASIC}	−0.048	−0.110; 0.014	0.131	0.012	−0.022; 0.045	0.491	−0.053	−0.100; −0.006	0.026	−0.057	−0.116; 0.002	0.060
	M_{ADJ}	−0.047	−0.110; 0.015	0.139	0.012	−0.022; 0.045	0.484	−0.053	−0.100; −0.007	0.025	−0.057	−0.116; 0.002	0.060
MUFA	M_{BASIC}	−0.004	−0.061; 0.053	0.888	0.020	−0.011; 0.050	0.211	−0.017	−0.060; 0.026	0.430	−0.026	−0.081; 0.028	0.342
	M_{ADJ}	−0.005	−0.062; 0.053	0.876	0.020	−0.011; 0.050	0.209	−0.018	−0.061; 0.025	0.420	−0.027	−0.081; 0.028	0.336
***n*-3 PUFA**	M_{BASIC}	−0.030	−0.078; 0.018	0.219	0.011	−0.015; 0.037	0.406	0.000	−0.036; 0.036	0.990	−0.032	−0.077; 0.014	0.173
	M_{ADJ}	−0.030	−0.079; 0.018	0.214	0.011	−0.015; 0.037	0.403	0.000	−0.036; 0.036	0.998	−0.032	−0.078; 0.014	0.169
***n*-6 PUFA**	M_{BASIC}	−0.020	−0.068; 0.028	0.409	0.019	−0.007; 0.044	0.148	0.016	−0.020; 0.052	0.390	−0.026	−0.071; 0.020	0.269
	M_{ADJ}	−0.021	−0.069; 0.027	0.397	0.020	−0.006; 0.046	0.124	0.014	−0.022; 0.050	0.446	−0.028	−0.074; 0.018	0.231
MALES													
SFA	M_{BASIC}	−0.029	−0.078; 0.019	0.237	−0.002	−0.031; 0.026	0.868	−0.016	−0.072; 0.04	0.577	0.002	−0.066; 0.070	0.962
	M_{ADJ}	−0.026	−0.075; 0.022	0.282	−0.003	−0.031; 0.026	0.863	−0.015	−0.072; 0.041	0.591	0.003	−0.065; 0.071	0.929
MUFA	M_{BASIC}	−0.021	−0.066; 0.025	0.372	0.000	−0.027; 0.027	0.975	−0.002	−0.055; 0.051	0.952	−0.001	−0.065; 0.063	0.972
	M_{ADJ}	−0.019	−0.065; 0.026	0.400	0.001	−0.026; 0.027	0.964	−0.002	−0.056; 0.051	0.927	−0.001	−0.065; 0.063	0.968
***n*-3 PUFA**	M_{BASIC}	−0.026	−0.064; 0.012	0.181	−0.003	−0.025; 0.02	0.818	0.005	−0.040; 0.049	0.826	0.001	−0.053; 0.055	0.971
	M_{ADJ}	−0.025	−0.063; 0.013	0.199	−0.001	−0.023; 0.022	0.952	0.003	−0.042; 0.048	0.891	0.000	−0.054; 0.054	0.992
***n*-6 PUFA**	M_{BASIC}	0.011	−0.029; 0.051	0.583	0.010	−0.013; 0.034	0.392	0.001	−0.045; 0.048	0.953	0.000	−0.056; 0.056	0.991
	M_{ADJ}	0.013	−0.027; 0.052	0.534	0.012	−0.011; 0.036	0.299	−0.001	−0.047; 0.046	0.975	−0.001	−0.057; 0.055	0.971

IQR = interquartile range; %EI = % of total energy intake; M_{BASIC} = single nutrient model adjusted for study, region, sex (not in sex-stratified models), exact age at blood sampling, BMI at 10 years, total daily energy intake at 10 years, screen-time at 10 years, fasting status at blood sampling, and lipid concentration at 10 years; M_{ADJ} = single nutrient model further adjusted for pubertal onset and parental education; Δ = change from age 10 to 15 years; Significant associations marked in bold (p-value < 0.05 for total population analyses, p-value < 0.025 for sex-stratified analyses—Bonferroni correction for multiple testing: 0.05/2).

Table 3. Substitution model: prospective associations of fatty acids and carbohydrates (CHO) (when replacing SFA) at age 10 years (per IQR increase in %EI), with changes in blood lipid concentrations (mmol/L) from age 10 to 15 years.

Substituting Nutrient		ΔLDL			ΔHDL			ΔTAG			ΔTOTAL:HDL		
		β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value
TOTAL													
MUFA	M_{BASIC}	−0.037	−0.085; 0.011	0.134	0.002	−0.025; 0.029	0.897	−0.041	−0.087; 0.005	0.077	−0.027	−0.083; 0.029	0.346
	M_{ADJ}	−0.034	−0.082; 0.014	0.163	0.002	−0.025; 0.029	0.894	−0.041	−0.086; 0.005	0.081	−0.025	−0.082; 0.031	0.378
***n*-3 PUFA**	M_{BASIC}	−0.027	−0.064; 0.011	0.164	−0.003	−0.024; 0.018	0.752	0.002	−0.033; 0.037	0.913	−0.008	−0.052; 0.036	0.715
	M_{ADJ}	−0.026	−0.063; 0.012	0.179	−0.003	−0.024; 0.018	0.775	0.002	−0.034; 0.037	0.916	−0.008	−0.052; 0.036	0.723
***n*-6 PUFA**	M_{BASIC}	0.018	−0.019; 0.056	0.341	0.016	−0.005; 0.037	0.143	0.019	−0.017; 0.055	0.303	−0.001	−0.045; 0.043	0.978
	M_{ADJ}	0.017	−0.021; 0.054	0.384	0.016	−0.005; 0.038	0.129	0.018	−0.018; 0.054	0.324	−0.002	−0.047; 0.042	0.916
CHO	M_{BASIC}	0.063	0.000; 0.127	0.050	−0.001	−0.036; 0.035	0.970	0.057	−0.003; 0.117	0.061	0.043	−0.031; 0.117	0.257
	M_{ADJ}	0.060	−0.004; 0.123	0.064	−0.001	−0.037; 0.034	0.947	0.057	−0.003; 0.117	0.063	0.041	−0.033; 0.115	0.278
FEMALES													
MUFA	M_{BASIC}	−0.053	−0.130; 0.024	0.175	0.005	−0.037; 0.046	0.825	−0.065	−0.122; −0.007	0.029	−0.054	−0.127; 0.020	0.153
	M_{ADJ}	−0.052	−0.129; 0.025	0.188	0.005	−0.037; 0.046	0.824	−0.064	−0.122; −0.007	0.029	−0.053	−0.127; 0.020	0.154
***n*-3 PUFA**	M_{BASIC}	−0.020	−0.080; 0.039	0.502	−0.004	−0.036; 0.028	0.822	0.002	−0.043; 0.047	0.928	−0.003	−0.060; 0.053	0.907
	M_{ADJ}	−0.020	−0.079; 0.039	0.510	−0.004	−0.036; 0.028	0.788	0.003	−0.042; 0.048	0.895	−0.002	−0.059; 0.055	0.942
***n*-6 PUFA**	M_{BASIC}	−0.004	−0.062; 0.054	0.896	0.015	−0.016; 0.046	0.341	0.034	−0.009; 0.078	0.125	−0.005	−0.060; 0.050	0.856
	M_{ADJ}	−0.005	−0.063; 0.053	0.868	0.017	−0.014; 0.048	0.292	0.032	−0.012; 0.075	0.155	−0.008	−0.064; 0.047	0.774
CHO	M_{BASIC}	**0.127**	**0.023; 0.231**	**0.017**	−0.003	−0.059; 0.053	0.916	**0.097**	**0.019; 0.175**	**0.015**	**0.114**	**0.014; 0.213**	**0.025**
	M_{ADJ}	**0.125**	**0.021; 0.229**	**0.019**	−0.004	−0.060; 0.052	0.891	**0.098**	**0.020; 0.176**	**0.014**	**0.115**	**0.015; 0.215**	**0.024**
MALES													
MUFA	M_{BASIC}	−0.027	−0.088; 0.033	0.373	−0.001	−0.036; 0.035	0.976	−0.015	−0.085; 0.055	0.668	−0.006	−0.091; 0.079	0.898
	M_{ADJ}	−0.024	−0.084; 0.036	0.435	−0.001	−0.037; 0.035	0.948	−0.015	−0.085; 0.056	0.683	−0.004	−0.089; 0.082	0.934
***n*-3 PUFA**	M_{BASIC}	−0.043	−0.091; 0.005	0.079	−0.008	−0.037; 0.020	0.570	0.006	−0.050; 0.062	0.833	−0.009	−0.076; 0.059	0.796
	M_{ADJ}	−0.043	−0.090; 0.005	0.081	−0.007	−0.035; 0.021	0.630	0.005	−0.050; 0.061	0.851	−0.009	−0.077; 0.058	0.788
***n*-6 PUFA**	M_{BASIC}	0.041	−0.009; 0.090	0.106	0.017	−0.012; 0.046	0.256	−0.004	−0.061; 0.054	0.898	0.001	−0.069; 0.071	0.985
	M_{ADJ}	0.041	−0.008; 0.090	0.103	0.018	−0.011; 0.048	0.224	−0.004	−0.062; 0.053	0.880	0.000	−0.070; 0.070	0.995
CHO	M_{BASIC}	0.017	−0.059; 0.093	0.658	−0.001	−0.046; 0.044	0.961	0.021	−0.068; 0.109	0.647	−0.008	−0.115; 0.099	0.884
	M_{ADJ}	0.012	−0.064; 0.088	0.755	0.000	−0.045; 0.045	0.991	0.020	−0.069; 0.108	0.663	−0.011	−0.118; 0.096	0.842

IQR = interquartile range; %EI = % of total energy intake; M_{BASIC} = substitution model adjusted for study, region, sex (not in sex-stratified models), exact age at blood sampling, BMI at 10 years, total daily energy intake at 10 years, screen-time at 10 years, fasting status at blood sampling, lipid concentration at 10 years, and all energy-bearing nutrients except SFA; M_{ADJ} = substitution model further adjusted for pubertal onset and parental education; Δ = change from age 10 to 15 years; Significant associations marked in bold (p-value < 0.05 for total population analyses, p-value < 0.025 for sex-stratified analyses—Bonferroni correction for multiple testing: 0.05/2).

4. Discussion

The present study used data from two large German birth cohorts to assess the prospective associations of fatty acid intakes with changes in blood lipid concentrations during adolescence. We found that higher intakes of SFA at age 10 years were associated with decreasing TAG between ages 10 and 15 years. Furthermore, we observed that the consumption of CHO at the expense of SFA in females was associated with increasing LDL, TAG and TOTAL:HDL.

4.1. Single Nutrient Model

Our findings regarding the prospective association of SFA intakes with reduced TAG concentrations are in line with existing literature in adults [35]. The observed relationship might be considered somewhat counterintuitive, given the suggestions for a possible detrimental role of SFA in the development of coronary heart disease [1,2]. Conflicting evidence has been reported in younger populations, although existing studies are scarce and heterogeneous in terms of design, statistical methods and outcome measurements. A study in children aged 6 to 12 years, reported a positive association between SFA and total cholesterol concentrations [36], whereas an inverse association was observed in a study in 15-year-olds [37]. Other studies have reported no association between dietary fatty acids and blood lipids in pre-pubertal and pubertal children [24,38]. The often-observed association of SFA consumption with increased LDL in adults [35] was not present in our adolescent population. In fact, we observed a negative relationship in our total study sample, although the association did not reach statistical significance (p-value = 0.068). Although the evidence for an association of SFA with LDL has been widely accepted, recent studies in adults have emerged, reporting no association between SFA and CVD risk [4]. Nonetheless, the present results should be interpreted in the context of possible correlations among different nutrients. In our dietary data, SFA was highly positively correlated with MUFA (r = 0.77), and also presented strong negative correlations with CHO (r = −0.81), which limits the ability to disentangle the individual effects of SFA [32]. In light of this and the inverse association observed between SFA and TAG levels in the current study, we speculate that increasing the intake of SFA might have led to decreasing TAG levels indirectly through a reduction in CHO intake. Indeed, CHO, in particular simple sugars, have been shown to have a detrimental impact on blood lipids through raising TAG levels [39]. This has been suggested to result mainly from increased hepatic secretion of very-low-density lipoprotein (VLDL) as well as impaired plasma TAG clearance, possibly induced by reduced insulin sensitivity [40].

4.2. Substitution Model

Results from our substitution analyses showed that replacing SFA with CHO was associated with increasing LDL, TAG and TOTAL:HDL in females. Our findings are in line with studies in adults which report a detrimental effect on blood lipids when substituting CHO for SFA [41]. However, the specific effects on blood lipid parameters differed from those observed in adults, who typically present lower LDL levels [9,42,43] occurring in parallel with decreasing HDL levels, and having no effect on the TOTAL:HDL ratio [44]. An increase in LDL is, however, plausible if we consider results from randomized controlled trials which have reported positive linear associations of CHO with small-dense LDL [45]. Other studies have shown positive relationships between dietary sugars with plasma LDL and TAG [46]. In agreement with this and other studies [8,9,41,44] females in our study population also presented positive associations between CHO and TAG. This relationship can be attributed to the increased secretion of VLDL and impaired plasma TAG clearance described above. The greater number of VLDL particles in the blood could also have led to the increase in the LDL production rate [40], which was further reflected by the lower TOTAL:HDL ratio observed amongst our findings. A previous study, including a subset of our study population, showed that the highest contribution towards total energy intake at age 10 years came from "refined grains" and "sugar-sweetened foods" [30], which might suggest that the CHO consumed by children in our study

population consisted largely of refined grains and sugars. Investigation into the effects of replacing SFA with different quality CHO is beyond the scope of the present study, but should be considered for further research in this age group.

Comparison of our results with existing evidence in adolescents is restricted due to the absence of studies carried out during this life period. One similar study observed theoretical effects of substituting MUFA or PUFA for SFA, and total fat for CHO from ages 8 to 11 years [25]. The study findings included slightly lowered total cholesterol when replacing SFA with MUFA or PUFA and higher HDL when replacing CHO with fat. Based on these findings, the authors suggested a similar effect of diet on serum lipids to that observed in adults [25]. Unfortunately, LDL, TAG and TOTAL:HDL were not included among the serum lipid measurements and so comparison with our study is limited. Nevertheless, our findings also seem to be to some extent comparable to observations in adult populations. Our results further suggest a sex-specific role of SFA (when replaced by CHO), acting mainly in female adolescents. The reasons for this gender discrepancy are unknown but we speculate that it could be related to possible sex differences in dietary patterns, hormones or pubertal stage. A greater proportion of girls in our study had entered pubertal onset at age 10 years (Table 1). It has been shown that physiological insulin resistance occurs during puberty [47], which may explain why females in the present study were more vulnerable than boys to the potentially adverse effects of carbohydrates. Additionally, girls in our study had slightly higher CHO intakes, which persisted at a high level, whilst they decreased in boys.

4.3. Strengths and Limitations

One of the main strengths of this study is its focus on the prospective role of dietary fatty acids on blood lipids during adolescence, a life period not often addressed and becoming increasingly relevant in terms of later disease development. Furthermore, we consider isocaloric replacements of SFA, which can contribute toward better understanding its independent role in the context of other nutrients. For our analyses, we benefited from a large homogenous population of females and males, providing data covering a five-year period from childhood to adolescence. The longitudinal nature of this study is a key aspect which allows us to add to the limited knowledge regarding fatty acid intake and prospective changes in markers of cardiometabolic risk during adolescence. Given the observational nature of the study, causality cannot be implied; nevertheless, the prospective analysis offers a temporal component which provides stronger grounds for a causal interpretation. Whether the observed effect sizes in this study can be considered clinically relevant might be a point for discussion. Furthermore, considering that children in the present study are not a high risk population and present normal blood lipid levels, the observation of associations at this stage provide only an indication of a possible early role of dietary nutrients in the long-term development of CVD risk factors. Nevertheless, given the increasing evidence for the progression of risk factors from childhood to adulthood, preventive measures might already consider this age group. Our findings provide a relevant indication of possible dietary targets which could support the development of recommendations for early disease prevention.

A main drawback in nutritional epidemiology is the high intercorrelation amongst different nutrients, which, if overlooked, can lead to incorrect conclusions. The use of substitution models can provide additional insight through the adjustment for other nutrients. However, the method can result in multicollinearity within statistical models, again generating misleading associations [32]. In our analyses, we tackle this problem by residualizing highly correlated variables, allowing the new variable to be included in the same model as the previously correlated nutrient, while avoiding multicollinearity [33]. A further limitation in the present study was non-random loss-to-follow-up, which meant that, for example, children of lower social classes might be underrepresented in our analyses. Therefore the generalizability of our findings is limited, as these cannot be considered representative of the study area (Table S1). Finally, we are aware of problems associated with misreporting of dietary intake with the use of FFQs. However, the FFQ was validated to estimate fatty acids and antioxidants in school-aged children. We observed plausible values in terms of energy intake

and believe that any misreporting was likely detected through extensive quality control, which was done at the expense of reducing the sample size, but with no substantial loss of power.

5. Conclusions

In conclusion, our findings suggest that higher SFA intakes might lead to reductions in TAG concentrations during adolescence. We highlight that observed associations in this context are not independent of other correlated nutrients. Furthermore, replacement of SFA with CHO in female children is associated with increasing levels of LDL, TAG and TOTAL:HDL during adolescence. Our findings confirm observations in adult populations, where detrimental aspects of increased consumption of CHO at the expense of SFA have been reported. Sex-specific determinants may however play a greater role during adolescence. It is important that recommendations to reduce SFA intakes do not overlook the possible effects of other nutrients consumed in their place.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/9/2/127/s1. Table S1. Comparison of descriptive characteristics of participants included in analyses (data for exposure at age 10 years and outcome at age 10 years and age 15 years) and participants lost to follow-up (data only for exposure and outcome at age 10 years).

Acknowledgments: The authors thank all the families for their participation in the GINIplus and LISAplus studies. Furthermore, we thank all members of the GINIplus and LISAplus Study Groups for their excellent work. The GINIplus Study Group consists of the following: Institute of Epidemiology I, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg (Heinrich J, Brüske I, Schulz H, Flexeder C, Zeller C, Standl M, Schnappinger M, Sußmann M, Thiering E, Tiesler C); Department of Pediatrics, Marien-Hospital, Wesel (Berdel D, von Berg A); Ludwig-Maximilians-University of Munich, Dr von Hauner Children's Hospital (Koletzko S); Child and Adolescent Medicine, University Hospital rechts der Isar of the Technical University Munich (Bauer CP, Hoffmann U); IUF-Environmental Health Research Institute, Düsseldorf (Hoffmann B, Link E, Klümper C). The GINIplus study was mainly supported for the first three years of the Federal Ministry for Education, Science, Research and Technology (interventional arm) and Helmholtz Zentrum Munich (former GSF) (observational arm). The 4 year, 6 year, 10 year and 15 year follow-up examinations of the GINIplus study were covered from the respective budgets of the five study centers (Helmholtz Zentrum Munich (former GSF), Research Institute at Marien-Hospital Wesel, LMU Munich, TU Munich and from 6 years onwards also from IUF—Leibniz Research-Institute for Environmental Medicine at the University of Düsseldorf) and a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). The LISAplus Study Group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Schnappinger M, Brüske I, Sußmann M, Lohr W, Schulz H, Zeller C, Standl M); Department of Pediatrics, Municipal Hospital "St. Georg", Leipzig (Borte M, Gnodtke E); Marien Hospital Wesel, Department of Pediatrics, Wesel (von Berg A, Berdel D, Stiers G, Maas B); Pediatric Practice, Bad Honnef (Schaaf B); Helmholtz Centre of Environmental Research—UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (Lehmann I, Bauer M, Röder S, Schilde M, Nowak M, Herberth G , Müller J, Hain A); Technical University Munich, Department of Pediatrics, Munich (Hoffmann U, Paschke M, Marra S); Clinical Research Group Molecular Dermatology, Department of Dermatology and Allergy, Technische Universität München (TUM), Munich (Ollert M). The LISAplus study was mainly supported by grants from the Federal Ministry for Education, Science, Research and Technology and in addition from Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research—UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Pediatric Practice, Bad Honnef for the first two years. The 4 year, 6 year, 10 year and 15 year follow-up examinations of the LISAplus study were covered from the respective budgets of the involved partners (Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research—UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Pediatric Practice, Bad Honnef, IUF—Leibniz-Research Institute for Environmental Medicine at the University of Düsseldorf) and in addition by a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). Further, the 15 years follow-up examination of the GINIplus and LISAplus studies was supported by the Commission of the European Communities, the 7th Framework Program: MeDALL project. The 15 year follow-up examination of the GINIplus study was additionally supported by the Mead Johnson and Nestlé companies. The work of BK is financially supported in part by the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG—no. 322605).

Author Contributions: Marie Standl, Joachim Heinrich, Carla Harris and Anette Buyken were involved in the conception and design of the study; Sibylle Koletzko, Andrea von Berg, Dietrich Berdel, Tamara Schikowski, Joachim Heinrich and Marie Standl in the data acquisition; Carla Harris and Marie Standl in the statistical analyses; Carla Harris, Marie Standl, Anette Buyken, Berthold Koletzko and Joachim Heinrich in the interpretation; Carla Harris drafted the manuscript; all authors revised it critically for important intellectual content, and approved the final version to be published.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kannel, W.B.; Dawber, T.R.; Kagan, A.; Revotskie, N.; Stokes, J., 3rd. Factors of risk in the development of coronary heart disease—Six year follow-up experience. The framingham study. *Ann. Intern. Med.* **1961**, *55*, 33–50. [CrossRef]
2. Keys, A.; Anderson, J.T.; Grande, F. Serum cholesterol response to changes in the diet: Iv. Particular saturated fatty acids in the diet. *Metab. Clin. Exp.* **1965**, *14*, 776–787. [CrossRef]
3. World Health Organization. *Diet, Nutrition and the Prevention of Chronic Diseases*; World Health Organization Technical Report Series; World Health Organization: Geneva, Switzerland, 2003; Volume 916, pp. 1–149.
4. Siri-Tarino, P.W.; Sun, Q.; Hu, F.B.; Krauss, R.M. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am. J. Clin. Nutr.* **2010**, *91*, 535–546. [CrossRef] [PubMed]
5. Mente, A.; de Koning, L.; Shannon, H.S.; Anand, S.S. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch. Intern. Med.* **2009**, *169*, 659–669. [CrossRef] [PubMed]
6. Chowdhury, R.; Warnakula, S.; Kunutsor, S.; Crowe, F.; Ward, H.A.; Johnson, L.; Franco, O.H.; Butterworth, A.S.; Forouhi, N.G.; Thompson, S.G.; et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: A systematic review and meta-analysis. *Ann. Intern. Med.* **2014**, *160*, 398–406. [CrossRef] [PubMed]
7. Hodson, L.; Skeaff, C.M.; Chisholm, W.A. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *Eur. J. Clin. Nutr.* **2001**, *55*, 908–915. [CrossRef] [PubMed]
8. Mensink, R.P.; Zock, P.L.; Kester, A.D.; Katan, M.B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to hdl cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.* **2003**, *77*, 1146–1155. [PubMed]
9. Food and Agriculture Organization. *Fats and Fatty Acids in human Nutrition*; Report of an Expert Consultation; FAO Food and Nutrition Paper; Food and Agriculture Organization: Rome, Italy, 2010; Volume 91, pp. 1–166.
10. WHO Expert Committee on Prevention in Childhood; Youth of Adult Cardiovascular Diseases World Health Organization. *Prevention in Childhood and Youth of Adult Cardiovascular Diseases: Time for Action/Report of a Who Expert Committee*; World Health Organization: Geneva, Switzerland, 1990.
11. Juhola, J.; Magnussen, C.G.; Viikari, J.S.; Kahonen, M.; Hutri-Kahonen, N.; Jula, A.; Lehtimaki, T.; Akerblom, H.K.; Pietikainen, M.; Laitinen, T.; et al. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: The cardiovascular risk in young finns study. *J. Pediatr.* **2011**, *159*, 584–590. [CrossRef] [PubMed]
12. Nicklas, T.A.; von Duvillard, S.P.; Berenson, G.S. Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: The bogalusa heart study. *Int. J. Sports Med.* **2002**, *23* (Suppl. 1), S39–S43. [CrossRef] [PubMed]
13. Webber, L.S.; Srinivasan, S.R.; Wattigney, W.A.; Berenson, G.S. Tracking of serum lipids and lipoproteins from childhood to adulthood. The bogalusa heart study. *Am. J. Epidemiol.* **1991**, *133*, 884–899. [PubMed]
14. Meredith, C.N.; Dwyer, J.T. Nutrition and exercise: Effects on adolescent health. *Annu. Rev. Public Health* **1991**, *12*, 309–333. [CrossRef] [PubMed]
15. Rogol, A.D.; Roemmich, J.N.; Clark, P.A. Growth at puberty. *J. Adolesc. Health* **2002**, *31*, 192–200. [CrossRef]
16. Praagman, J.; de Jonge, E.A.; Kiefte-de Jong, J.C.; Beulens, J.W.; Sluijs, I.; Schoufour, J.D.; Hofman, A.; van der Schouw, Y.T.; Franco, O.H. Dietary saturated fatty acids and coronary heart disease risk in a dutch middle-aged and elderly population. *Arterioscler. Thromb. Vasc. Biol.* **2016**, *36*, 2011–2018. [CrossRef] [PubMed]
17. Li, Y.; Hruby, A.; Bernstein, A.M.; Ley, S.H.; Wang, D.D.; Chiuve, S.E.; Sampson, L.; Rexrode, K.M.; Rimm, E.B.; Willett, W.C.; et al. Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: A prospective cohort study. *J. Am. Coll. Cardiol.* **2015**, *66*, 1538–1548. [CrossRef] [PubMed]
18. Jakobsen, M.U.; O'Reilly, E.J.; Heitmann, B.L.; Pereira, M.A.; Balter, K.; Fraser, G.E.; Goldbourt, U.; Hallmans, G.; Knekt, P.; Liu, S.; et al. Major types of dietary fat and risk of coronary heart disease: A pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr.* **2009**, *89*, 1425–1432. [CrossRef] [PubMed]

19. Lambert, J.; Agostoni, C.; Elmadfa, I.; Hulshof, K.; Krause, E.; Livingstone, B.; Socha, P.; Pannemans, D.; Samartin, S. Dietary intake and nutritional status of children and adolescents in europe. *Br. J. Nutr.* **2004**, *92* (Suppl. 2), S147–S211. [CrossRef] [PubMed]
20. Luepker, R.V.; Perry, C.L.; McKinlay, S.M.; Nader, P.R.; Parcel, G.S.; Stone, E.J.; Webber, L.S.; Elder, J.P.; Feldman, H.A.; Johnson, C.C.; et al. Outcomes of a field trial to improve children's dietary patterns and physical activity. The child and adolescent trial for cardiovascular health. Catch collaborative group. *J. Am. Med. Assoc.* **1996**, *275*, 768–776. [CrossRef]
21. Cheng, H.H.; Wen, Y.Y.; Chen, C. Serum fatty acid composition in primary school children is associated with serum cholesterol levels and dietary fat intake. *Eur. J. Clin. Nutr.* **2003**, *57*, 1613–1620. [CrossRef] [PubMed]
22. Bel-Serrat, S.; Mouratidou, T.; Huybrechts, I.; Labayen, I.; Cuenca-Garcia, M.; Palacios, G.; Breidenassel, C.; Molnar, D.; Roccaldo, R.; Widhalm, K.; et al. Associations between macronutrient intake and serum lipid profile depend on body fat in european adolescents: The healthy lifestyle in europe by nutrition in adolescence (helena) study. *Br. J. Nutr.* **2014**, *112*, 2049–2059. [CrossRef] [PubMed]
23. Niinikoski, H.; Pahkala, K.; Ala-Korpela, M.; Viikari, J.; Rönnemaa, T.; Lagström, H.; Jokinen, E.; Jula, A.; Savolainen, M.J.; Simell, O.; et al. Effect of repeated dietary counseling on serum lipoproteins from infancy to adulthood. *Pediatrics* **2012**, *129*, e704–e713. [CrossRef] [PubMed]
24. Ku, C.Y.; Gower, B.A.; Nagy, T.R.; Goran, M.I. Relationships between dietary fat, body fat, and serum lipid profile in prepubertal children. *Obes. Res.* **1998**, *6*, 400–407. [CrossRef] [PubMed]
25. Nicklas, T.A.; Dwyer, J.; Feldman, H.A.; Luepker, R.V.; Kelder, S.H.; Nader, P.R. Serum cholesterol levels in children are associated with dietary fat and fatty acid intake. *J. Am. Diet. Assoc.* **2002**, *102*, 511–517. [CrossRef]
26. von Berg, A.; Kramer, U.; Link, E.; Bollrath, C.; Heinrich, J.; Brockow, I.; Koletzko, S.; Grubl, A.; Filipiak-Pittroff, B.; Wichmann, H.E.; et al. Impact of early feeding on childhood eczema: Development after nutritional intervention compared with the natural course—The giniplus study up to the age of 6 years. *Clin. Exp. Allergy* **2010**, *40*, 627–636.
27. Heinrich, J.; Bolte, G.; Holscher, B.; Douwes, J.; Lehmann, I.; Fahlbusch, B.; Bischof, W.; Weiss, M.; Borte, M.; Wichmann, H.E. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur. Respir. J.* **2002**, *20*, 617–623. [CrossRef] [PubMed]
28. Stiegler, P.; Sausenthaler, S.; Buyken, A.E.; Rzehak, P.; Czech, D.; Linseisen, J.; Kroke, A.; Gedrich, K.; Robertson, C.; Heinrich, J. A new FFQ designed to measure the intake of fatty acids and antioxidants in children. *Public Health Nutr.* **2010**, *13*, 38–46. [CrossRef] [PubMed]
29. Willett, W. *Nutritional Epidemiology*; Oxford University Press: Oxford, UK, 1998.
30. Harris, C.; Flexeder, C.; Thiering, E.; Buyken, A.; Berdel, D.; Koletzko, S.; Bauer, C.P.; Bruske, I.; Koletzko, B.; Standl, M. Changes in dietary intake during puberty and their determinants: Results from the giniplus birth cohort study. *BMC Public Health* **2015**, *15*, 841. [CrossRef] [PubMed]
31. Hartmann, B.M.; Bell, S.; Vásquez-Caicedo, A.L.; Götz, A.; Erhardt, J.; Brombach, C. *Der Bundeslebensmittelschlüssel*. German Nutrient Database; Federal Research Centre for Nutrition and Food (BfEL): Karlsruhe, Germany, 2005.
32. Reed, D.; McGee, D.; Yano, K.; Hankin, J. Diet, blood pressure, and multicollinearity. *Hypertension* **1985**, *7*, 405–410. [PubMed]
33. Dormann, C.F.; Elith, J.; Bacher, S.; Buchmann, C.; Carl, G.; Carré, G.; Marquéz, J.R.G.; Gruber, B.; Lafourcade, B.; Leitão, P.J.; et al. Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography* **2013**, *36*, 27–46. [CrossRef]
34. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2016.
35. Wolfram, G.; Bechthold, A.; Boeing, H.; Ellinger, S.; Hauner, H.; Kroke, A.; Leschik-Bonnet, E.; Linseisen, J.; Lorkowski, S.; Schulze, M.; et al. Evidence-based guideline of the german nutrition society: Fat intake and prevention of selected nutrition-related diseases. *Ann. Nutr. Metab.* **2015**, *67*, 141–204. [CrossRef] [PubMed]
36. Guillaume, M.; Lapidus, L.; Lambert, A. Differences in associations of familial and nutritional factors with serum lipids between boys and girls: The luxembourg child study. *Am. J. Clin. Nutr.* **2000**, *72*, 384–388. [PubMed]
37. Samuelson, G.; Bratteby, L.E.; Mohsen, R.; Vessby, B. Dietary fat intake in healthy adolescents: Inverse relationships between the estimated intake of saturated fatty acids and serum cholesterol. *Br. J. Nutr.* **2001**, *85*, 333–341. [CrossRef] [PubMed]

38. Altwaijri, Y.A.; Day, R.S.; Harrist, R.B.; Dwyer, J.T.; Ausman, L.M.; Labarthe, D.R. Sexual maturation affects diet–blood total cholesterol association in children: Project heartbeat! *Am. J. Prev. Med.* **2009**, *37*, S65–S70. [CrossRef] [PubMed]
39. Siri-Tarino, P.W.; Chiu, S.; Bergeron, N.; Krauss, R.M. Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment. *Ann. Rev. Nutr.* **2015**, *35*, 517–543. [CrossRef] [PubMed]
40. Parks, E.J.; Hellerstein, M.K. Carbohydrate-induced hypertriacylglycerolemia: Historical perspective and review of biological mechanisms. *Am. J. Clin. Nutr.* **2000**, *71*, 412–433. [PubMed]
41. Siri-Tarino, P.W.; Sun, Q.; Hu, F.B.; Krauss, R.M. Saturated fat, carbohydrate, and cardiovascular disease. *Am. J. Clin. Nutr.* **2010**, *91*, 502–509. [CrossRef] [PubMed]
42. Panel on Dietetic Products, Nutrition and Allergies. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J.* **2010**, *8*. [CrossRef]
43. Mensink, R.P.; Katan, M.B. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler. Thromb.* **1992**, *12*, 911–919. [CrossRef] [PubMed]
44. Sacks, F.M.; Katan, M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. *Am. J. Med.* **2002**, *113* (Suppl. 9B), 13S–24S. [CrossRef]
45. Krauss, R.M.; Blanche, P.J.; Rawlings, R.S.; Fernstrom, H.S.; Williams, P.T. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. *Am. J. Clin. Nutr.* **2006**, *83*, 1025–1031. [PubMed]
46. Te Morenga, L.A.; Howatson, A.J.; Jones, R.M.; Mann, J. Dietary sugars and cardiometabolic risk: Systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *Am. J. Clin. Nutr.* **2014**, *100*, 65–79. [CrossRef] [PubMed]
47. Amiel, S.A.; Sherwin, R.S.; Simonson, D.C.; Lauritano, A.A.; Tamborlane, W.V. Impaired insulin action in puberty. *N. Engl. J. Med.* **1986**, *315*, 215–219. [CrossRef] [PubMed]

Paper 2: Supplementary Material

Supplementary Materials: Dietary Fatty Acids and Changes in Blood Lipids during Adolescence: The Role of Substituting Nutrient Intakes

Carla Harris, Anette Buyken, Sibylle Koletzko, Andrea von Berg, Dietrich Berdel, Tamara Schikowski, Berthold Koletzko, Joachim Heinrich and Marie Standl

Table S1. Comparison of descriptive characteristics of participants included in analyses (data for exposure at age 10 years and outcome at age 10 years and age 15 years) and participants lost to follow-up (data only for exposure and outcome at age 10 years).

Variables	Included	Lost to follow-up	*p*-Value
N	1606	555	
BMI at 10 years [kg/m²] [b]	16.8 (2.2)	17.1 (2.5)	0.008
Fasting at 10 years [yes] [a]	17.0	17.8	0.648
screen-time at 10 years [high] [a]	10.0	11.5	0.331
Energy intake at 10 years [kcal] [b]	1971.3 (548.2)	1932.3 (570.1)	0.160
Sex [female] [a]	48.7	50.8	0.403
Study [a]			
GINI observation	30.9	31.7	
GINI intervention	34.2	29.7	
LISA	34.9	38.6	0.122
Region [a]			
Munich	57.0	57.7	
Leipzig	8.9	7.2	
Bad Honnef	4.7	5.8	
Wesel	29.4	29.4	0.509
Parental education level [high] [a]	70.9	66.6	0.065
Pubertal onset 10 years [yes] [a]	71.6	67.6	0.092

[a] Presented as percentage and tested by Fisher's exact test (binary variables) or Pearson's Chi-squared test (variables with more than 2 levels); [b] Presented as mean (standard deviation) and tested by *t*-test.

6 Paper 3: Serum Fatty Acids and Low-grade Inflammation in Children

(Harris et al. European Journal of Clinical Nutrition, 2017)

Original title:	Associations between fatty acids and low-grade inflammation in children from the LISAplus birth cohort study
Authors:	C Harris, H Demmelmair, A von Berg, I Lehmann, C Flexeder, B Koletzko, J Heinrich, M Standl
Journal:	European Journal of Clinical Nutrition
Volume:	71
Pages:	1303–1311
Year:	2017
DOI:	10.1038/ejcn.2017.73

Associations between fatty acids and low-grade inflammation in children from the LISAplus birth cohort study

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Running title:

Fatty acids and low-grade inflammation in children

Conflict of interest:

The authors declare no conflict of interest.

Abstract

Background: Assessing fatty acid (FA) composition in relation to inflammatory markers can shed light on the role of different FA and their metabolism in low-grade inflammation. Existing exploratory studies in children are scarce, and findings inconsistent. We hence aim to analyse associations of FA with common inflammatory markers, high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6), in 10-year-old children.

Methods: Complete data were available for 958 participants from the 10-year follow-up of the LISAplus birth cohort study. FA composition was assessed in serum glycerophospholipids. Hs-CRP and IL-6 were categorized into 3 levels. Associations of FA with inflammatory markers were assessed using multinomial logistic regression, adjusting for potential confounders. Additionally, sex-stratified analyses were carried out.

Results: FA exposures associated with significantly higher low-grade inflammation, as indicated by higher hs-CRP or IL-6 levels, included: palmitic acid (PA) (IL-6: $p<0.001$, 95% confidence interval: 1.30;2.43), arachidonic acid (AA) (hs-CRP: $p=0.002$, 1.07;1.31), n-6 highly-unsaturated FA (HUFA) (hs-CRP: $p=0.002$, 1.06;1.27), ratio of AA to linoleic acid (AA/LA) (hs-CRP: $p<0.001$, 1.16;1.62), and total saturated FA (SFA) (IL-6: $p<0.001$, 1.77;3.15). FA exposures associated with reduced levels of inflammatory markers included: linoleic acid (LA) (hs.CRP: $p=0.001$, 0.84;0.96, IL-6: $p<0.001$, 0.69;0.90) and total polyunsaturated FA (PUFA) ($p<0.001$, 0.57;0.78).

Conclusions: These findings suggest that higher SFA and minor n-6 HUFA, namely PA and AA, are associated with increased low-grade inflammation in children; whereas the major dietary n-6 PUFA and total PUFA are associated with reduced inflammation. Elevated desaturase activity, estimated by the ratio AA/LA, may be associated with higher inflammation, particularly in boys.

Introduction

A state of chronic low-grade inflammation, characterised by raised concentrations of circulating inflammatory markers, is known to underlie metabolic conditions such as atherosclerosis (1, 2) and obesity (3, 4). C-reactive protein (CRP) is an acute phase protein synthesised primarily in response to circulating proinflammatory cytokine interleukin-6 (IL-6) (5). Elevated concentrations of both these inflammatory markers have been observed in association with arterial changes in children (6, 7), suggesting a possible role of low-grade inflammation in the pathogenesis of early atherosclerosis.

Some dietary components have the capacity to influence inflammatory processes (8), thereby signifying potential modifiable targets for the prevention of low-grade inflammation and associated diseases. It is now recognised that lipid-derived mediators, produced from long-chain fatty acids (FA), are greatly involved in the metabolic mechanisms of inflammation (9). Long-chain n-3 polyunsaturated FA (PUFA), have been shown to have anti-inflammatory properties, partly by reducing levels of arachidonic acid (AA), a known source of pro-inflammatory eicosanoids in immune cell membranes (10). FA composition, often measured in plasma or serum lipids (11, 12), reflects both dietary FA intake and endogenous FA metabolism (13). Especially the major dietary n-6 PUFA, linoleic acid (LA), and the long-chain n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are well reflected in serum phospholipids. A number of studies analysing FA composition in relation to inflammatory markers in adults, have shed light on the possible involvement of different FA and their metabolism in low-grade inflammation (14-17). Evidence on the relationship between FA composition and low-grade inflammation in children is however limited to few studies with inconsistent findings (18, 19). Despite their valuable contributions, there is still insufficient evidence to draw definitive conclusions regarding FA in the modulation of inflammatory processes in children.

Therefore, the aim of this exploratory study was to analyse the associations between different FA measured in serum glycerophospholipids assumed to play relevant roles in inflammatory processes, with common markers of inflammation in 10-year-old children, namely high-sensitivity CRP (hs-CRP) and IL-6.

Methods

Data was obtained from the 10-year follow-up assessment of the ongoing LISAplus (Influence of *L*ifestyle-Related Factors on the *I*mmune *S*ystem and the Development of *A*llergies in Childhood *plus* the Influence of Traffic Emissions and Genetics) birth cohort study (20). The study design, recruitment and exclusion criteria have been described previously (20). In brief, between the end of 1997 and beginning of 1999, healthy full-term new-borns were recruited from obstetric clinics within four German cities. Information was collected using identical questionnaires and at physical

examinations. During the 10-year follow-up physical examination, venous blood samples were collected in serum separator tubes and centrifuged at 3000U/min for 10 minutes at 4°C. Serum was aliquoted and stored at -80°C for later analysis of fatty acids and inflammatory markers.
Approval by the local ethics committee (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participants' families were obtained.

Inflammatory markers: hs-CRP and IL-6

Serum concentrations of hs-CRP were measured using the Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitive assay; and concentrations of IL-6 by flow cytometry using a cytometric bead array (BD CBA Human Soluble Flex Set system; Becton Dickinson, Heidelberg, Germany), according to manufacturer instructions. Measured hs-CRP and IL-6 concentrations were highly skewed, with many observations below the detection limit. Given this non-normal distribution, data categorisation was required for analyses. Both inflammatory markers were hence categorized into 3 levels separately for girls and boys, considering all children with available measurements (n=1083, see Figure 1). Categories of hs-CRP were defined similarly to those published in the recent study on fatty acids and hs-CRP in European children (19) to ease comparison: (I) hs-CRP < 0.02mg/dl; (II) hs-CRP ≥ 0.02mg/dl and < 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (< 0.11mg/dl in girls; < 0.09mg/dl in boys); and (III) hs-CRP ≥ 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (≥ 0.11mg/dl in girls; ≥ 0.09mg/dl in boys). IL-6 was categorized with reference to the minimal detectable concentration (1.5pg/ml): (I) IL-6 ≤ 1.5pg/ml; (II) IL-6 > 1.5pg/ml and < 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (< 4.26pg/ml in girls; < 3.93pg/ml in boys); and (III) IL-6 ≥ 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (≥ 4.26pg/ml in girls; ≥ 3.93pg/ml in boys).

Fatty acid status

Serum glycerophospholipid FA concentrations were measured by a high-throughput method developed with plasma samples, and successfully applied previously for analyses of FA in serum from cord blood and blood samples collected at ages 2, 6 and 10 years in the LISAplus study (21-23). Full details on sample preparation and analysis have been described elsewhere (24). The following FA were analysed in the present study: palmitic acid (PA), oleic acid (OA), LA, γ-linoleic acid (GLA), dihomo-γ-linoleic acid (DHGLA), AA, α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), total SFA, total monounsaturated FA (MUFA) and total PUFA. Additionally, we included FA groups and ratios which have previously been proposed to play a role in inflammation. Highly unsaturated n-6 and n-3 FA (HUFA: ≥20 carbons and ≥3 double bonds) are known precursors of chemical messengers

involved in inflammation (25). Since n-6 and n-3 PUFA compete for the same desaturase enzymes (Δ5 and Δ6 desaturase) for long-chain PUFA synthesis, it has been discussed that a low ratio of n-6 to n-3 PUFA (n6/n3) could reduce inflammation by favouring conversion of dietary n-3 PUFA to EPA, and limiting AA availability (26). On the other hand, this has not been confirmed, and accumulating evidence indicates no role for n6/n3 in modulating inflammation (27, 28). EPA and DHA have been reported to inhibit AA metabolism and to form potent anti-inflammatory lipid mediators (9, 10); their respective ratios (EPA/AA and DHA/AA) have also proven relevant in the reduction of inflammatory cytokine release (29, 30). Finally, greater desaturase activity has been suggested to promote inflammation by increasing availability of eicosanoid precursors (31). Since practical reasons prevent the measurement of desaturase activity directly, product-to-precursor ratios, such as AA/LA or AA/DHGLA, can be used as surrogate measures to estimate overall and Δ5 desaturase activity, respectively (32). Full names of abbreviations of exposure variables and the FAs encompassed under umbrella terms (HUFA, n-6/n-3, SFA, MUFA and PUFA), are listed in supplementary Table S1. For use in our main analyses, proportions of each FA relative to total FA (%FA) were calculated. In an additional sensitivity analysis we analysed FA concentrations.

Adjustment variables

Variables used for adjusting statistical models included sex, recruitment region (Munich; Wesel; Bad Honnef; Leipzig), exact age at physical examination (years), maternal education level (highest level achieved: low: <10thgrade; medium: 10thgrade; high: >10thgrade), BMI (in kg/m^2, calculated from height and weight measurements obtained at the physical examination), screen time (low: ≤1h in winter and ≤2h in summer; medium: >1h in winter or >2h in summer; high: >1h in winter and >2h in summer), onset of puberty (yes: estradiol >18.4pmol/L in females; testosterone >0.09nmol/L in males), and whether the child was ever breastfed (yes: ≥1 month).

Statistical analysis

Differences in characteristics between girls and boys were tested by Student's t-test (means) or by Wilcoxon rank sum test (medians) for continues variables, and by Pearson's Chi-squared test for categorical variables. A two-sided α-level of 5% was considered significant. Associations of %FA with hs-CRP and IL-6 were assessed using multinomial logistic regression, given that the outcome variables hs-CRP and IL-6 were both categorised into 3 levels (ordinal logistic regression could not be applied, as the assumption of proportional odds was not satisfied). Results were presented as odds ratios with corresponding 95% confidence intervals [OR (95% CI)], with the lowest level (I) as the reference category. A basic model (M1) and a fully adjusted model (M2) were used, adjusting for: (M1) sex, region, age and maternal education level; and (M2) further adjusting for BMI, screen time, onset of puberty and whether the child was ever breastfed. Sensitivity analyses were run

stratified by sex. In order to avoid chance findings resulting from the large number of regression models, we corrected for multiple testing using Bonferroni correction: the alpha level was divided by twenty (the number of tests performed). This yielded a corrected two-sided α-level of 0.0025 (0.05/20 = 0.0025). For sex-stratified analyses, the p-value was further divided by two, accounting for the analysis at two levels (0.0025/2=0.00125). Finally, we reran our analyses using FA concentrations, including adjustment for total FA. To avoid problems of multicollinearity, we used FA residuals calculated by regressing individual FA concentrations on total FA. All analyses were conducted using R, version 3.3.0 (https://www.R-project.org/) (33), with code available upon request. Multinomial logistic regression was calculated using the multinom() function in package "nnet" (34).

Results

Complete information on FA, hs-CRP, IL-6 and adjustment variables was available for 958 participants (Figure 1). Subjects with hs-CRP values > 1mg/dl (35) or IL-6 values > 20pg/ml (36) were considered as outliers and excluded from the analysis (7 subjects with hs-CRP levels from 1.03-4.37mg/dl and 6 subjects with IL-6 levels from 32.9-4384.0pg/ml). Only participants with complete data for both exposure and outcome measurements were included (n=1054). Participants were further excluded who were lacking data for adjustment variables (78 subjects), who reported an illness affecting diet (4 subjects), or presented outlying values in exposure measurements (14 subjects). The resulting sample size (n=958) was considered adequate for multinomial logistic regression analyses, based on reports from simulation studies (37, 38).

Basic characteristics of the study population are displayed in Table 1. About half of the study participants were from Munich with a high maternal education level. Almost all children were breastfed and most reported low screen-time. Girls had significantly lower screen-time than boys and about two thirds of them had entered onset of puberty, compared to just 27% of boys. Girls also had higher hs-CRP and IL-6 levels than boys.

Results from the multinomial logistic regression models (M1: basic model and M2: fully adjusted model) are presented in Table 2. Associations observed in the fully adjusted model (M2) are displayed in Figure 2. FA exposures associated with significantly higher low-grade inflammation, as indicated by higher hs-CRP or IL-6 levels, included: PA [IL-6 III vs. I: OR=1.78 (95% CI=1.30;2.43)], AA [hs-CRP II vs. I: 1.18 (1.07;1.31)], n-6 HUFA [hs-CRP II vs. I: 1.16 (1.06;1.27)], ratio AA/LA [hs-CRP II vs. I: 1.38 (1.16;1.62)], and total SFA [IL-6 III vs. I: 2.36 (1.77;3.15)]. FA exposures associated with reduced levels of inflammatory markers included: LA [hs-CRP II vs. I: 0.90 (0.84;0.96); IL-6 III vs. I: 0.79 (0.69;0.90)], and total PUFA [IL-6 III vs. I: 0.67 (0.57;0.78)]. Sex-stratified sensitivity analyses results are displayed in Supplementary Tables S2a and S2b, for males and females respectively. As in the total population, both sexes presented a

positive association of SFA, and an inverse association of total PUFA with IL-6. Males additionally presented a significant direct association between AA/LA and hs-CRP. Results from the sensitivity analysis using FA concentrations did not differ from those obtained using %FA (data not shown).

Discussion

This exploratory study assessed the associations between FA measured in serum glycerophospholipids and common markers of inflammation (hs-CRP and IL-6) in 10-year-old children. Amongst our main findings, PA, total SFA, AA, n-6 HUFA, and AA/LA were associated with increased low-grade inflammation, as indicated by at least one inflammatory marker. On the other hand, LA and total PUFA were inversely associated with low-grade inflammation.

Few studies exist which describe fatty acid status and markers of inflammation in children, and these differ in terms of study design, methods, location, and age of subjects. In order to aid comparison, an overview of existing studies in both adults and children is presented in supplementary Table S3. In line with the present findings, González-Gil et al. reported increased hs-CRP concentrations with higher AA, n-6 HUFA, and AA/LA in a large sample of European children (19). Given that n-6 HUFA, particularly AA, are known sources of pro-inflammatory eicosanoids, and that these may increase with greater desaturase activity (estimated by product-to-precursor ratio AA/LA) (31, 32), the observed associations with increased levels of inflammation makers are not unexpected. Interestingly, in our study none of the above-mentioned FA exposures presented an association with IL-6, which is the primary CRP regulator (39, 40). This might indicate the involvement of other circulating cytokines. Indeed, interleukin-1β is known to strongly up-regulate IL-6-induced CRP production (41, 42). On the other hand, it is possible that differences in hs-CRP were more readily detected given the high sensitivity and stability of this marker, often deeming it first choice for the assessment of low-grade inflammation (2, 5). Although the associations observed with hs-CRP did not indicate a dose-response relationship in the fully adjusted model, the basic model indicated significant associations for n-6 HUFA and AA/LA with both hs-CRP levels II and III relative to level I. By including adjustment variables one-by-one in the model, it was evident that BMI was the strongest determinant of hs-CRP, as has been observed previously (19, 43).

Following our sex-stratified analysis the association between AA/LA and hs-CRP remained significant only in males. This is in contrast to findings by González-Gil et al., who reported this association only in females (19). Previous authors (17) have attributed sex differences to the presence of oestrogen, which enhances the elongation of fatty acids to longer-chain derivatives, such as EPA and DHA (44, 45), which can be anti-inflammatory (46). Children in the European study were aged 2-9 years (19), whereas our study was carried out in 10-year-olds, among which

about two thirds of the females had entered onset of puberty. The discrepancy between findings could hence be related to age and in turn hormonal differences.

An association between SFA and low-grade inflammation, as indicated by the present study results, has been previously observed in adults (16, 47). In particular, PA has been shown to induce the expression of IL-6 through activation of nuclear factor-kB (48, 49), a protein complex involved in cytokine production. Klein Platat et al. reported a positive association between SFA in plasma phospholipids and IL-6 in overweight adolescents (18). Like us, the authors observed no association with hs-CRP. Additionally, neither palmitic acid nor total SFA showed significant associations with hs-CRP in the recent European study population (19). To our knowledge, the present findings are the first to indicate a role of SFA, namely PA, in triggering pro-inflammatory responses in otherwise healthy children, irrespective of BMI.

We observed inverse associations with low-grade inflammation for LA, the main dietary n-6 PUFA, and for total PUFA. Although the anti-inflammatory role of n-3 PUFA has been more extensively investigated, a number of studies in both adults and children have reported reduced concentrations of inflammatory markers with higher total n-6 PUFA levels (14, 16, 17), and specifically with LA (15, 19, 50). There is some evidence suggesting that the presence of double bonds, regardless of the position of the bond (n-3 or n-6), may play a relevant role in reducing inflammation (51). In contrast to these findings, it has been argued that high LA consumption could induce inflammation through its endogenous conversion to AA, which can act as substrate for synthesis of proinflammatory molecules (26, 52). However, little evidence currently supports a proinflammatory role of LA in humans (28, 53). It has been shown that AA production from LA is tightly regulated (54), and tissue AA content is barely altered by LA intake (55), even in the context of a high n6/n3 ratio (56). Furthermore, LA and AA are known to produce both proinflammatory and resolving metabolites and could therefore contribute to anti-inflammatory responses as well (9). Our results do not support the theoretical role of n6/n3 in modulating inflammation, proposed on the basis that LA can diminish the conversion of ALA to EPA (26). Although true to some extent (57), the conversion of dietary n-3 PUFA to long chain derivatives in humans is low (58), and small changes are likely not highly relevant in terms of the overall inflammatory process. Furthermore, achieving a lower n6/n3 ratio by limiting intakes of n-6 PUFA has not consistently resulted in improved CVD risk (59). In this context, and in line with existing literature, our findings suggest that elevated LA in serum phospholipids, within the ranges observed in the current study, is not detrimental in terms of inflammatory processes in children; rather, both LA and total PUFA seem to promote an anti-inflammatory response (60).

Strengths and limitations

The present study adds to the limited literature on associations of FA composition with markers of inflammation in children, and benefits from a large, homogenous study population. A main strength in our study is the analysis of FA status, reflecting individual dietary FA intake and endogenous metabolism. FA measured in serum phospholipids have been shown to reflect FA intake over a period of weeks to months (60), making them acceptable markers of habitual FA intake. Among the different lipid fractions, phospholipids contain the highest percentages of DHA and AA, hence allowing a more precise analysis of FA composition (61). Although sample alterations during handling and storage cannot be completely excluded, serum samples obtained in our study were frozen directly after sampling and stored at –80°C until analysis. Furthermore, time until centrifugation was short and haemolysis was minimal, thereby limiting the probability of exchange of phospholipids between cells and serum.

Our findings are based on the analysis of percentage of FA relative to total FA (%FA). Despite its use in most studies, this method is limited by the inability to account for actual FA concentrations (62). However, additional analyses in our study sample indicated similar results for both methods. Given the exploratory nature of the current study, a large number of FA exposures were assessed. The multiple tests and possible correlation between FA exposures, increases the probability of occurrence of chance findings. We therefore applied a rather conservative approach to correct for multiple testing. Observed significant associations were in line with existing literature and the directions of associations for both inflammatory outcomes were generally consistent, suggesting our findings are unlikely to have arisen by chance. Furthermore, we are aware that the AA/LA ratio assessed in our study represents an indirect measurement of desaturase activity. However, it has been shown that single-nucleotide polymorphisms and haplotypes of the genes coding for desaturase enzymes are associated with relative proportions of serum phospholipid FA (63), and the use of the AA/LA ratio as a marker of overall desaturase activity is widespread (31, 32, 64).

As often occurring in cohort studies, children of lower social classes were underrepresented in the present analyses. Although we adjusted for parental education in our analysis, our findings may not be representative of the study area. Additional assessment of other cytokine measurements, which unfortunately were not available from the LISAplus cohort, would have been useful to strengthen our conclusions and better understand the possible inflammatory pathways involved. Finally, it must be kept in mind that our findings are based on cross-sectional analyses, and hence the observed associations between serum FA and inflammatory markers do not necessarily infer causality.

Conclusion

The results of this exploratory study suggest that higher SFA and n-6 HUFA, namely PA and AA, are associated with increased levels of low-grade inflammation in children, as indicated by the inflammatory markers IL-6 and hs-CRP. In contrast, the major dietary n-6 PUFA and total PUFA are associated with reduced levels of low-grade inflammation. Elevated desaturase activity, estimated by the ratio AA/LA, may be associated with increased inflammation, particularly in boys. Sex might play a relevant role in the underlying inflammatory mechanisms in children, and should be kept in mind for future studies.

Authorship

CH, JH and MS were involved in the conception and design of the study; BK, HD, IL, AvB and JH in the data acquisition; CH, MS and CF in the statistical analyses; CH, MS, HD and JH in the interpretation; CH drafted the manuscript; all authors revised it critically for important intellectual content, and approved the final version to be published.

Acknowledgements

The authors thank all the families for their participation in the LISAplus studies. Furthermore, we thank all members of the LISAplus Study Groups for their excellent work.

The LISAplus Study group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Schnappinger M, Brüske I, Sußmann M, Lohr W, Schulz H, Zeller C, Standl M); Department of Pediatrics, Municipal Hospital "St. Georg", Leipzig (Borte M, Gnodtke E); Marien Hospital Wesel, Department of Pediatrics, Wesel (von Berg A, Berdel D, Stiers G, Maas B); Pediatric Practice, Bad Honnef (Schaaf B); Helmholtz Centre of Environmental Research – UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (Lehmann I, Bauer M, Röder S, Schilde M, Nowak M, Herberth G , Müller J, Hain A); Technical University Munich, Department of Pediatrics, Munich (Hoffmann U, Paschke M, Marra S); Clinical Research Group Molecular Dermatology, Department of Dermatology and Allergy, Technische Universität München (TUM), Munich (Ollert M).

The LISAplus study was mainly supported by grants from the Federal Ministry for Education, Science, Research and Technology and in addition from Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research - UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Pediatric Practice, Bad Honnef for the first 2 years. The 4 year, 6 year, and 10 year follow-up examinations of the LISAplus study were covered from the respective budgets of the involved partners (Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental

Research - UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Pediatric Practice, Bad Honnef, IUF – Leibniz-Research Institute for Environmental Medicine at the University of Düsseldorf) and in addition by a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). The work of BK is financially supported in part by the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG – no.322605).

Conflict of interest

The authors declare no conflict of interest.

Supplementary information

Supplementary Information accompanies the paper on the EJCN website (http://www.nature.com/ejcn)

References

1. Libby P. Inflammation in Atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012;32(9): 2045-51.

2. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. *Am J Med* 2004;116 Suppl 6A:9s-16s.

3. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature reviews Immunology* 2006;6(10):772-83.

4. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999;282(22):2131-5.

5. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003;111(12):1805-12.

6. Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arterioscler Thromb Vasc Biol* 2002;22(8):1323-8.

7. Kapiotis S, Holzer G, Schaller G, Haumer M, Widhalm H, Weghuber D, et al. A Proinflammatory State Is Detectable in Obese Children and Is Accompanied by Functional and Morphological Vascular Changes. *Arterioscler Thromb Vasc Biol* 2006;26(11):2541-6.

8. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, et al. Inflammatory disease processes and interactions with nutrition. *Br J Nutr* 2009;101 Suppl 1:S1-45.

9. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie* 2009;91(6):791-5.

10. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol* 2013;75(3):645-62.

11. Fekete K, Marosvölgyi T, Jakobik V, Decsi T. Methods of assessment of n–3 long-chain polyunsaturated fatty acid status in humans: a systematic review. *Am J Clin Nutr* 2009;89(6):2070S-84S.

12. Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. *Eur J Clin Nutr* 2000;54(5):367-72.

13. Nakamura MT, Nara TY. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annu Rev Nutr* 2004;24:345-76.

14. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, et al. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* 2006;91(2):439-46.

15. Steffen BT, Steffen LM, Tracy R, Siscovick D, Hanson NQ, Nettleton J, et al. Obesity modifies the association between plasma phospholipid polyunsaturated fatty acids and markers of inflammation: the Multi-Ethnic Study of Atherosclerosis. *Int J Obes (Lond)* 2012;36(6):797-804.

16. Kaikkonen JE, Kresanov P, Ahotupa M, Jula A, Mikkila V, Viikari JS, et al. High serum n6 fatty acid proportion is associated with lowered LDL oxidation and inflammation: the Cardiovascular Risk in Young Finns Study. *Free Radic Res* 2014;48(4):420-6.

17. Muka T, Kiefte-de Jong JC, Hofman A, Dehghan A, Rivadeneira F, Franco OH. Polyunsaturated fatty acids and serum C-reactive protein: the Rotterdam study. *Am J Epidemiol* 2015;181(11):846-56.

18. Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. *Am J Clin Nutr* 2005;82(6):1178-84.

19. González-Gil EM, Santabárbara J, Siani A, Ahrens W, Sioen I, Eiben G, et al. Whole-blood fatty acids and inflammation in European children: the IDEFICS Study. *Eur J Clin Nutr* 2016;70(7):819-23.

20. Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I, Fahlbusch B, et al. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J* 2002;20(3):617-23.

21. Glaser C, Rzehak P, Demmelmair H, Klopp N, Heinrich J, Koletzko B, et al. Influence of FADS Polymorphisms on Tracking of Serum Glycerophospholipid Fatty Acid Concentrations and Percentage Composition in Children. *PLoS ONE* 2011;6(7):e21933.

22. Glaser C, Demmelmair H, Sausenthaler S, Herbarth O, Heinrich J, Koletzko B. Fatty acid composition of serum glycerophospholipids in children. *J Pediatr* 2010;157(5):826-31.e1.

23. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. *Br J Nutr* 2014;111(11):2024-31.

24. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. *J Lipid Res* 2010;51(1):216-21.

25. Lands B. Consequences of essential fatty acids. *Nutrients* 2012;4(9):1338-57.

26. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental biology and medicine (Maywood)*. 2008;233(6):674-88.

27. Marventano S, Kolacz P, Castellano S, Galvano F, Buscemi S, Mistretta A, et al. A review of recent evidence in human studies of n-3 and n-6 PUFA intake on cardiovascular disease, cancer, and depressive disorders: does the ratio really matter? *Int J Food Sci Nutr* 2015;66(6):611-22.

28. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al. Omega-6 Fatty Acids and Risk for Cardiovascular Disease. A Science Advisory From the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* 2009;119(6):902-7.

29. Ohnishi H, Saito Y. Eicosapentaenoic acid (EPA) reduces cardiovascular events: relationship with the EPA/arachidonic acid ratio. *J Atheroscler Thromb* 2013;20(12):861-77.

30. Cotogni P, Trombetta A, Muzio G, Maggiora M, Canuto RA. The Omega-3 Fatty Acid Docosahexaenoic Acid Modulates Inflammatory Mediator Release in Human Alveolar Cells Exposed to Bronchoalveolar Lavage Fluid of ARDS Patients. *BioMed Research International* 2015;2015:11.

31. Martinelli N, Girelli D, Malerba G, Guarini P, Illig T, Trabetti E, et al. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. *Am J Clin Nutr* 2008;88(4):941-9.

32. Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of Fatty acids and insulin action. *Ann N Y Acad Sci* 2002;967:183-95.

33. R Core Team. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria 2016.

34. Venables WN, Ripley BD. Modern Applied Statistics with S. Fourth ed: Springer, New York; 2002.

35. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta* 1981;117(1):13-23.

36. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine Levels in the Serum of Healthy Subjects. *Mediators of Inflammation* 2013;2013:6.

37. Vittinghoff E, McCulloch CE. Relaxing the Rule of Ten Events per Variable in Logistic and Cox Regression. *Am J Epidemiol* 2007;165(6):710-8.

38. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* 1996;49(12):1373-9.

39. Black S, Kushner I, Samols D. C-reactive Protein. *J Biol Chem* 2004;279(47):48487-90.

40. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989;242(2):237-9.

41. Bode JG, Albrecht U, Häussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins–regulation by IL-6-and IL-1-type cytokines involving STAT3 and its crosstalk with NF-κB-dependent signaling. *Eur J Cell Biol* 2012;91(6):496-505.

42. Ganter U, Arcone R, Toniatti C, Morrone G, Ciliberto G. Dual control of C-reactive protein gene expression by interleukin-1 and interleukin-6. *EMBO J* 1989;8(12):3773-9.

43. Ford ES. C-reactive protein concentration and cardiovascular disease risk factors in children: findings from the National Health and Nutrition Examination Survey 1999-2000. *Circulation* 2003;108(9):1053-8.

44. Decsi T, Kennedy K. Sex-specific differences in essential fatty acid metabolism. *Am J Clin Nutr* 2011;94(6 Suppl):1914S-9S.

45. Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* 2002;88(4):411-20.

46. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 2000;71(1 Suppl):343s-8s.

47. King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. *Am J Cardiol* 2003;92(11):1335-9.

48. Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Haring HU, et al. Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor-kappaB. *J Biol Chem* 2004;279(23):23942-52.

49. Ajuwon KM, Spurlock ME. Palmitate Activates the NF-κB Transcription Factor and Induces IL-6 and TNFα Expression in 3T3-L1 Adipocytes. *J Nutr* 2005;135(8):1841-6.

50. Enzenbach C, Kroger J, Zietemann V, Jansen EH, Fritsche A, Doring F, et al. Erythrocyte membrane phospholipid polyunsaturated fatty acids are related to plasma C-reactive protein and adiponectin in middle-aged German women and men. *European journal of nutrition* 2011;50(8):625-36.

51. De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. *Am J Clin Nutr* 2000;71(1 Suppl):213s-23s.

52. Lands B. Historical perspectives on the impact of n-3 and n-6 nutrients on health. *Prog Lipid Res* 2014;55:17-29.

53. Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. *Journal of the Academy of Nutrition and Dietetics* 2012;112(7):1029-41, 41.e1-15.

54. Mohrhauer H, Holman RT. THE EFFECT OF DOSE LEVEL OF ESSENTIAL FATTY ACIDS UPON FATTY ACID COMPOSITION OF THE RAT LIVER. *J Lipid Res* 1963;4:151-9.

55. Rett BS, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. *Nutrition & Metabolism* 2011;8:36.

56. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 2012;95(5):1003-12.

57. Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J Nutr* 2007;137(4):945-52.

58. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Current opinion in clinical nutrition and metabolic care* 2002;5(2):127-32.

59. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO food and nutrition paper. 2010;91:1-166.

60. Riboli E, Ronnholm H, Saracci R. Biological markers of diet. *Cancer Surv* 1987;6(4):685-718.

61. Rise P, Eligini S, Ghezzi S, Colli S, Galli C. Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. *Prostaglandins Leukot Essent Fatty Acids* 2007;76(6):363-9.

62. Sergeant S, Ruczinski I, Ivester P, Lee TC, Morgan TM, Nicklas BJ, et al. Impact of methods used to express levels of circulating fatty acids on the degree and direction of associations with blood lipids in humans. *Br J Nutr* 2016;115(2):251-61.

63. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* 2006;15(11):1745-56.

64. Steffen LM, Vessby B, Jacobs DR, Jr., Steinberger J, Moran A, Hong CP, et al. Serum phospholipid and cholesteryl ester fatty acids and estimated desaturase activities are related to overweight and cardiovascular risk factors in adolescents. *International Journal of Obesity* 2008;32(8):1297-304.

Figure Legends

Fig. 1 Study participants

[a]hs-CRP > 1mg/dl or IL-6 > 20pg/ml. [b]Adjustment variables: sex, region, age, maternal education level, BMI, screen time, onset of puberty and whether the child was ever breastfed. [c]Illness affecting diet: e.g. diabetes, anorexia, coeliac disease, cancer.

Fig. 2 Odds ratio (OR) and 95% confidence interval (95% CI) of the associations between fatty acids and categories of hs-CRP and IL-6 (reference: category I). Multinomial logistic regression model (M2) adjusted for sex, region, age, maternal education level, BMI, sedentary behaviour and whether the child was ever breastfed. Significant associations are marked with *.

Table1. Descriptive characteristics of study participants

	All N=958	**Males** N=520	**Females** N=438	**P-value[g]**
	n (%)	**n (%)**	**n (%)**	
Region				
Munich	487 (50.8)	269 (51.7)	218 (49.8)	0.944
Leipzig	245 (25.6)	130 (25.0)	115 (26.3)	
Bad Honnef	135 (14.1)	72 (13.8)	63 (14.4)	
Wesel	91 (9.5)	49 (9.4)	42 (9.6)	
Mother's education level[a]				
Low	68 (7.1)	39 (7.5)	29 (6.6)	0.455
Medium	339 (35.4)	175 (33.7)	164 (37.4)	
High	551 (57.5)	306 (58.8)	245 (55.9)	
Breast feeding (yes)[b]	927 (96.8)	502 (96.5)	425 (97.0)	0.805
Screen-timc[c]				
Low	642 (67.0)	327 (62.9)	315 (71.9)	**0.011**
Medium	213 (22.2)	128 (24.6)	85 (19.4)	
High	103 (10.8)	65 (12.5)	38 (8.7)	
Onset of Puberty (yes)[d]	429 (44.8)	139 (26.7)	290 (66.2)	**<0.001**
hs-CRP groups[e]				
CRP I	416 (43.4)	265 (51.0)	151 (34.5)	**<0.001**
CRP II	412 (43.0)	198 (38.1)	214 (48.9)	
CRP III	130 (13.6)	57 (11.0)	73 (16.7)	
IL-6 groups[f]				
IL-6 I	751 (78.4)	425 (81.7)	326 (74.4)	**0.019**
IL-6 II	161 (16.8)	72 (13.8)	89 (20.3)	
IL-6 III	46 (4.8)	23 (4.4)	23 (5.3)	
	mean (sd) or median (25th;75th perc.)	**mean (sd) or median (25th;75th perc.)**	**mean (sd) or median (25th;75th perc.)**	
Age (years)	10.2 (10.1; 10.3)	10.2 (10.1; 10.3)	10.2 (10.1; 10.3)	0.900
BMI (kg/m^2)	16.6 (15.5; 18.3)	16.6 (15.6; 18.3)	16.6 (15.4; 18.3)	0.756
hs-CRP (mg/dl)	0.02 (0.01; 0.05)	0.02 (0.01; 0.04)	0.03 (0.02; 0.07)	**<0.001**
IL-6 (pg/ml)	1.5 (1.5; 1.5)	1.5 (1.5; 1.5)	1.5 (1.5; 1.52)	**0.006**
PA (% of total FA)	26.8 (1.1)	26.7 (1.1)	26.9 (1.1)	**0.004**
OA (% of total FA)	12.1 (11.3; 13.1)	12.1 (11.3; 13.1)	12.2 (11.3; 13.1)	0.530
LA (% of total FA)	23.3 (2.3)	23.1 (2.4)	23.4 (2.2)	0.080
GLA (% of total FA)	0.12 (0.09; 0.16)	0.12 (0.1; 0.16)	0.12 (0.09; 0.15)	**0.020**
DHGLA (% of total FA)	3.24 (0.59)	3.29 (0.59)	3.19 (0.6)	**0.007**
AA (% of total FA)	10 (1.6)	10.2 (1.6)	9.83 (1.53)	**0.002**
ALA (% of total FA)	0.24 (0.2; 0.31)	0.25 (0.2; 0.32)	0.24 (0.19; 0.3)	**0.044**
EPA (% of total FA)	0.6 (0.49; 0.75)	0.62 (0.51; 0.77)	0.59 (0.47; 0.72)	**0.008**
DPA (% of total FA)	0.93 (0.8; 1.05)	0.95 (0.81; 1.08)	0.89 (0.78; 1.01)	**<0.001**
DHA (% of total FA)	2.78 (2.29; 3.33)	2.78 (2.33; 3.31)	2.77 (2.28; 3.35)	0.804
n-3 HUFA (% of total FA)	4.37 (3.83; 5.1)	4.42 (3.86; 5.08)	4.32 (3.78; 5.1)	0.162
n-6 HUFA (% of total FA)	14.1 (1.9)	14.3 (1.9)	13.8 (1.8)	**<0.001**
DHA/AA	0.27 (0.24; 0.32)	0.27 (0.24; 0.32)	0.28 (0.24; 0.33)	0.120
EPA/AA	0.06 (0.05; 0.08)	0.06 (0.05; 0.08)	0.06 (0.05; 0.08)	0.359
AA/LA	0.42 (0.37; 0.5)	0.43 (0.37; 0.51)	0.42 (0.37; 0.48)	**0.012**
AA/DHGLA	3.11 (2.65; 3.63)	3.12 (2.68; 3.63)	3.11 (2.63; 3.61)	0.754
n6/n3	8.2 (1.76)	8.13 (1.72)	8.29 (1.8)	0.171
SFA (% of total FA)	42.2 (1.2)	42.1 (1.3)	42.4 (1.1)	**0.003**
MUFA (% of total FA)	14.4 (13.5; 15.3)	14.3 (13.5; 15.3)	14.5 (13.6; 15.4)	0.183
PUFA (% of total FA)	42.8 (2.2)	43 (2.2)	42.6 (2.1)	**0.017**

Values are presented as counts (%) for categorical variables, mean (standard deviation) for normally distributed continuous variables, and median (25th;75th percentile) for non-normally distributed continuous variables. [a]Highest level achieved (low: <10th grade; medium: 10th grade; high: >10th grade). [b]Whether the child was ever breastfed (yes: ≥1 month). [c]Self-reported h/day spent on screen activities (Low: ≤1h in winter and ≤2h in summer; Medium: >1h in winter or >2h in summer; High: >1h in winter and >2h in summer). [d]Females: estradiol >18.4pmol/L; Males: testosterone >0.09nmol/L. [e](I) hs-CRP < 0.02mg/dl; (II) hs-CRP ≥ 0.02mg/dl and < 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (< 0.11mg/dl in girls; < 0.09mg/dl in boys); and (III) hs-CRP ≥ 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (≥ 0.11mg/dl in girls; ≥ 0.09mg/dl in boys). [f](I) IL-6 ≤ 1.5pg/ml; (II) IL-6 > 1.5pg/ml and < 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (< 4.26pg/ml in girls; < 3.93pg/ml in boys); and (III) IL-6 ≥ 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (≥ 4.26pg/ml in girls; ≥ 3.93pg/ml in boys). [g]Comparison between males and females: tested by Student's t-test (means) or by Wilcoxon rank sum test (medians) for continues variables, and by Pearson's Chi-squared Test for categorical variables. Significant p-values are marked in bold (p<0.05)

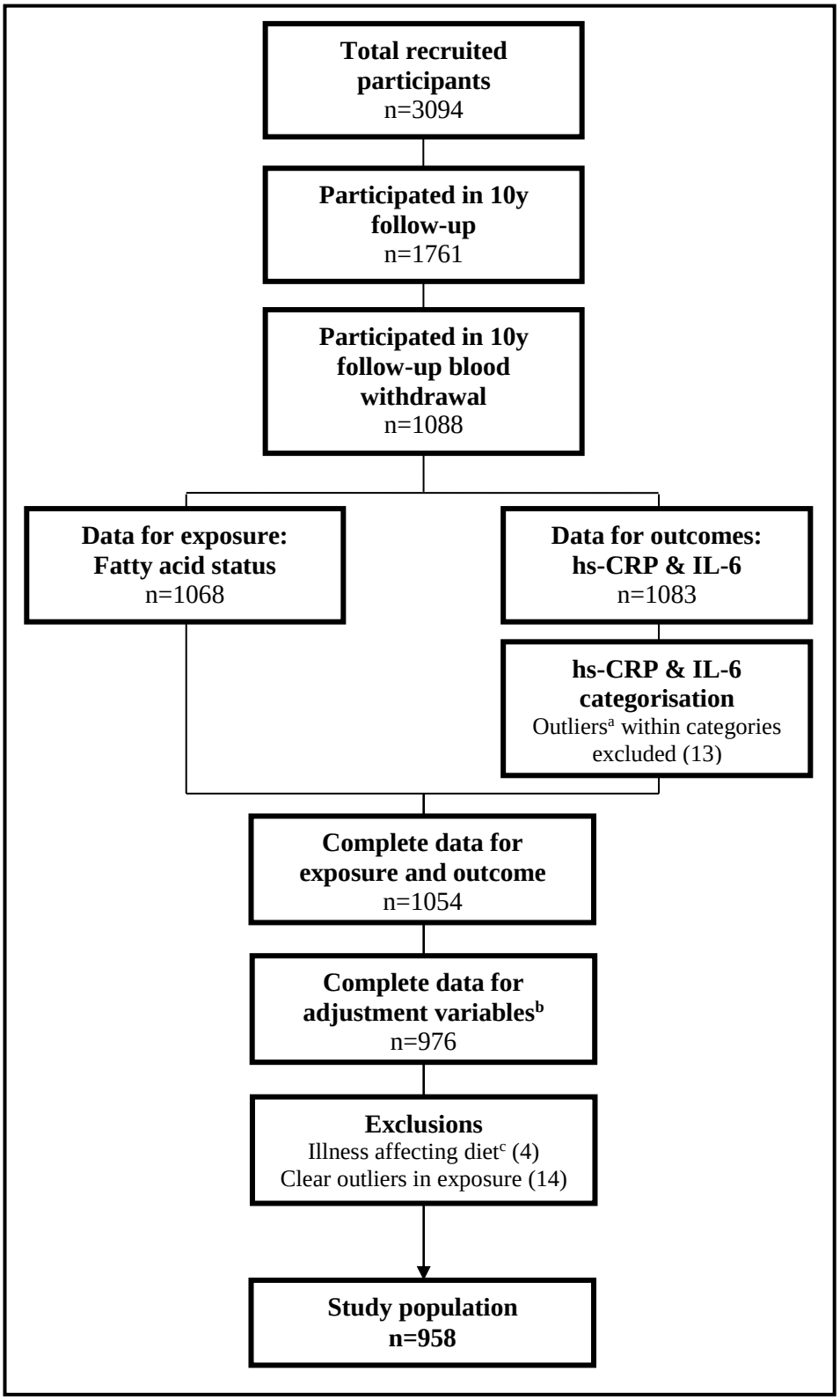
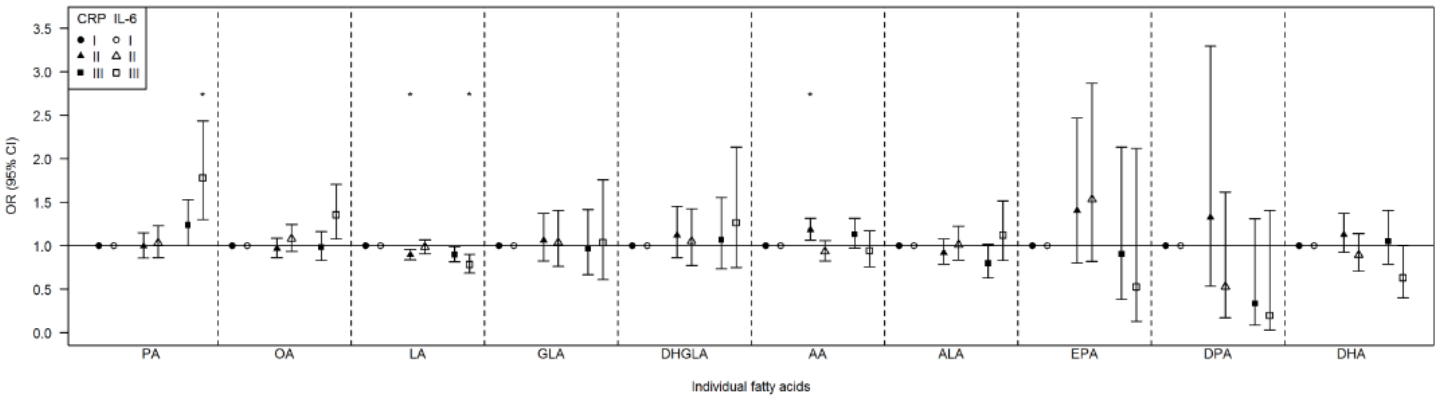

Total recruited participants
n=3094
Participated in 10y follow-up
n=1761
Participated in 10y follow-up blood withdrawal
n=1088
Data for exposure: Fatty acid status
n=1068
Data for outcomes: hs-CRP & IL-6
n=1083
hs-CRP & IL-6 categorisation
Outliers[a] within categories excluded (13)
Complete data for exposure and outcome
n=1054
Complete data for adjustment variables[b]
n=976
Exclusions
Illness affecting diet[c] (4)
Clear outliers in exposure (14)
Study population
n=958

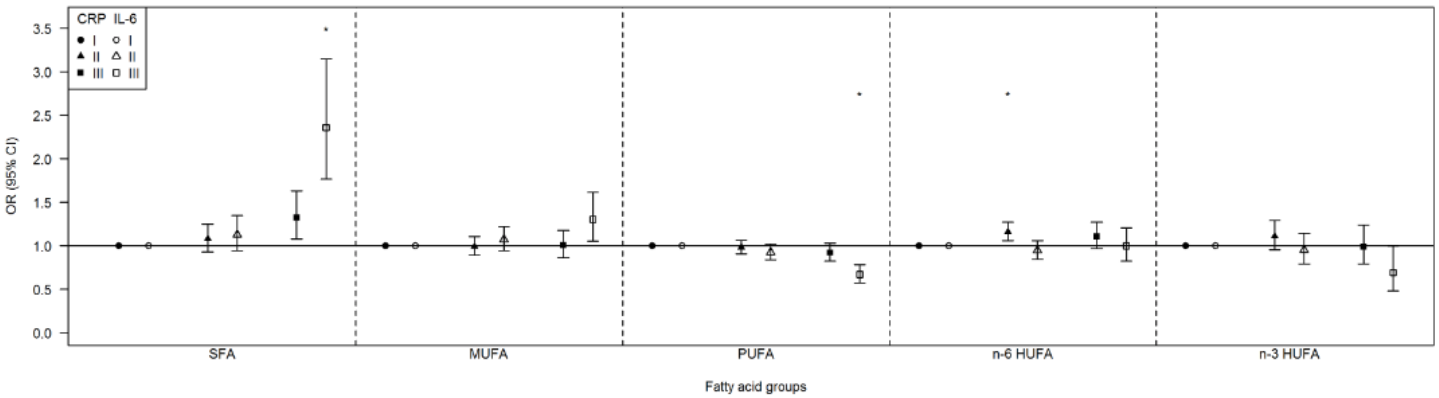
Figure 1

Table 2. Odds ratio and 95% confidence interval assessing the association of fatty acid exposures with hs-CRP and IL-6 categories. Multinomial logistic regression models adjusting for: (M1) sex, region, age and maternal education level; and (M2) further adjusting for BMI, screen-time, onset of puberty and whether the child was ever breastfed.

Fatty acids	**hs-CRP category II vs. I**			**hs-CRP category III vs. I**			**IL-6 category II vs. I**			**IL-6 category III vs. I**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
PA (%FA)												
M1	1.04	0.91;1.20	0.560	1.33	1.09;1.62	0.006	1.07	0.90;1.27	0.460	1.74	1.28;2.36	**<0.001**
M2	0.99	0.86;1.15	0.935	1.24	1.00;1.53	0.051	1.03	0.86;1.23	0.728	1.78	1.30;2.43	**<0.001**
OA (%FA)												
M1	0.92	0.83;1.03	0.148	0.91	0.78;1.07	0.257	1.05	0.92;1.21	0.481	1.38	1.10;1.73	0.006
M2	0.97	0.86;1.09	0.599	0.98	0.83;1.16	0.837	1.08	0.94;1.24	0.293	1.35	1.08;1.71	0.010
LA (%FA)												
M1	0.85	0.80;0.91	**<0.001**	0.82	0.75;0.90	**<0.001**	0.95	0.88;1.03	0.207	0.82	0.72;0.94	0.003
M2	0.90	0.84;0.96	**0.001**	0.90	0.82;0.99	0.030	0.99	0.91;1.07	0.709	0.79	0.69;0.90	**<0.001**
GLA[a] (%FA)												
M1	1.22	0.96;1.55	0.102	1.22	0.87;1.73	0.254	1.11	0.82;1.49	0.506	0.98	0.58;1.66	0.947
M2	1.06	0.82;1.37	0.642	0.97	0.67;1.41	0.875	1.04	0.77;1.41	0.811	1.04	0.61;1.76	0.899
DHGLA (%FA)												
M1	1.35	1.06;1.73	0.016	1.47	1.04;2.09	0.029	1.16	0.87;1.56	0.322	1.16	0.69;1.93	0.577
M2	1.12	0.87;1.45	0.386	1.07	0.73;1.55	0.731	1.05	0.78;1.43	0.747	1.27	0.75;2.13	0.376
AA (%FA)												
M1	1.24	1.12;1.37	**<0.001**	1.22	1.05;1.40	0.008	0.96	0.85;1.09	0.558	0.92	0.75;1.14	0.460
M2	1.18	1.07;1.31	**0.002**	1.13	0.97;1.32	0.111	0.93	0.83;1.06	0.281	0.94	0.76;1.17	0.605
ALA[a] (%FA)												
M1	0.95	0.82;1.10	0.488	0.83	0.66;1.05	0.121	1.02	0.85;1.23	0.834	1.12	0.83;1.52	0.447
M2	0.92	0.79;1.08	0.291	0.80	0.63;1.02	0.068	1.01	0.84;1.23	0.909	1.12	0.83;1.52	0.455
EPA (%FA)												
M1	2.12	1.23;3.65	0.007	1.77	0.81;3.88	0.151	1.80	0.99;3.29	0.055	0.45	0.11;1.78	0.253
M2	1.40	0.80;2.47	0.239	0.90	0.38;2.13	0.816	1.53	0.82;2.87	0.182	0.53	0.13;2.12	0.368
DPA (%FA)												
M1	1.53	0.65;3.62	0.333	0.51	0.14;1.84	0.302	0.60	0.20;1.78	0.354	0.19	0.03;1.32	0.093
M2	1.33	0.53;3.29	0.545	0.34	0.09;1.31	0.116	0.53	0.17;1.62	0.264	0.20	0.03;1.41	0.106
DHA (%FA)												
M1	1.15	0.95;1.38	0.154	1.11	0.85;1.46	0.437	0.93	0.73;1.18	0.536	0.62	0.39;0.98	0.039
M2	1.13	0.92;1.37	0.240	1.05	0.79;1.41	0.733	0.90	0.70;1.14	0.375	0.63	0.40;1.01	0.054
n-3 HUFA (%FA)												
M1	1.16	1.00;1.34	0.044	1.09	0.883;1.344	0.422	0.99	0.83;1.18	0.918	0.67	0.47;0.96	0.031
M2	1.11	0.95;1.29	0.180	0.99	0.79;1.241	0.930	0.95	0.79;1.15	0.609	0.69	0.48;1.00	0.047
n-6 HUFA (%FA)												
M1	1.23	1.13;1.35	**<0.001**	1.23	1.08;1.39	**0.002**	0.99	0.88;1.10	0.799	0.97	0.80;1.17	0.735
M2	1.16	1.06;1.27	**0.002**	1.11	0.97;1.27	0.134	0.95	0.85;1.06	0.344	1.00	0.82;1.21	0.978
DHA/AA[a]												
M1	0.90	0.74;1.09	0.281	0.89	0.67;1.18	0.430	0.95	0.75;1.21	0.688	0.63	0.39;1.03	0.067
M2	0.93	0.76;1.14	0.458	0.91	0.67;1.22	0.528	0.95	0.74;1.22	0.684	0.63	0.39;1.04	0.069
EPA/AA[a]												
M1	1.37	0.82;2.29	0.232	1.34	0.64;2.80	0.440	1.75	0.98;3.13	0.061	0.54	0.15;2.01	0.359
M2	1.01	0.59;1.73	0.973	0.83	0.37;1.84	0.638	1.56	0.85;2.84	0.149	0.61	0.16;2.25	0.455
AA/LA[a]												
M1	1.54	1.31;1.80	**<0.001**	1.58	1.27;1.98	**<0.001**	1.00	0.83;1.21	0.998	1.19	0.88;1.62	0.263
M2	1.38	1.16;1.62	**<0.001**	1.31	1.04;1.67	0.023	0.92	0.76;1.12	0.426	1.28	0.93;1.76	0.129
AA/DHGLA[a]												
M1	1.01	0.99;1.03	0.483	1.01	0.98;1.03	0.588	0.99	0.97;1.01	0.283	0.98	0.94;1.03	0.461
M2	1.01	0.99;1.03	0.204	1.02	0.99;1.05	0.195	0.99	0.97;1.01	0.391	0.98	0.94;1.03	0.404
n-6/n-3[a]												
M1	0.99	0.98;1.00	0.005	0.99	0.98;1.00	0.167	1.00	0.99;1.01	0.883	1.01	0.99;1.02	0.493
M2	0.99	0.98;1.00	0.039	1.00	0.99;1.01	0.710	1.00	0.99;1.01	0.722	1.00	0.99;1.02	0.639
SFA (% of total FA)												
M1	1.20	1.04;1.38	0.012	1.53	1.26;1.85	**<0.001**	1.19	1.00;1.42	0.046	2.17	1.65;2.86	**<0.001**
M2	1.08	0.93;1.25	0.312	1.32	1.08;1.63	0.008	1.13	0.94;1.35	0.187	2.36	1.77;3.15	**<0.001**
MUFA (% of total FA)												
M1	0.97	0.87;1.07	0.516	0.97	0.84;1.13	0.711	1.06	0.93;1.21	0.362	1.32	1.07;1.63	0.010
M2	0.99	0.89;1.10	0.875	1.01	0.86;1.18	0.931	1.07	0.94;1.22	0.291	1.31	1.05;1.62	0.015
PUFA (% of total FA)												
M1	0.97	0.90;1.04	0.369	0.90	0.81;1.00	0.040	0.92	0.83;1.01	0.067	0.68	0.58;0.79	**<0.001**
M2	0.98	0.91;1.06	0.657	0.92	0.82;1.03	0.146	0.93	0.84;1.02	0.118	0.67	0.57;0.78	**<0.001**

[a]OR for these variables refer to changes of 0.1 units. Significant associations are marked in bold (Bonferroni corrected p-value <0.0025)


CRP IL-6
I I
II II
III III
OR (95% CI)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PA
OA
LA
GLA
DHGLA
AA
ALA
EPA
DPA
DHA
Individual fatty acids


CRP IL-6
I I
II II
III III
OR (95% CI)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SFA
MUFA
PUFA
n-6 HUFA
n-3 HUFA
Fatty acid groups

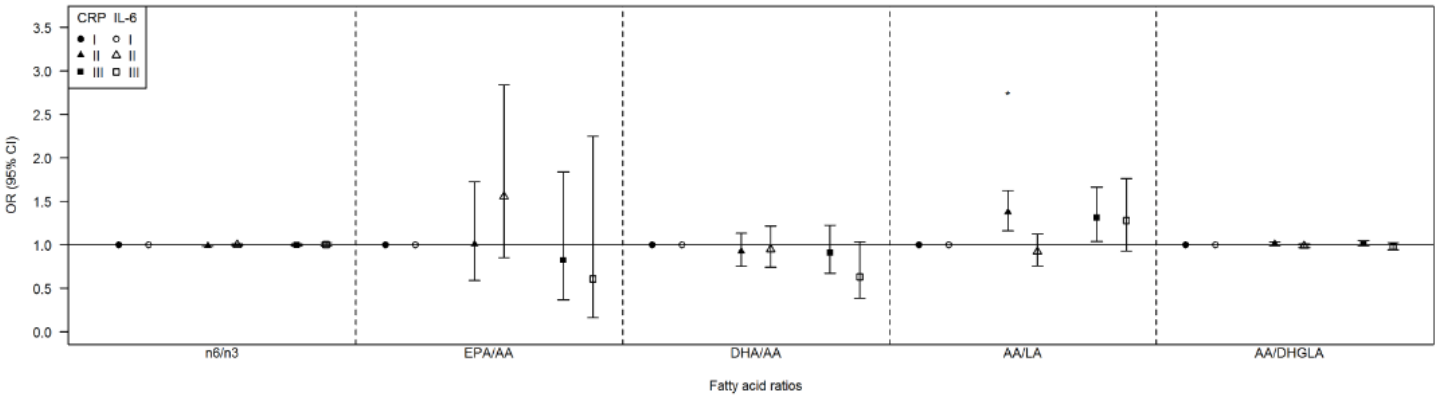

CRP IL-6
I I
II II
III III
OR (95% CI)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
n6/n3
EPA/AA
DHA/AA
AA/LA
AA/DHGLA
Fatty acid ratios

Figure 2

Paper 3: Supplementary Material

Table S1 Full names of abbreviations and lists of fatty acids encompassed under umbrella terms

Abbreviations	**Full names of fatty acids**
PA	Palmitic acid
OA	Oleic acid
LA	Linoleic acid
GLA	γ-linoleic acid
DHGLA	Dihomo- γ-linoleic acid
AA	Arachidonic acid
ALA	α-linoleic acid
EPA	Eicosapentaenoic acid
DPA	Docosapentaenoic acid
DHA	Docosahexaenoic acid
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
HUFA (≥20 carbons and ≥3 double bonds)	Highly-unsaturate fatty acid
	FAs encompassed under umbrella terms
n-3 HUFA	Eicosatrienoic acid +Eicosapentaenoic acid +Docosapentaenoic acid +Docosahexaenoic acid
n-6 HUFA	Dihomo-γ-linoleic acid +Arachidonic acid +Adrenic acid +Osbond acid
DHA/AA	Docosahexaenoic acid / Arachidonic acid
EPA/AA	Eicosapentaenoic acid / Arachidonic acid
AA/LA	Arachidonic acid / Linoleic acid
AA/DHGLA	Arachidonic acid / Dihomo-γ-linoleic acid
Ratio n6/n3	(Linoleic acid +γ-linoleic acid +Eicosadienoic acid +Dihomo-γ-linoleic acid +Arachidonic acid +Adrenic acid +Osbond acid) / (Alpha-linolenic acid +Eicosatrienoic acid +Eicosapentaenoic acid +Docosapentaenoic acid +Docosahexaenoic acid)
SFA	Mystiric acid +Palmitic acid +Margeric acid +Stearic acid
MUFA	Pentadecenoic acid +Palmitoleic acid +Oleic acid +Vaccenic +Gondoic
PUFA	Linoleic +γ-linoleic +Alpha-linolenic +Eicosadienoic +Mead +Dihomo-γ-linoleic +Arachidonic +Eicosatrienoic +Eicosapentaenoic +Adrenic +Osbond +Docosapentaenoic +Docosahexaenoic

Table S2a. Odds ratio and 95% confidence interval assessing the association of fatty acids with hs-CRP and IL-6 categories in **males**. Multinomial logistic regression models adjusting for: (M1) region, age and maternal education level; and (M2) further adjusting for BMI, screen-time, onset of puberty and whether the child was ever breastfed.

Fatty acids	**hs-CRP category II vs. I**			**hs-CRP category III vs. I**			**IL-6 category II vs. I**			**IL-6 category III vs. I**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
PA (% of total FA)												
M1	1.08	0.90;1.30	0.387	1.45	1.093;1.935	0.010	1.16	0.907;1.482	0.237	1.51	1.002;2.27	0.049
M2	1.03	0.85;1.25	0.737	1.28	0.941;1.734	0.117	1.10	0.856;1.416	0.454	1.54	0.998;2.373	0.051
OA (% of total FA)												
M1	0.84	0.72;0.98	0.023	0.93	0.742;1.173	0.553	1.00	0.817;1.231	0.981	1.46	1.068;2.003	0.018
M2	0.87	0.74;1.02	0.089	0.99	0.774;1.27	0.947	1.02	0.826;1.252	0.874	1.42	1.031;1.961	0.032
LA (% of total FA)												
M1	0.84	0.77;0.91	**<0.001**	0.79	0.7;0.901	**<0.001**	0.96	0.866;1.07	0.476	0.86	0.724;1.019	0.081
M2	0.88	0.8;0.96	0.005	0.89	0.773;1.017	0.085	1.00	0.89;1.113	0.936	0.82	0.684;0.985	0.034
GLA[a] (% of total FA)												
M1	1.26	0.94;1.69	0.117	1.14	0.721;1.801	0.577	1.28	0.885;1.855	0.190	1.09	0.552;2.139	0.811
M2	1.11	0.81;1.51	0.515	0.89	0.536;1.479	0.655	1.24	0.846;1.813	0.271	1.17	0.588;2.33	0.655
DHGLA (% of total FA)												
M1	1.29	0.93;1.79	0.126	1.42	0.858;2.346	0.173	1.25	0.813;1.909	0.313	1.07	0.508;2.238	0.866
M2	1.12	0.80;1.58	0.512	1.05	0.6;1.831	0.869	1.17	0.753;1.814	0.487	1.17	0.548;2.483	0.690
AA (% of total FA)												
M1	1.31	1.15;1.50	**<0.001**	1.26	1.032;1.546	0.024	0.94	0.791;1.123	0.507	0.87	0.649;1.165	0.348
M2	1.24	1.08;1.43	0.003	1.15	0.93;1.428	0.194	0.91	0.763;1.092	0.320	0.90	0.667;1.224	0.512
ALA[a] (% of total FA)												
M1	0.87	0.723;1.06	0.164	0.76	0.549;1.04	0.085	1.02	0.785;1.318	0.898	1.28	0.898;1.821	0.174
M2	0.86	0.702;1.05	0.131	0.74	0.528;1.032	0.076	1.03	0.791;1.333	0.842	1.25	0.866;1.8	0.234
EPA (% of total FA)												
M1	2.14	1.08;4.24	0.029	2.16	0.801;5.826	0.128	1.77	0.808;3.862	0.154	0.40	0.055;2.905	0.366
M2	1.43	0.704;2.91	0.322	1.06	0.346;3.259	0.916	1.55	0.69;3.503	0.287	0.50	0.068;3.635	0.490
DPA (% of total FA)												
M1	1.96	0.64;6.00	0.239	0.71	0.121;4.14	0.701	0.74	0.161;3.39	0.698	0.09	0.006;1.337	0.080
M2	1.81	0.55;5.954	0.330	0.49	0.073;3.308	0.466	0.66	0.14;3.112	0.599	0.09	0.005;1.349	0.081
DHA (% of total FA)												
M1	1.24	0.965;1.604	0.092	1.29	0.876;1.899	0.198	0.76	0.524;1.087	0.131	0.72	0.39;1.342	0.304
M2	1.24	0.948;1.631	0.116	1.20	0.786;1.843	0.393	0.71	0.487;1.034	0.074	0.76	0.404;1.422	0.388
n-3 HUFA (% of total FA)												
M1	1.23	1.012;1.482	0.037	1.21	0.91;1.62	0.187	0.90	0.694;1.174	0.446	0.72	0.441;1.179	0.193
M2	1.18	0.966;1.448	0.103	1.09	0.791;1.5	0.600	0.86	0.652;1.128	0.272	0.75	0.454;1.237	0.260
n-6 HUFA (% of total FA)												
M1	1.27	1.13;1.425	**<0.001**	1.25	1.044;1.485	0.015	0.98	0.845;1.143	0.822	0.91	0.713;1.171	0.476
M2	1.20	1.058;1.35	0.004	1.12	0.926;1.347	0.249	0.95	0.813;1.112	0.529	0.95	0.734;1.231	0.702
DHA/AA[a]												
M1	0.92	0.704;1.207	0.554	1.04	0.693;1.569	0.840	0.74	0.494;1.098	0.134	0.83	0.436;1.588	0.578
M2	0.98	0.738;1.31	0.908	1.07	0.695;1.658	0.750	0.72	0.479;1.079	0.111	0.83	0.425;1.624	0.588
EPA/AA[a]												
M1	1.37	0.715;2.631	0.341	1.51	0.578;3.951	0.400	1.72	0.8;3.709	0.165	0.59	0.101;3.466	0.560
M2	1.03	0.521;2.039	0.931	0.91	0.309;2.669	0.861	1.57	0.715;3.454	0.261	0.65	0.106;3.947	0.638
AA/LA[a]												
M1	1.61	1.316;1.973	**<0.001**	1.66	1.24;2.232	**<0.001**	0.96	0.744;1.243	0.766	1.13	0.757;1.677	0.557
M2	1.43	1.155;1.767	**0.001**	1.33	0.969;1.823	0.078	0.89	0.68;1.168	0.403	1.24	0.815;1.882	0.316
AA/DHGLA[a]												
M1	1.02	0.992;1.047	0.179	1.01	0.965;1.05	0.764	0.97	0.939;1.011	0.170	0.98	0.914;1.04	0.438
M2	1.02	0.993;1.05	0.150	1.02	0.969;1.062	0.533	0.98	0.939;1.013	0.190	0.98	0.914;1.041	0.450
n-6/n-3[a]												
M1	0.99	0.973;0.996	0.010	0.99	0.967;1.003	0.104	1.01	0.99;1.021	0.514	1.01	0.979;1.031	0.722
M2	0.99	0.975;1	0.043	0.99	0.973;1.013	0.486	1.01	0.993;1.025	0.282	1.00	0.976;1.03	0.867
SFA (% of total FA)												
M1	1.36	1.13;1.624	**0.001**	1.57	1.199;2.054	**0.001**	1.33	1.057;1.68	0.015	1.80	1.262;2.556	**0.001**
M2	1.22	1.013;1.479	0.036	1.32	0.98;1.784	0.067	1.28	1.003;1.632	0.047	1.93	1.326;2.814	**<0.001**
MUFA (% of total FA)												
M1	0.88	0.764;1.011	0.071	1.01	0.821;1.248	0.908	1.01	0.833;1.217	0.942	1.39	1.037;1.859	0.028
M2	0.89	0.769;1.032	0.124	1.02	0.817;1.283	0.839	1.01	0.831;1.219	0.949	1.36	1.012;1.837	0.041
PUFA (% of total FA)												
M1	0.97	0.877;1.071	0.539	0.87	0.748;1.015	0.076	0.90	0.787;1.033	0.137	0.69	0.551;0.854	**<0.001**
M2	0.99	0.894;1.104	0.909	0.91	0.773;1.079	0.286	0.92	0.798;1.054	0.223	0.68	0.542;0.851	**<0.001**

[a]OR for these variables refer to changes of 0.1 units. Significant associations are marked in bold (Bonferroni corrected p-value <0.00125)

Table S2b. Odds ratio and 95% confidence interval assessing the association of fatty acids with hs-CRP and IL-6 categories in **females**. Multinomial logistic regression models adjusting for: (M1) region, age and maternal education level; and (M2) further adjusting for BMI, screen-time, onset of puberty and whether the child was ever breastfed.

Fatty acids	**hs-CRP category II vs. I**			**hs-CRP category III vs. I**			**IL-6 category II vs. I**			**IL-6 category III vs. I**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
PA (% of total FA)												
M1	0.96	0.775;1.199	0.740	1.23	0.913;1.667	0.171	1.01	0.784;1.295	0.952	2.04	1.274;3.278	0.003
M2	0.93	0.74;1.165	0.522	1.19	0.861;1.644	0.291	0.98	0.757;1.276	0.895	2.06	1.281;3.304	0.003
OA (% of total FA)												
M1	1.03	0.867;1.215	0.760	0.90	0.716;1.133	0.371	1.08	0.892;1.315	0.419	1.29	0.919;1.821	0.140
M2	1.11	0.931;1.329	0.240	1.01	0.79;1.284	0.956	1.14	0.934;1.391	0.198	1.27	0.9;1.804	0.173
LA (% of total FA)												
M1	0.87	0.787;0.967	0.009	0.86	0.745;0.984	0.029	0.95	0.84;1.063	0.346	0.78	0.638;0.96	0.019
M2	0.91	0.818;1.017	0.099	0.92	0.793;1.067	0.269	0.98	0.865;1.105	0.718	0.75	0.604;0.931	0.009
GLA[a] (% of total FA)												
M1	1.22	0.79;1.89	0.367	1.29	0.731;2.265	0.382	0.83	0.503;1.37	0.466	0.82	0.345;1.93	0.644
M2	1.05	0.664;1.665	0.830	1.03	0.557;1.904	0.926	0.74	0.44;1.251	0.264	0.80	0.328;1.951	0.624
DHGLA (% of total FA)												
M1	1.52	1.038;2.211	0.031	1.65	0.99;2.733	0.055	1.13	0.745;1.705	0.572	1.25	0.607;2.568	0.547
M2	1.17	0.779;1.758	0.450	1.14	0.662;1.971	0.633	0.97	0.624;1.495	0.877	1.38	0.637;2.982	0.415
AA (% of total FA)												
M1	1.15	0.985;1.343	0.078	1.15	0.931;1.417	0.197	0.97	0.814;1.152	0.715	1.00	0.731;1.361	0.989
M2	1.11	0.945;1.3	0.207	1.10	0.878;1.367	0.421	0.94	0.784;1.122	0.484	1.00	0.735;1.369	0.984
ALA[a] (% of total FA)												
M1	1.06	0.828;1.365	0.630	0.92	0.651;1.31	0.655	1.02	0.766;1.349	0.910	0.83	0.474;1.437	0.498
M2	0.99	0.763;1.291	0.957	0.83	0.569;1.199	0.315	0.98	0.732;1.31	0.888	0.86	0.498;1.489	0.592
EPA (% of total FA)												
M1	2.12	0.845;5.336	0.109	1.26	0.353;4.471	0.724	1.79	0.681;4.72	0.237	0.49	0.066;3.672	0.490
M2	1.44	0.56;3.677	0.452	0.54	0.131;2.184	0.383	1.41	0.503;3.94	0.514	0.60	0.079;4.53	0.619
DPA (% of total FA)												
M1	0.98	0.249;3.836	0.974	0.27	0.04;1.841	0.182	0.42	0.084;2.06	0.283	0.44	0.026;7.457	0.573
M2	0.72	0.168;3.039	0.649	0.13	0.017;1.021	0.052	0.33	0.063;1.742	0.193	0.56	0.031;9.971	0.691
DHA (% of total FA)												
M1	1.03	0.78;1.365	0.825	0.97	0.659;1.439	0.895	1.09	0.798;1.5	0.577	0.51	0.256;1.019	0.057
M2	1.00	0.744;1.335	0.982	0.88	0.583;1.331	0.547	1.06	0.769;1.471	0.710	0.53	0.262;1.059	0.072
n-3 HUFA (% of total FA)												
M1	1.07	0.858;1.334	0.547	0.96	0.705;1.313	0.807	1.07	0.837;1.375	0.579	0.62	0.365;1.05	0.075
M2	1.02	0.806;1.277	0.901	0.84	0.602;1.173	0.307	1.03	0.796;1.336	0.816	0.64	0.375;1.097	0.105
n-6 HUFA (% of total FA)												
M1	1.19	1.031;1.373	0.018	1.20	0.99;1.462	0.063	0.98	0.835;1.149	0.800	1.05	0.793;1.395	0.724
M2	1.11	0.958;1.291	0.163	1.10	0.895;1.354	0.364	0.93	0.788;1.102	0.409	1.07	0.801;1.428	0.650
DHA/AA[a]												
M1	0.87	0.658;1.152	0.331	0.80	0.537;1.202	0.287	1.16	0.845;1.592	0.358	0.45	0.206;0.97	0.042
M2	0.87	0.643;1.164	0.338	0.76	0.493;1.158	0.199	1.17	0.844;1.616	0.350	0.46	0.208;0.999	0.050
EPA/AA[a]												
M1	1.38	0.588;3.248	0.457	1.09	0.34;3.515	0.881	1.79	0.713;4.491	0.215	0.48	0.069;3.396	0.466
M2	1.04	0.432;2.498	0.932	0.58	0.162;2.1	0.410	1.52	0.576;3.989	0.399	0.58	0.081;4.106	0.583
AA/LA[a]												
M1	1.43	1.094;1.857	0.009	1.47	1.033;2.103	0.033	1.02	0.762;1.362	0.899	1.27	0.788;2.054	0.325
M2	1.30	0.984;1.706	0.065	1.28	0.881;1.867	0.194	0.94	0.698;1.271	0.695	1.33	0.811;2.184	0.258
AA/DHGLA[a]												
M1	0.99	0.969;1.02	0.664	1.00	0.969;1.04	0.824	0.99	0.964;1.023	0.661	0.99	0.941;1.049	0.804
M2	1.00	0.977;1.032	0.777	1.02	0.98;1.055	0.370	1.00	0.968;1.028	0.870	0.99	0.937;1.047	0.740
n-6/n-3[a]												
M1	0.99	0.98;1.004	0.204	1.00	0.982;1.015	0.822	1.00	0.981;1.009	0.476	1.01	0.984;1.033	0.522
M2	1.00	0.982;1.008	0.462	1.01	0.988;1.023	0.565	1.00	0.983;1.012	0.728	1.01	0.98;1.031	0.665
SFA (% of total FA)												
M1	0.98	0.782;1.235	0.878	1.51	1.112;2.044	0.008	1.06	0.815;1.378	0.665	2.93	1.842;4.655	**<0.001**
M2	0.88	0.69;1.118	0.291	1.36	0.98;1.879	0.066	0.99	0.751;1.295	0.920	3.17	1.933;5.183	**<0.001**
MUFA (% of total FA)												
M1	1.08	0.922;1.262	0.345	0.95	0.765;1.171	0.613	1.10	0.92;1.32	0.293	1.25	0.907;1.712	0.174
M2	1.13	0.962;1.338	0.134	1.02	0.813;1.275	0.878	1.14	0.949;1.371	0.161	1.24	0.898;1.715	0.190
PUFA (% of total FA)												
M1	0.97	0.86;1.084	0.557	0.92	0.786;1.072	0.278	0.93	0.809;1.064	0.281	0.67	0.535;0.839	**<0.001**
M2	0.97	0.856;1.089	0.568	0.91	0.775;1.078	0.285	0.93	0.806;1.065	0.283	0.66	0.525;0.831	**<0.001**

[a]OR for these variables refer to changes of 0.1 units. Significant associations are marked in bold (Bonferroni corrected p-value <0.00125)

Table S3 Overview of existing studies on fatty acids and markers of low-grade inflammation

Author, year	Study population: age, country	Study N (pooled/sex – stratified)	Diet/blood: Fatty acids	Adjustment (fully adjusted model)	Results			
					(+) association with CRP or IL-6	(+) association with other pro-inflammatory markers	(-) association with CRP or IL-6	(-) association with other pro-inflammatory markers
Pischon et al., 2003	M: 40-75y, F: 25-72y, US	859 (pooled)	Diet (FFQ):EPA+DHA, ALA, LA	age, gender, smoking, physical activity, alcohol, anti-inflammatory drugs, BMI, caloric intake, protein, SFA, MUFA, cholesterol, remaining PUFAs.	x	x	*CRP:* EPA+DHA ($p<0.1$)	EPA+DHA
Lopez-Garcia et al., 2004	43-69y, US	727 (only F)	Diet (FFQ): ALA, EPA, DHA, total n-3	age, BMI, physical activity, smoking, alcohol, intakes of LA and SFA, vitamin E, dietary fibre, trans FA, hormone therapy.	x	x	*CRP and IL-6:* ALA, total n-3	ALA, EPA+DHA, total n-3
Klein-Platat et al., 2005	12y, Eastern France	120 [60 NW, 60 OW] (pooled)	Blood: LA, ALA, EPA, DHA ,SFA, PUFA/SFA	sex, sexual maturity, physical activity, body fat, WHR	*IL-6:* SFA (only in OW subjects)	NA	*CRP:* ALA, EPA (only in OW subjects) *IL-6:* PUFA/SFA (only in OW subjects)	NA
Ferrucci et al, 2006	20-98y, Italy	1123 (pooled)	Blood: LA, AA, ALA, EPA, DHA, AA/EPA, total n-3, total n-6, n-6/n-3	age, sex, education, intake of energy/proteins/carbohydrates, physical activity, BMI, LDL, HDL, TG, hypertension, diabetes, CHD, heart failure, stroke, arterial disease, drug treatment	x	n-6/n-3	*CRP:* ALA *IL-6:* AA, EPA, DHA, total n-3, total n-6	AA, ALA, DHA, total n-3, total n-6
Steffen et al., 2012	52-72y, US (multi-ethnic: African American, Asian, Hispanic, White)	2848 (pooled)	Blood: LA, GLA, DHGLA, AA, ALA, EPA, DPA, DHA	age, gender, race/ethnicity, field centre, education, smoking, physical activity and energy intake, BMI, HDL, LDL, TG	*CRP:* DHGLA *IL-6* GLA, DHGLA	GLA, DHGLA,	*CRP:* LA, DPA *IL-6* EPA, DPA	LA, EPA
Kaikkonen et al., 2014	24-39y, Finland	2196 (pooled)	Blood: PUFA, n-3 , n-6, n-6/n-3, MUFA,SFA, PUFA/SFA, double bonds per FA	age, physical activity, use of contraceptives, vitamin E intake, smoking, BMI, alcohol intake, glucose, insulin, systolic blood pressure, LDL.	*CRP:* MUFA, SFA	NA	*CRP:* PUFA, n-3, n-6, PUFA/SFA, FA double bonds	NA
Muka et al., 2015	≥55y, Netherlands	4707 (pooled and stratified)	Diet (FFQ): total PUFA, total n-6, total n-3, n-3/n-6	age, sex, education, income, cholesterol intake, physical activity, BMI, smoking, use of anti-inflammatory drugs, DHD index, prevalent chronic diseases, serum total cholesterol, HDL, systolic blood pressure, n-3 or n-6 PUFA (in n-6 and n-3 PUFA analyses respectively)	*CRP:* Stratified (F): total n-3, n-3/n-6*	NA	*CRP:* pooled: total PUFA, total n-6. Stratified (F): total PUFA, total n-6	NA
González-Gil et al., 2016	2-9y, 6 European countries	1401 (stratified)	Blood: PA, OA, LA, GLA, AA, ALA, EPA, DPA, DHA, n-3 HUFA, n-6 HUFA, %n-3 of total HUFA, DHA/AA, EPA/AA, AA/LA, AA/DHGLA, n-6/n-3, total n-3, total n-6, SFA, MUFA, PUFA	age, mother education, country, BMI, whether child was ever breastfed, physical activity.	*CRP:* F: AA, n-6 HUFA, AA/LA M: x	NA	*CRP:* F: EPA/AA M: LA, total n-6	NA
Harris et al.	10y, Germany	1003 (pooled and stratified)	Blood: PA, OA, LA, GLA, DHGLA, AA, ALA, EPA, DPA, DHA, n-3 HUFA, n-6 HUFA, DHA/AA, EPA/AA, AA/LA, n-6/n-3, AA/DHGLA, SFA, MUFA, PUFA	sex, region, age, maternal education, BMI, screen-time, whether child was ever breastfed.	*CRP:* pooled: AA, n-6 HUFA, AA/LA stratified (M): AA/LA *IL-6:* pooled: PA, SFA stratified (M & F): SFA	NA	*CRP:* pooled: LA *IL-6:* pooled: LA, PUFA M & F: PUFA	NA

HUFA= highly unsaturated FA, M=males, F=females, NW=normal weight, OW=overweight, WHR=waist/hip ratio, NA=not reported, x=no association

7 Paper 4: Changes in Dietary Intake During Puberty and their Determinants

(Harris et al. BMC Public Health, 2015)

Original title:	Changes in dietary intake during puberty and their determinants: results from the GINIplus birth cohort study
Authors:	C Harris, C Flexeder, E Thiering, A Buyken, D Berdel, S Koletzko, C-P Bauer, I Brüske, B Koletzko, M Standl
Journal:	BMC Public Health
Volume:	15
Pages:	841
Year:	2015
DOI:	10.1186/s12889-015-2189-0

Harris et al. BMC Public Health (2015) 15:841
DOI 10.1186/s12889-015-2189-0

BMC Public Health

RESEARCH ARTICLE Open Access

Changes in dietary intake during puberty and their determinants: results from the GINIplus birth cohort study

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Abstract

Background: Understanding changes in dietary intake during puberty could aid the mapping of dietary interventions for primary prevention. The present study describes dietary changes from childhood to adolescence, and their associations with parental education, family income, child education, body mass index (BMI), pubertal onset and screen-time sedentary behaviour.

Methods: Dietary data ($n = 1232$) were obtained from food frequency questionnaires at the 10- and 15-year follow-ups of the GINIplus birth cohort study. Intakes of 17 food groups, macronutrients and antioxidant vitamins, were described by a) paired Wilcoxon rank sum tests, comparing average intakes at each time-point, and b) Cohen's kappa "tracking" coefficients, measuring stability of intakes (maintenance of relative tertile positions across time). Further, associations of changes (tertile position increase or decrease vs. tracking) with parental education, family income, child education, pubertal onset, BMI, and screen-time, were assessed by logistic regression and multinomial logistic regression models stratified by baseline intake tertile.

Results: Both sexes increased average intakes of water and decreased starchy vegetables, margarine and dairy. Females decreased meat and retinol intakes and increased vegetables, grains, oils and tea. Males decreased fruit and carbohydrates and increased average intakes of meat, caloric drinks, water, protein, fat, polyunsaturated fatty acids (PUFAs), vitamin C and alpha-tocopherol. Both sexes presented mainly "fair" tracking levels [$\kappa_w = 0.21–0.40$]. Females with high (vs. low) parental education were more likely to increase their nut intake [OR = 3.8; 95 % CI = (1.7;8.8)], and less likely to decrease vitamin C intakes [0.2 (0.1;0.5)], while males were less likely to increase egg consumption [0.2 (0.1;0.5)] and n3 PUFAs [0.2 (0.1;0.5)]. Females with a higher (vs. low) family income were more likely to maintain medium wholegrain intakes [0.2 (0.1;0.7) for decrease vs. tracking, and 0.1 (0.0;0.5) for increase vs. tracking], and were less likely to decrease vitamin C intakes [0.2 (0.1;0.6)]. Males with high education were less likely to increase sugar-sweetened foods [0.1 (0.1;0.4)]. Finally, BMI in females was negatively associated with decreasing protein intakes [0.7 (0.6;0.9)]. In males BMI was positively associated with increasing margarine [1.4 (1.1;1.6)] and vitamin C intakes [1.4 (1.1;1.6)], and negatively associated with increasing n3 PUFA.

Conclusions: Average dietary intakes changed significantly, despite fair tracking levels, suggesting the presence of trends in dietary behaviour during puberty. Family income and parental education predominantly influenced intake changes. Our results support the rationale for dietary interventions targeting children, and suggest that sex-specific subpopulations, e.g. low socio-economic status, should be considered for added impact.

Keywords: Puberty, Dietary intake, Dietary changes, Tracking, Determinants, Epidemiology

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Background

Public health interventions, aimed at the primary prevention of chronic diseases through diet, typically focus on education and facilitation towards the development of healthier eating habits [1–3]. Children are often targeted, due to the underlying evidence that the physiological risk of chronic diseases can develop early in childhood [4]. However, newly adopted health conducts in children may not be maintained throughout adolescence, as behaviour during this stage is often erratic and prone to changes [5]. Understanding food intake changes during the transition into adolescence can hence help guide the mapping of dietary interventions for primary prevention. Aside from general dietary alterations occurring at the population level, knowledge regarding the stability of individual diet during puberty could help answer questions such as *when* to introduce dietary interventions to ensure optimal adoption and maintenance. Furthermore, evaluating which factors may determine particular dietary changes could help to identify possible subpopulations as important targets for dietary interventions.

The maintenance of food intake behaviour over time, relative to the rest of the population, is referred to as "dietary tracking" [6]. The presence and strength of dietary tracking, or lack thereof, can reflect the level of stability of individual long-term eating behaviours. A 2012 review [7], summarizing the results of studies assessing tracking levels of dietary patterns from childhood to adolescence [8–11], reported weak to moderate tracking of intakes including fruit and vegetables, total energy, macronutrients, meat and oils. These findings indicate that although some children maintain a relatively stable dietary behaviour during pubertal maturation, others might notably alter their intakes. Nevertheless, only one of the included studies attempted to identify possible determinants of dietary changes during this time period, where, family income, urban-rural residence and mother education were found to be potential predictors of meat, vegetable, fruit and oil intake changes over 6 years [11]. A review on determinants of fruit and vegetable intakes in children and adolescents reported consistent positive associations with family income, parental education, parental intake and home accessibility; a negative association with age; and higher intakes in girls than in boys. However, most of the included studies were based on cross-sectional data and the authors recognised the need for longitudinal analyses [12]. A 2012 longitudinal study testing the association between parental education and intakes of fruit, vegetables, snacks, soft drinks and squash over 20 months, reported that increases in sugar-sweetened beverages were more likely in children with low parental education [13]. Gebremariam et al. assessed the associations of sedentary behaviour on changing intakes of fruits, vegetables, soft drinks, sugar and snacks, and found evidence that high screen-time sedentary behaviour was longitudinally associated with increased consumption of soft drinks and sweets and lower intakes of vegetables [14]. Early onset of puberty was associated with the development of unhealthy lifestyles, such as lower rates of breakfast routines, in a study assessing longitudinal effects of pubertal timing on health behaviours [15]. Additionally, a study in low income adolescents, observed that overweight adolescents were more likely to reduce their energy, fibre and snack food intakes over time [16].

The currently available longitudinal studies suggest that socio-economic environment as well as individual characteristics and behaviours, play an important role in determining food intake changes throughout pubertal maturation. Nevertheless, the available literature is scarce and knowledge in this area is still limited. The need for longitudinal studies assessing differences in dietary behaviours of subjects of both sexes and from different segments of the population has been suggested [12, 17]. To our knowledge, no longitudinal cohort study has yet provided a comprehensive description of habitual dietary intake before and after puberty, assessing both environmental and personal factors as potential determinants of observed changes. Our study aim was hence to examine overall changes in intakes of 17 different food groups representative of total dietary intake, as well as macronutrients and antioxidant vitamins, during this time period; to evaluate the stability of individuals' intakes over time, and to determine whether specific changes in diet can be predicted by parental education, family income, child education, BMI, pubertal onset and screen-time sedentary behaviour.

Methods

Study participants

The present analysis was based on data collected at the 10- and 15-year follow-ups of the ongoing German birth cohort study GINIplus (*G*erman *I*nfant *N*utritional *I*ntervention *plus* environmental and genetic influences on allergy development). Details on the GINIplus study design, recruitment and exclusion criteria have been described previously and can be found elsewhere [18]. In short, healthy full-term new-borns ($n = 5991$) were recruited from obstetric clinics in two different regions of Germany (Munich and Wesel). Infants were allocated to the study intervention arm (randomized to one of three hydrolysed formulae or to conventional cow's milk) or to the non-intervention arm. Data on health outcomes and covariates were collected by means of identical questionnaires, completed by parents of all children at various time-points. Information on the relevant exposure variables and covariates is given below. To aid

reporting of results, the 10-year time-point is hence forth referred to as baseline, and the 15-year time-point as follow-up.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the local ethics committees (Bavarian Board of Physicians, Board of Physicians of North-Rhine-Westphalia). Written informed consent was obtained from all subjects.

Dietary intake

Dietary assessment at baseline and follow-up was carried out using a self-administered FFQ, designed and validated to measure 10-year-old children's usual food and nutrient intake over the past year, and more specifically to estimate energy, fatty acid and antioxidant intake [19]. Due to the uncertain quality of dietary information collected from young children, the FFQ at baseline was addressed to the parents, who completed it along with their children. This was done in order to maximise accuracy by obtaining mutual impact from both the child and the parent [19]. At follow-up, the FFQ was addressed directly to the participants, who were asked to complete it themselves with support of whoever cooked at home, if needed. The FFQ comprised of eighty food items accompanied by several questions about preferred fat and energy contents, preparation methods, diets and food preferences, buying habits and dietary supplement use. To estimate how often food was consumed over the previous year, subjects could choose one of nine frequency categories, including 'never', 'once a month', '2-3 times a month', 'once a week', '2-3 times a week', '4-6 times a week', 'once a day', '2-3 times a day' and 'four times a day or more'. In addition, common portion sizes were assigned for each food item to enable an estimation of quantities. For food items that are difficult to describe in common household measures, coloured photographs from the EPIC (European Prospective Investigation into Cancer and Nutrition) study showing three different portion sizes were included [20]. The 80 FFQ food items were allocated into 41 groups and combined to form 17 major food groups. The categorization systems of a number of sources were compared [21–26] and adapted to the food items present in the FFQ. A list of the resulting food groups is displayed in Table 1. Further details on the development of the FFQ, including food item selection, dietary vitamins, supplement use, and validation methods, have been previously described [19, 27].

A quality control procedure was developed and applied to the FFQ data at both time-points (Fig. 1). This was done based on recommendations by Willett et al. for data cleaning in nutritional epidemiology [28]. Subjects were excluded if a complete block of food items, presented together under the same subheading, was empty (144 at baseline and 134 at follow-up). For each food item, if the intake frequency was provided, but portion size was missing, portion size was replaced by the median obtained from the remaining sex-specific populations. Subjects were excluded if responses to more than 40 food items (50 % of the FFQ) were missing (16 at baseline and 4 at follow-up). Intake frequencies and amounts were then combined to calculate average consumption in grams per day (g/d). Evidence suggests that the presence of intermittent blanks in an otherwise carefully completed FFQ, are best considered as no consumption of the missing food item [28]. Therefore, any remaining missing information on frequency of intake was regarded as "never", and intake of the specific food item was defined as 0 g/d. Based on the German Food Code and Nutrient Database (BLS) version II.3.1 [29], the corresponding energy and nutrient content per daily grams of intake were calculated for each food item. Total daily energy and nutrient intake was obtained by the sum of daily energy and nutrients of all food items respectively. Intakes relative to total daily energy intake were calculated as the ratio of energy from each food item or macronutrient to the total daily energy intake, and multiplied by 100 to obtain percentage contributions towards total energy intake (%EI). Due to the lack of energy content of water and tea, these food groups were presented in g/day. Furthermore, vitamin intakes were presented in mg/day. Subjects were excluded if total daily energy intake was outside 500-3500 kcal or 800-4000 kcal for females and males respectively (38 subjects at baseline and 126 at follow-up), ranges suggested by Willett et al. in order to avoid substantial loss to follow-up [28]. Further exclusions were made if provided values for %EI of specific food items were implausible (1 subject at follow-up due to extreme rice values: 57 % of total daily energy intake from rice or 620 g/d). Only participants who completed the FFQ at both time-points were included ($n = 1304$). After excluding participants presenting extreme values for co-variables (1 subject), or reporting an illness affecting diet (22 subjects) or medical dietary indications (49 subjects), 1232 participants remained for inclusion in the analyses. Due to the extensive quality control applied at both time-points, the FFQ data in the present study differs from that in previously published papers using only the GINIplus 10-year follow-up dietary data [19, 27].

Socio-economic environment

Parental education and family income

Parental education and family income were used as proxies for socio-economic status (SES). Parental education was defined by the highest level achieved by either the mother or the father, according to the German education system. Children were grouped by low (10 years of education or less) or

Table 1 Food groups and list of corresponding food items

Major food group	Food groups	FFQ Food items
1. Fruit	Whole fruit	Apples, Pears
		Tropical fruits
	Berries	Berries
2. Vegetables (excl. potatoes)	Green Leafy	Spinach, chard
		Cruciferous vegetables
		Lettuce
	Red/Orange	Carrots
		Peppers
3. Starchy vegetables	Potatoes	Boiled-, jacket-potato
	Fried potatoes	Chips, croquettes
4. Whole grains	Wholegrain bread	Wholegrain bread/toast
	Wholegrain cereals	Muesli, cereals
5. Refined grains	White breads	White bread/toast
		Bread roll, Pretzel
	Sweet breads	Raisin bread
		Croissant, chocolate bread
	Brown bread	Brown-, rye-, multi-grain
	Refined cereals	Cornflakes
	Pasta	Pasta, noodles
	Rice	Rice
	Pizza	Pizza
	Salty snacks	Snack mixes
6. Meat	Red meat	Pork
		Beef, veal
	Offal	Offal
	Processed meat	Salami
		Leberwurst
		Cold meat
		Bratwurst
		Sausage, Wiener-, pork-sausage
	Poultry	Poultry meat
	Ready-to-eat meals	Ready-to-eat meals with meat
7. Fish	Fresh fish	Freshwater fish
		Salt-water fish
	Canned fish	Bismarck herring, matie
		Canned fish
	Breaded fish	Fish fingers
8. Egg	Egg	Eggs, scrambled/fried
9. Nuts, seeds	Nuts	Nuts
	Seeds	Pumpkin-, pine, sunflower-seed
10. Butter	Butter	Butter
		Butter (in cooking)
11. Margarine	Margarine	Margarine, sunflower spread
		Margarine (in cooking)

Table 1 Food groups and list of corresponding food items *(Continued)*

	Low-fat margarine	Low-fat margarine
		Low-fat margarine (in cooking)
12. Oils	High MUFA oils	Olive oil
	High PUFA oils	Safflower oil
		Sunflower oil
		Maize germ oil
		Walnut oil
		Vegetable oil
13. Dairy	Milk and milk products	Milk
		Cream cheese, quark (curd)
		Buttermilk, whey
		Hard cheese
		Soft cheese
		Cream, crème fraiche
		Yoghurt, fruit yoghurt
14. Sugar-sweetened foods	Cakes and biscuits	Cream tart
		Pastries
		Biscuits, cookies
		Sponge cake
		Pie
	Chocolate	Chocolate
		Chocolate bars
	Sweets and sugars	Choco-hazelnut spread
		Sugar beet molasses
		Gummy bears
	Dairy products with added sugars	Cocoa, milkshake
		Semolina pudding, rice pudding
		Ice cream
15. Caloric drinks	Sugar-sweetened-drinks	Lemonade, coke, ice tea
		Sport-, energy-drinks
	Fruit and vegetable juices	Squash, fruit nectar
		Fruit juice
		Vegetable juice
		Diluted juice
16. Water	Water	Mineral-, tap water
17. Tea	Tea	Tea

high (more than 10 years of education) parental education. Family income was categorized by tertiles (low, medium and high), assigned separately and then merged, for the two study centres due to differences in salaries and living costs.

Individual characteristics and behaviours

BMI, pubertal onset, child education level, and screen-time

The focus of the present study was on identifying factors present at childhood, associated with the development of dietary behaviours, and hence only exposure variables measured at baseline were required for the analyses. BMI [kg/m^2] at baseline was used as a continuous variable, calculated from parental-reported weight and height measurements obtained from the 10-year follow-up questionnaire. Data on pubertal onset (yes/no) were obtained from the 10-year questionnaire, defined as "yes" if parents stated the presence of any of the following: acne or spots, pubic or axillary hair, breast development, menstruation, penis or

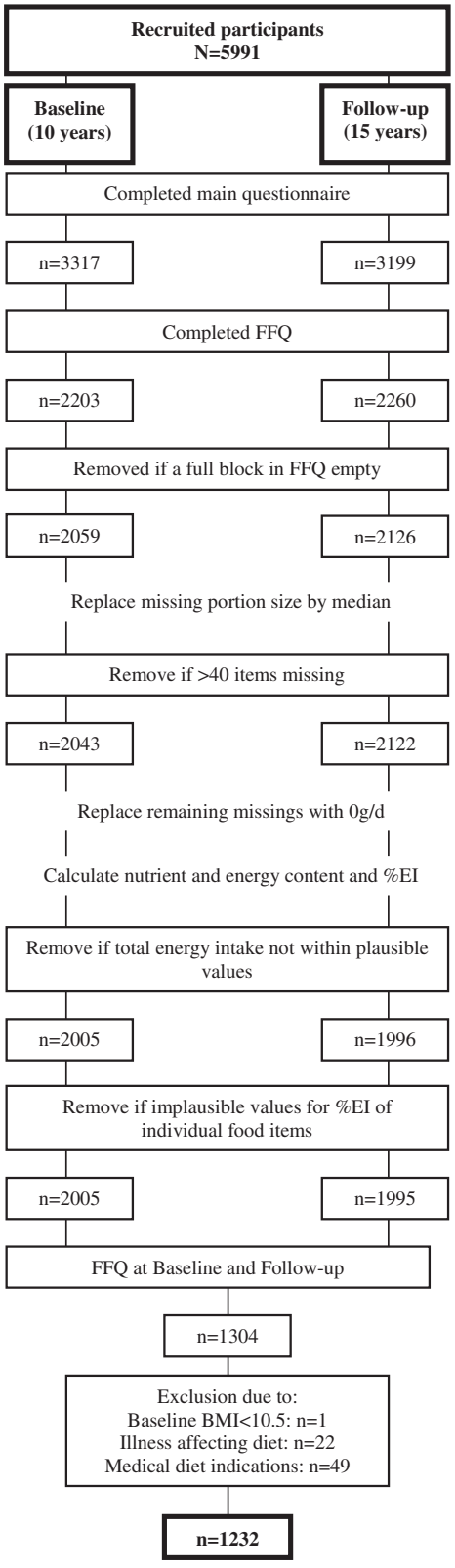

Fig. 1 Study population and quality control procedure

testicle enlargement, or any other signs of pubertal onset. Data on pubertal stage at follow-up was obtained from a self-rating pubertal development scale [30], and children were categorised into "pre-", "early-", "mid-", "late-" and "post-" pubertal. As the study focus is on changes during puberty, pubertal stage at follow-up was presented for reference, but it must be kept in mind that it is not analogous to the 10-year variable, and hence not comparable. Child education level was defined by the highest level achievable in the secondary school type they attended according to the German education system. Children were grouped analogous to the definition used for parental education, as "low" (schooling programme finalized in 10 years or less) or "high" (schooling programme finalized in more than 10 years). Children who could not be grouped by school type were not included in the analyses. Screen-time was measured at the 10-year follow-up by the amount of time typically spent in front of a screen (television, computer, etc.), reported in 4 categories (ranging from "less than 1 h" to "5 or more") and categorized as low (≤ 2 h) or high (> 2 h).

Statistical analysis

To test for differences due to attrition bias, we compared characteristics of participants lost to follow-up (data only at baseline) to those included in the present study analyses, who adhered at follow-up (data at both baseline and follow-up). Categorical variables, presented as percentages, were tested by Fisher's exact test (binary variables) or Pearson's Chi-squared test (variables with more than 2 levels). Continuous variables, presented as means (standard deviation), were tested by Student's *t*-test.

The basic characteristics of the study population were described by means (standard deviation) and percentages, separately for females and males. Female and male characteristics were compared using Pearson's Chi-squared Test or Student's *t*-test for categorical and continuous variables respectively. All further statistical analyses were performed stratified for females and males in order to identify sex-specific differences in dietary behaviours.

Average dietary changes

Due to deviation from the normal distribution, food group intake data at baseline and follow-up are presented by the median %EI and 25^{th} and 75^{th} percentiles. Statistically significant differences from baseline to follow-up were tested using the paired Wilcoxon signed rank test.

Dietary tracking

Dietary tracking refers to the maintenance of food intake behaviour over time [6]. Each food group was categorized into sex-specific tertiles at baseline and at follow-

up: T1 (lowest tertile), T2 (medium tertile) and T3 (highest tertile). Individuals remaining within the same relative tertile of %EI, at baseline and follow-up, were regarded as "tracking" i.e. suggesting stable dietary intakes over time. [11, 16, 31, 32] Tracking coefficients were calculated for each food group by Cohen's kappa statistic (a measure of agreement between two observations) using linear weights (κ_w) for kappa values [33]. Coefficients were interpreted based on the following cut-off values as suggested by Landis and Koch [33, 34]: ≤ 0 = poor, 0.01–0.20 = slight, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial and 0.81–1 = almost perfect. To test whether individuals tracked significantly within a food group, an exact binomial test was used. Here, the observed percentage of individuals remaining in the same tertile over time (i.e. tracking) was compared to that expected (33.3 %) assuming independence.

Associations with dietary changes

In order to avoid false assumptions of linear effects, associations with dietary changes were evaluated categorically using the previously defined tertiles. Possible changes in intakes were identified relative to baseline tertiles: individuals in the lowest tertile T1 at baseline either remained in T1 at follow-up ("tracking in T1"), or increased to tertiles T2 or T3; individuals in the highest baseline tertile T3 either remained in T3 at follow-up ("tracking in T3"), or decreased to T1 or T2. Only individuals in the medium tertile T2 at baseline could either remain in T2 at follow-up ("tracking in T2"), decrease to T1 or increase to T3. Therefore, three regression models were fitted, one for each baseline intake tertile: 1) model for baseline tertile T1 ("increase" vs "tracking in T1"); 2) model for baseline tertile T2 ("increase" and "decrease" vs "tracking in T2"); 3) model for baseline tertile T3 ("decrease" vs "tracking in T3"). The models 1 and 3 were logistic regression models and model 2 was a multinomial logistic regression model. The results are presented as odds ratios with corresponding 95 % confidence interval [OR (95 % CI)]. These regression models tested the associations of dietary changes with parental education level (high vs. low), family income (medium and high vs. low), child education level (high vs. low), pubertal onset at baseline (yes vs. no), baseline BMI, and baseline screen-time (high vs. low). Models were adjusted for possible confounders including age at baseline, baseline energy intake (total daily energy intake [kcal] at 10-year follow-up), diet changes between baseline and follow-up (e.g. starting or stopping a diet in between assessments), study centre (Munich or Wesel), and study intervention arm (assigned to milk formula intervention or control group upon birth). Due to lack of sufficient data in specific cases, certain multinomial regressions were modelled differently: male models for baseline tertile T2 intakes of vegetables, starchy vegetables, refined grain, meat, egg, nuts, butter, margarine and protein, were not adjusted for diet changes; furthermore, the model for T2 starchy vegetable intake in males did not include pubertal onset. For a more thorough interpretation of the regression analyses, we also considered associations between the exposure variables and baseline food intake tertiles, using Pearson's χ^2 test for categorical variables, and one-way analysis of variance for continuous variables (See Additional file 1: Tables S1a and S1b).

Statistical significance was defined by a two-sided alpha level of 5 %. For the regression analyses we corrected for multiple testing using Bonferroni correction: the alpha level was divided by six, because data were analysed both by sex (two) and baseline intake categories (three) which yields a corrected two sided alpha level of 0.0083 (0.05/(2*3) = 0.0083). All analyses were performed using R version 3.1.0 (https://www.R-project.org/) [35]. Weighted kappa was calculated using the cohen.kappa() function in package "psych" [36], and multinomial regression analysis was performed using the function multinom() in package "nnet" [37].

Results

In the present analysis 1232 participants (643 females and 589 males) were included with complete FFQ information at both time-points (Fig. 1). Participation at both time-points, compared to participation at baseline only, was higher amongst female subjects, with higher education, subjects with a higher parental education, with medium family income level, with a lower baseline screen-time, or subjects living in Munich (Additional file 2: Table S2).

Study population

Basic characteristics of the study population stratified by sex are displayed in Table 2. Parental education was mostly high, especially in females (71.4 and 62.9 in females and males respectively). More females (46.4 %) than males (10.9 %) had reached the onset of puberty at baseline, and pubertal development at follow-up was more advanced in females then in males. Mean baseline energy intake was significantly higher in males than females (2105.4 kcal/d (standard deviation = 567.7 kcal/d) in males and 1831.4 kcal/d (488.1 kcal/d) in females), with similar macronutrient proportions in both sexes. Follow-up energy intake was also higher in males, but protein and fat intake was greater in males whereas females consumed more carbohydrates. More females

Table 2 Basic characteristics of the study population

	Females		Males		*p*-value[a]
	n	% or mean (SD)	n	% or mean (SD)	
N	643		589		
Parental education level[b]	623		568		
Low (≤ 10 years)	178	28.6	211	37.1	0.002*
High (> 10 years)	445	71.4	357	62.9	
Family income level[c]	592		536		
Low	168	28.4	166	31.0	0.609
Medium	229	38.7	196	36.6	
High	195	32.9	174	32.5	
Child education level	596		552		
Low (≤ 10 years)	210	35.2	215	38.9	0.215
High (> 10 years)	386	64.8	337	61.1	
Pubertal onset at BL	633		579		
Yes	294	46.4	63	10.9	<0.001*
No	339	53.6	516	89.1	
Pubertal onset at FU	553		490		
Pre-pubertal	0	0	6	1.2	<0.001*
Early puberty	0	0	20	4.1	
Mid-puberty	22	4	174	35.5	
Late puberty	450	81.4	286	58.4	
Post-pubertal	81	14.6	4	0.8	
BMI [kg/m^2]	589	16.7 (2.3)	527	16.8 (2.3)	0.508
Screen-time[d]	631		584		
Low (≤ 2 h)	578	91.6	523	89.6	0.261
High (> 2 h)	53	8.4	61	10.4	
Age at BL [y]	641	11 (0.5)	588	11 (0.5)	0.169
Age at FU [y]	643	15.5 (0.3)	589	15.5 (0.3)	0.961
Energy intake at BL [kcal/day]	643	1831.4 (488.1)	589	2105.4 (562.3)	<0.001*
% Protein at BL	643	14.7	589	14.8	0.597
% Fat at BL	643	30.4	589	31	0.052
% Carbohydrate at BL	643	54.9	589	54.2	0.067
Energy intake at FU [kcal/day]	643	1784.1 (568)	589	2387.4 (657.7)	<0.001*
% Protein at FU	643	14.8	589	15.3	0.001*
% Fat at FU	643	30.1	589	31.3	0.001*
% Carbohydrate at FU	643	55.1	589	53.4	<0.001*
Diet start/stop between BL and FU	630		572		
Yes	86	13.7	36	6.3	<0.001*
No	544	86.3	536	93.7	
Study center	643		589		
Munich	334	51.9	313	53.1	0.717
Wesel	309	48.1	276	46.9	

Table 2 Basic characteristics of the study population *(Continued)*

Study arm	643		589		
Control group	348	54.1	329	55.9	0.579
Infant intervention	295	45.9	260	44.1	

SD standard deviation, *BL* baseline, *FU* follow-up
[a]tested by Pearson's Chi^2 test (categorical variables) or by Student's *t*-test; *p-value < 0.05
[b]Highest level achieved by mother or father
[c]Tertiles stratified by study centre and merged
[d]Hours spent on screen-time behaviours.)

(13.7 %) than males (6.3 %) started or stopped a diet between assessments.

Average dietary changes

The median (25th percentile; 75th percentile) intakes of food groups (in %EI; in ml/d for tea and water), macronutrients (in %EI), PUFAs (in %EI), and antioxidant vitamins (in mg/d), at baseline and follow-up are presented in Table 3. From baseline to follow-up, females significantly increased their average intakes of vegetables, whole grain, refined grain, oils, tea and water; and decreased their intake of starchy vegetables, meat, margarine, dairy and retinol. However, when excluding females who became vegetarian or vegan ($n = 25$)

Table 3 Changes in average intakes of food groups, macronutrients and vitamins in females and males

	Females				Males			
	Baseline[a]	Follow-up[a]	Change	p-value[b]	Baseline[a]	Follow-up[a]	Change	p-value[b]
Fruit	4.2 (2.7;6.1)	3.9 (2.3;6.4)		0.568	3.3 (1.9;4.9)	2.2 (1.1;3.8)	(-)	<0.001
Vegetables	1.6 (1.0;2.4)	1.9 (1.1;3.0)	(+)	<0.001	1.2 (0.7;1.8)	1.2 (0.6;1.8)		0.427
Starchy vegetables	2.2 (1.4;3.5)	1.9 (1.2;3.2)	(-)	<0.001	2.1 (1.4;3.3)	1.8 (1.2;2.9)	(-)	<0.001
Whole grains	2.4 (0.7;7.2)	3.0 (0.9;7.6)	(+)	0.026	2.1 (0.3;6.5)	2.4 (0.5;6.0)		0.767
Refined grains	27.8 (23.1;33.9)	28.8 (23.2;35.6)	(+)	0.021	27.4 (21.5;33)	26.7 (21.1;33.3)		0.616
Meat	11.3 (7.7;15.8)	11.1 (6.9;15.4)	(–)	0.043	12.8 (9.3;17.3)	13.7 (10;18.8)	(+)	<0.001
Fish	1.1 (0.6;1.8)	1.1 (0.5;1.8)		0.124	1.3 (0.7;1.9)	1.3 (0.7;2.0)		0.885
Eggs	0.6 (0.3;1.0)	0.6 (0.3;1.0)		0.440	0.5 (0.3;0.9)	0.5 (0.3;1.0)		0.729
Nuts and seeds	0.3 (0.1;0.9)	0.4 (0.0;0.8)		0.940	0.3 (0.1;0.8)	0.3 (0.0;0.9)		0.287
Butter	0.6 (0.1;2.3)	0.7 (0.1;2.4)		0.209	0.6 (0.0;2.3)	0.8 (0.1;2.3)		0.380
Margarine	0.3 (0.0;1.3)	0.2 (0.0;1.1)	(–)	0.013	0.3 (0.0;1.3)	0.2 (0.0;0.9)	(–)	<0.001
Oils	1.2 (0.6;2.4)	1.4 (0.6;2.6)	(+)	0.023	1.1 (0.5;2.3)	1.2 (0.6;2.1)		0.863
Dairy	10.4 (6.8;15.0)	9.2 (5.6;13.6)	(–)	<0.001	10.8 (6.6;16.7)	9.1 (5.5;14.2)	(–)	<0.001
Sugar-sweetened foods	15.7 (9.9;21.8)	15.1 (9.5;21.5)		0.611	15.4 (10.3;22.2)	15.6 (10.3;21.7)		0.996
Caloric drinks	7.1 (2.9;13.0)	6.1 (2.5;12.8)		0.819	8.0 (3.4;14.5)	10.5 (4.5;16.8)	(+)	<0.001
Tea [ml/d]	21.1 (2;89.5)	25.8 (4.4;133.6)	(+)	<0.001	10.1 (0.0;60)	10.2 (0.0;50.8)		0.612
Water [ml/d]	651.0 (339.6;939.1)	906.7 (575.4;1355)	(+)	<0.001	634.4 (277.8;1046)	944.9 (376.3; 1530)	(+)	<0.001
Protein	14.5 (13.0;16.2)	14.6 (12.8;16.2)		0.229	14.8 (13.2;16.3)	15.2 (13.4;17.0)	(+)	<0.001
Fat	29.8 (26.2;34.0)	29.7 (26.5;33.6)		0.195	30.5 (27.5;34.3)	30.8 (27.4;35.3)	(+)	<0.001
Carbohydrate	55.4 (50.9;59.5)	55.5 (50.9;59.5)		0.062	54.8 (49.8;58.8)	53.5 (48.5;58.3)	(–)	<0.001
n3 PUFA	0.6 (0.5;0.6)	0.6 (0.5;0.7)		0.721	0.5 (0.5;0.6)	0.6 (0.5;0.7)	(+)	<0.001
n6 PUFA	3.8 (3.3;4.5)	3.9 (3.3;4.7)		0.818	3.9 (3.3;4.6)	4.0 (3.3;4.7)	(+)	<0.001
Retinol [mg/d]	0.4 (0.3;0.5)	0.3 (0.2;0.5)	(–)	<0.001	0.4 (0.3;0.7)	0.5 (0.3;0.7)		0.053
Beta Carotene [mg/d]	4.0 (2.6;5.9)	3.9 (2.4;5.8)		0.752	3.5 (2.2;5.4)	3.3 (2.0;5.2)		0.076
Vitamin C [mg/d]	99.4 (71.3;136.8)	97.7 (69.0;146.1)		0.264	98 (68.3;130.7)	102.2 (72.4;140.9)	(+)	0.019
alpha tocopherol [mg/d]	7.8 (6.1;9.8)	7.9 (6.0;10.4)		0.130	8.2 (6.4;10.4)	9.0 (7.1;11.5)	(+)	<0.001

[a]Median (25th percentile; 75th percentile), presented in %EI unless stated otherwise
[b]Paired Wilcoxon rank sum test; (+) = significant increase from baseline to follow-up: p-value < 0.05; (–) = significant decrease from baseline to follow-up: p-value < 0.05

between baseline and follow-up, the decrease in meat intake was no longer significant. Males significantly increased their average intake of meat, caloric drinks, water, protein, fat, n3 and n6 PUFAs, vitamin C and alpha-tocopherol; and decreased their average fruit, starchy vegetable, margarine, dairy and carbohydrate intakes.

Dietary tracking

Tracking coefficients and percentages of individuals tracking are shown for females and males in Table 4. Based on the kappa coefficients, both sexes presented fair tracking for most food groups, macronutrients, PUFAs and vitamins (κ = 0.21-0.4). Exceptions in both sexes were butter, margarine and tea, which showed moderate tracking levels (κ = 0.41-0.6). Furthermore, oil, fat, carbohydrates and retinol, in females, and alpha-tocopherol in males showed only slight tracking levels (κ = 0.01-0.20). Both females and males tracked significantly for all food groups, macronutrients, PUFAs and vitamins (i.e. significantly more subjects remained in the same relative tertile from baseline to follow-up than expected by chance).

Table 4 Tracking coefficients and percentage of individuals tracking in females and males

	Females		Males	
	Coefficient (κ_w)[a]	%[b]	Coefficient (κ_w)[a]	%[b]
Expected[c]		33.3		33.3
Fruit	0.259 (0.20;0.32)	45.9	0.389 (0.33;0.45)	54.2
Vegetables	0.311 (0.25;0.37)	49.8	0.309 (0.25;0.37)	47.7
Starchy vegetables	0.371 (0.31;0.43)	52.1	0.313 (0.25;0.37)	48.9
Whole grains	0.245 (0.18;0.31)	46.8	0.263 (0.20;0.33)	46.2
Refined grains	0.238 (0.18;0.30)	44.8	0.221 (0.16;0.28)	44.0
Meat	0.273 (0.21;0.33)	46.3	0.259 (0.20;0.32)	46.0
Fish	0.287 (0.23;0.35)	47.3	0.286 (0.22;0.35)	46.3
Egg	0.224 (0.16;0.28)	44.0	0.259 (0.20;0.32)	46.9
Nuts and seeds	0.217 (0.16;0.28)	43.1	0.298 (0.23;0.36)	48.7
Butter	0.451 (0.40;0.51)	57.9	0.481 (0.42;0.54)	60.3
Margarine	0.469 (0.41;0.52)	59.3	0.455 (0.40;0.51)	58.6
Oils	0.185 (0.12;0.25)	42.8	0.263 (0.20;0.33)	47.5
Dairy	0.252 (0.19;0.31)	46.3	0.286 (0.22;0.35)	48.0
Sugar sweetened foods	0.259 (0.20;0.32)	47.0	0.240 (0.18;0.30)	46.2
Caloric drinks	0.315 (0.25;0.37)	50.1	0.389 (0.33;0.45)	53.8
Tea	0.428 (0.37;0.48)	56.8	0.432 (0.37;0.49)	56.0
Water	0.311 (0.25;0.37)	48.5	0.391 (0.33;0.45)	54.0
Protein	0.220 (0.16;0.28)	43.2	0.259 (0.20;0.32)	46.0
Fat	0.196 (0.14;0.26)	41.4	0.225 (0.16;0.29)	44.3
Carbohydrate	0.189 (0.13;0.25)	40.6	0.240 (0.18;0.30)	45.2
n3 PUFA	0.238 (0.18;0.30)	45.7	0.217 (0.15;0.28)	43.6
n6 PUFA	0.224 (0.16;0.28)	44.6	0.240 (0.18;0.30)	45.5
Retinol [mg/d]	0.196 (0.13;0.26)	44.3	0.313 (0.25;0.37)	49.1
Beta Carotene [mg/d]	0.304 (0.24;0.36)	49.5	0.332 (0.27;0.39)	49.9
Vitamin C [mg/d]	0.259 (0.20;0.32)	47.1	0.202 (0.14;0.27)	43.6
alpha tocopherol [mg/d]	0.206 (0.15;0.27)	42.8	0.126 (0.06;0.19)	38.0

[a]Tracking coefficient of weighted Cohen's Kappa (95 % CI)
[b]Individuals (%) remaining in the same relative tertile from baseline to follow-up
[c]Expected (%) individuals remaining in the same tertile assuming unity

Associations with dietary changes

Dietary changes presenting significant associations (change vs. tracking) with any of parental education level, family income, child education level, pubertal onset, BMI and screen-time are shown in Tables 5 and 6 for females and males respectively. Results for the regression analyses on the remaining food groups, macronutrients, PUFAs, or vitamins are presented in Additional file 3: Table S3.

Females with higher compared to lower parental education level, and with low (T1) baseline nut intakes, were more likely to increase nut intake over time [OR = 3.8; 95 % CI = (1.7, 8.8)]. Similarly, high parental education females were less likely to reduce medium (T2) vitamin C intakes [0.2 (0.1, 0.5)]. Females with medium (T2) baseline whole grain intakes and medium family income, were less likely to reduce their intakes [0.2 (0.1, 0.7)] than females with a low family income; whereas those with high family income were less likely to increase their whole grain intakes [0.1 (0.0, 0.5)]. Females with medium family income and medium (T2) baseline retinol intake were less likely to decrease their intakes [0.2 (0.1, 0.6)]. Furthermore, high family income level females with high (T3) vitamin C intakes were less likely to reduce their intakes over time [0.2 (0.1, 0.6)]. Finally, BMI in females was negatively associated with decreasing high (T3) protein intakes [0.7 (0.6, 0.9)], i.e. higher BMI females were more likely to maintain high protein intakes at follow-up than to reduce them.

Compared to low parental education, males with high parental education, and low (T1) baseline egg intakes, were less likely to increase their egg consumption [0.2 (0.1, 0.5)]. Similarly, those with low n3 PUFA intakes were less likely to increase their intakes [0.2 (0.1, 0.5)]. Children with high education level and low (T1) baseline sugar-sweetened food intakes were less likely to increase their intakes [0.1 (0.1, 0.4)]. BMI in males was positively associated with increased margarine [1.3 (1.1, 1.6)] and vitamin C intakes [1.3 (1.1, 1.6)], when baseline intakes were low (T1); whilst a negative association was seen with increasing n3 PUFA [0.7 (0.6, 0.9)], i.e. higher BMI males were more likely to increase low baseline margarine and vitamin C intakes, and to maintain low n3 PUFA intakes at follow-up.

Table 5 Associations[a] with dietary intake changes stratified by baseline intake tertile in females

Reference	Tracking in T1[b]	Tracking in T2[c]		Tracking in T3[d]
Change	Increase	Increase	Decrease	Decrease
Whole grains				
ParEdu high	1.8 (0.7;4.2)	1.1 (0.4;3.1)	0.6 (0.2;1.6)	0.7 (0.3;1.9)
Income med	1.2 (0.5;2.8)	0.3 (0.1;0.8)	0.2 (0.1;0.7)*	0.4 (0.2;1.1)
Income high	0.6 (0.2;1.7)	0.1 (0.0;0.5)*	0.3 (0.1;1.0)	1.2 (0.4;3.1)
ChildEdu high	0.9 (0.4;2.0)	1.7 (0.6;4.7)	1.7 (0.6;4.4)	0.6 (0.2;1.3)
Puberty yes	0.9 (0.4;1.8)	0.9 (0.4;2.1)	1.1 (0.5;2.5)	0.8 (0.4;1.6)
BMI	0.9 (0.8;1.1)	1.0 (0.8;1.2)	1.1 (0.9;1.3)	1.1 (0.9;1.3)
Screen high	0.3 (0.1;1.1)	0.2 (0.0;2.2)	2.2 (0.6;7.7)	3.0 (0.5;16.9)
Nuts				
ParEdu high	3.8 (1.7;8.8)*	1.8 (0.6;5.4)	0.8 (0.3;2.1)	0.6 (0.2;1.4)
Income med	0.5 (0.2;1.1)	1.9 (0.7;5.2)	3.2 (1.0;9.8)	1.8 (0.7;4.4)
Income high	0.4 (0.1;1.1)	0.6 (0.2;1.8)	2.1 (0.6;6.7)	1.4 (0.5;3.8)
ChildEdu high	0.8 (0.4;1.7)	0.8 (0.3;2.0)	1.1 (0.4;3.0)	1.4 (0.6;3.1)
Puberty yes	1.1 (0.6;2.3)	1.1 (0.5;2.6)	1.3 (0.6;3.1)	0.5 (0.3;1.0)
BMI	0.9 (0.8;1.1)	1.0 (0.9;1.3)	0.9 (0.8;1.1)	0.9 (0.8;1.1)
Screen high	0.4 (0.1;1.2)	1.4 (0.3;7.3)	2.2 (0.5;10.4)	0.5 (0.1;2.1)
Protein				
ParEdu High	1.5 (0.7;3.4)	1.0 (0.4;2.7)	1.1 (0.4;2.9)	0.3 (0.1;0.9)
Income med	0.9 (0.4;2.1)	0.4 (0.1;1.1)	0.7 (0.2;2.1)	0.6 (0.2;1.6)
Income high	1.3 (0.6;3.3)	0.9 (0.3;2.7)	1.8 (0.6;5.9)	0.6 (0.2;2.1)
ChildEdu high	0.6 (0.3;1.3)	0.7 (0.3;1.8)	0.5 (0.2;1.2)	0.9 (0.4;2.2)
Puberty yes	1.0 (0.5;2.1)	1.4 (0.6;3.2)	1.1 (0.5;2.5)	1.1 (0.5;2.3)
BMI	1.0 (0.8;1.2)	1.0 (0.9;1.2)	1.0 (0.8;1.2)	0.7 (0.6;0.9)*
Sed high	0.8 (0.3;2.2)	1.0 (0.2;4.5)	0.2 (0.0;2.0)	2.5 (0.7;9.1)
Retinol				
ParEdu High	0.7 (0.3;1.7)	1.2 (0.5;3.1)	1.3 (0.5;3.7)	1.1 (0.5;2.4)
Income med	0.8 (0.3;2.0)	0.6 (0.2;1.8)	0.2 (0.1;0.6)*	0.6 (0.2;1.4)
Income high	1.2 (0.5;3.4)	0.7 (0.2;2.4)	0.2 (0.1;0.7)	0.8 (0.3;2.1)
ChildEdu high	0.9 (0.4;2.2)	1.1 (0.4;2.8)	0.3 (0.1;0.8)	0.6 (0.3;1.4)
Puberty yes	2.1 (1.0;4.3)	0.7 (0.3;1.7)	0.3 (0.1;0.9)	0.9 (0.5;1.9)
BMI	1.0 (0.9;1.2)	1.0 (0.8;1.2)	1.0 (0.8;1.2)	1.2 (1.0;1.4)
Sed high	0.6 (0.2;1.9)	0.8 (0.2;2.8)	0.2 (0.0;1.0)	0.2 (0.0;1.1)

Table 5 Associations[a] with dietary intake changes stratified by baseline intake tertile in females *(Continued)*

Vitamin C				
ParEdu High	1.0 (0.4;2.3)	0.6 (0.2;1.7)	0.2 (0.1;0.5)*	1.4 (0.6;3.3)
Income med	0.5 (0.2;1.1)	1.5 (0.5;4.5)	1.0 (0.3;2.9)	0.3 (0.1;0.8)
Income high	0.4 (0.2;1.1)	3.2 (0.9;10.6)	1.3 (0.4;4.6)	0.2 (0.1;0.6)*
ChildEdu high	1.5 (0.7;3.3)	0.6 (0.3;1.5)	0.8 (0.3;2.0)	2.2 (0.9;5.2)
Puberty yes	1.5 (0.7;3.2)	0.4 (0.2;0.9)	0.7 (0.3;1.9)	1.3 (0.6;2.5)
BMI	1.0 (0.8;1.1)	1.2 (1.0;1.5)	1.2 (1.0;1.4)	1.1 (1.0;1.3)
Sed high	0.8 (0.3;2.3)	0.9 (0.2;5.0)	1.2 (0.2;6.5)	1.7 (0.5;6.2)

ParEdu high: parental education (high vs. low); Income med/high: family income (medium/high vs. low); ChildEdu high: child education (high vs. low); Puberty yes: pubertal onset at baseline (yes vs. no); Screen high: screen-time at baseline (high vs. low).

*p-value < 0.0083 (Bonferroni correction for multiple testing: 0.05/6)

[a]Odds ratio (95 % CI)

[b]Logistic regression (increase vs. tracking in lowest tertile)

[c]Multinomial logistic regression (increase or decrease vs. tracking in medium tertile)

[d]Logsitic regression (decrease vs. tracking in highest tertile)

Table 6 Associations[a] with dietary intake changes stratified by baseline intake tertile in males

Reference	Tracking in T1[b]	Tracking in T2[c]		Tracking in T3[d]
Change	Increase	Increase	Decrease	Decrease
Egg[e]				
ParEdu high	0.2 (0.1;0.5)*	0.6 (0.2;1.9)	0.7 (0.3;2.1)	0.7 (0.3;1.7)
Income med	2.2 (0.8;6.0)	1.2 (0.4;3.6)	1.0 (0.4;3.0)	1.2 (0.5;3.0)
Income high	2.1 (0.7;6.4)	0.4 (0.1;1.5)	0.8 (0.3;2.4)	1.6 (0.6;4.1)
ChildEdu high	1.0 (0.4;2.5)	3.0 (1.0;9.2)	1.2 (0.4;3.1)	1.4 (0.6;3.4)
Puberty yes	2.3 (0.6;8.7)	7.3 (1.3;39.9)	3.5 (0.6;19.9)	1.8 (0.6;5.1)
BMI	0.9 (0.8;1.1)	1.0 (0.8;1.2)	0.9 (0.8;1.1)	1.0 (0.9;1.1)
SedBeh high	0.5 (0.2;1.3)	1.3 (0.3;5.3)	0.7 (0.1;3.3)	1.4 (0.4;4.4)
Margarine[e]				
ParEdu high	0.5 (0.2;1.5)	0.9 (0.3;2.8)	3.8 (0.9;15.2)	1.0 (0.4;2.5)
Income med	1.5 (0.5;4.6)	0.5 (0.2;1.5)	0.9 (0.3;3.3)	1.3 (0.5;3.4)
Income high	1.3 (0.4;3.8)	0.7 (0.2;2.3)	0.7 (0.2;2.9)	3.6 (1.1;11.5)
ChildEdu high	0.8 (0.3;2.3)	0.4 (0.1;1.2)	0.2 (0.1;0.9)	0.6 (0.2;1.3)
Puberty yes	0.9 (0.2;3.2)	1.5 (0.4;6.3)	2.1 (0.5;9.4)	1.6 (0.4;6.1)
BMI	1.3 (1.1;1.6)*	0.8 (0.7;1.0)	0.9 (0.8;1.2)	1.0 (0.9;1.2)
SedBeh high	1.5 (0.4;6.4)	0.9 (0.3;3.4)	1.2 (0.3;5.0)	2.8 (0.9;8.8)
Sugar-sweetened foods				
ParEdu high	1.9 (0.7;5.3)	0.6 (0.2;1.9)	2.5 (0.6;10.7)	1.1 (0.4;2.7)
Income med	1.6 (0.5;4.5)	3.0 (0.9;10.0)	5.5 (1.4;22.5)	1.2 (0.5;2.9)
Income high	2.4 (0.7;7.5)	0.9 (0.3;3.0)	0.8 (0.2;3.3)	0.8 (0.3;2.3)
ChildEdu high	0.1 (0.1;0.4)*	2.5 (0.8;7.6)	2.4 (0.6;8.9)	0.7 (0.3;1.6)
Puberty yes	1.3 (0.4;3.8)	0.3 (0.0;1.7)	0.5 (0.1;3.5)	11.3 (1.3;98.4)
BMI	0.9 (0.7;1.0)	0.8 (0.6;1.0)	1.0 (0.8;1.3)	0.9 (0.8;1.1)
SedBeh high	2.1 (0.5;8.6)	2.1 (0.5;9.0)	4.0 (0.7;23.9)	1.0 (0.4;2.8)
n3 PUFA				
ParEdu high	0.2 (0.1;0.5)*	0.5 (0.1;1.6)	1.0 (0.3;3.4)	1.0 (0.4;2.4)
Income med	1.4 (0.5;3.9)	1.5 (0.4;5.1)	0.9 (0.3;3.0)	1.4 (0.6;3.4)
Income high	1.6 (0.6;4.4)	1.4 (0.4;5.0)	1.4 (0.4;4.8)	1.5 (0.6;4.1)
ChildEdu high	0.6 (0.2;1.7)	0.9 (0.3;2.5)	0.8 (0.3;2.1)	1.1 (0.5;2.5)
Puberty yes	1.2 (0.3;4.5)	1.0 (0.3;4.0)	0.5 (0.1;2.1)	0.8 (0.3;2.7)
BMI	0.7 (0.6;0.9)*	0.8 (0.7;1.0)	1.0 (0.8;1.2)	1.1 (0.9;1.3)
SedBeh high	0.3 (0.1;0.9)	1.3 (0.3;5.7)	0.6 (0.1;3.0)	1.1 (0.3;3.8)

Table 6 Associations[a] with dietary intake changes stratified by baseline intake tertile in males *(Continued)*

Vitamin C				
ParEdu high	0.6 (0.2;1.5)	1.0 (0.3;3.1)	0.7 (0.2;2.0)	2.1 (0.8;5.5)
Income med	1.1 (0.5;2.8)	0.7 (0.2;2.3)	0.5 (0.2;1.5)	1.5 (0.6;4.2)
Income high	1.3 (0.5;3.7)	0.7 (0.2;2.6)	0.7 (0.2;2.3)	1.5 (0.5;4.0)
ChildEdu high	1.6 (0.7;4.1)	1.3 (0.5;3.5)	1.4 (0.5;3.7)	1.2 (0.5;3.2)
Puberty yes	1.2 (0.3;4.1)	0.7 (0.1;4.2)	1.6 (0.4;6.9)	1.7 (0.5;5.6)
BMI	1.3 (1.1;1.6)*	1.0 (0.8;1.2)	1.0 (0.8;1.2)	0.9 (0.8;1.1)
SedBeh high	0.5 (0.2;1.5)	1.6 (0.5;5.2)	0.8 (0.2;3.0)	4.2 (0.9;19.3)

ParEdu high: parental education (high vs. low); Income med/high: family income (medium/high vs. low); ChildEdu high: child education (high vs. low); Puberty yes: pubertal onset at baseline (yes vs. no); Screen high: screen-time at baseline (high vs. low)

*p-value < 0.0083 (Bonferroni correction for multiple testing: 0.05/6)

[a]Odds ratio (95 % CI)

[b]Logistic regression (increase vs. tracking in lowest tertile)

[c]Multinomial logistic regression (increase or decrease vs. tracking in medium tertile)

[d]Logsitic regression (decrease vs. tracking in highest tertile)

[e]Multinomial regression not adjusted for diet change

Discussion

In the present study we evaluated changes in intakes of 17 food groups, as well as macronutrients, and antioxidant vitamins, using repeated FFQ data from the 10- and 15-year follow-up assessments of the German GINIplus birth cohort study. We observed overall dietary intake changes occurring within the study population, evaluated individual levels of dietary stability (tracking), and identified socio-economic factors, and individual characteristics and behaviours which may be associated with specific dietary changes during the transition from childhood to adolescence.

The few studies available describing habitual dietary intake during puberty, differ in terms of study design, follow-up period, data collection methods, age of subjects, and study location [11, 13, 31, 32, 38, 39]. Dietary behaviours observed, range from specific food items or food groups [13, 31, 38–40] to broader dietary patterns, including a range of foods [11, 16, 41]. Comparison with other studies is hence limited, especially because available longitudinal studies are scarce; however, despite these differences, some similarities and inconsistencies between our and previous study findings, are worth mentioning.

Average changes in dietary intake

Average intakes of food groups changed significantly in both males and females. Both sexes presented a decrease in intakes of starchy vegetables, dairy and margarine, and an increase in total water intake. Meat intake increased in males and decreased in females (mainly due to subjects changing towards a vegetarian or vegan diet). Males also reduced fruit intake and increased caloric drinks, while females increased intakes of whole and refined grains, vegetables, oils and tea. As in our study, a study in Swedish adolescents aged 15 at baseline, and followed up at ages 17 and 21, reported that changes in food group intakes in males were less frequent than in females, suggesting a greater tendency in females to modify their diet during pubertal maturation and throughout adolescence [38]. Nevertheless, these changes did not seem to impact the overall intakes of macronutrients and vitamins in females, who presented only decreased retinol intakes. As meat and dairy are sources of this vitamin [42], the reduced consumption of these food groups in females might explain the lower retinol intakes. Males however, significantly increased protein and fat intakes, as well as n3 and n6 PUFAs, vitamin C and alpha-tocopherol, and decreased carbohydrates. Furthermore, food groups presenting changes in the previously mentioned study were similar to those in our study: females decreased fat spread, milk and meat intakes, and increased pasta intake from 15 to 17 years. At 21 years females had further reduced their meat intake and males had reduced fruit intake [38]. An increased consumption of caloric drinks in adolescence has also been observed previously in Norwegian [13] and German populations, especially in males [40].

Dietary tracking

Dietary tracking assessed the stability of food intakes within the study population. Females and males presented "fair" levels of tracking for all food groups, except for butter, margarine, and tea, which revealed stronger tracking; and oil, fat, carbohydrate and retinol in females and alpha-tocopherol in males, which showed only slight tracking. Previous studies on tracking of dietary behaviour in females and males during puberty have reported similar (slight to moderate) tracking levels for food groups such as fruit and vegetables [13, 16], caloric drinks [13, 31], dairy [31] or meat [11], among others. The present results suggest a possible overlap of dietary behaviours observed in other countries, although this may be limited due to sociocultural differences. We noted that food groups indicating greater stability were also amongst those presenting highly significant changes in average intakes. For example, average margarine intake decreased significantly over time, but margarine also presented the highest tracking coefficients in both females and males. These results are not necessarily contradictory as it is possible for a child to significantly modify his/her intake of a specific food group, while remaining in the same position relative to others in the sample (indicative of tracking). We performed further sensitivity analyses to determine if specifically non-tracking participants were responsible for the observed changes, but this was not the case. These results suggest that in our study sample, average intake changes observed during puberty in food groups such as margarine, starchy vegetables, fruit and caloric drinks, follow sex-specific secular trends, where the "order of the children by intake" remains but the overall median intakes are altered.

Associations with dietary changes

In the present study, the association of dietary intake changes, with selected indicators of socio-economic status and individual characteristics differed amongst females and males for different food groups, macronutrients and vitamins, and according to baseline intake levels. Studies on the determinants of changes in dietary intake during puberty are limited. Wang et al. [11], reported that children's dietary intake patterns can be predicted by family income, urban-rural residence, maternal education and baseline dietary intakes. In our study we observed significant associations of dietary intake with parental education, family income, child education and BMI. Given that the consumption of nuts, whole grains, vitamin C and retinol are frequently associated with

health-benefits [42–44], our findings suggest that higher SES in females, represented by higher parental education and family income, may promote an increased consumption (nuts) or at least the maintenance of higher intakes (whole grain, vitamin C and retinol) of certain healthier foods and nutrients during puberty. On the other hand, our results also indicate that females with lower family income were more likely to increase whole grain intakes than those with high income. Despite typically being more expensive [45], increasing whole grain products may be an attainable goal in children with less resources making efforts to improve their diet as they grow older. In males, higher parental education was associated with maintenance of low egg and n3 PUFA intakes as opposed to increasing intakes. Egg intake has been previously associated with unhealthy lipid profiles in humans [46]. Adolescent males with higher educated parents may be more informed with regards to dietary advice [47], and eggs may hence be eaten sparingly. Eggs are also a source of n3 PUFA, which may in turn remain low in the same male subgroup of parental education, even though n3 PUFA has been associated with beneficial health effects [48]. Higher child education in males was associated with tracking low intakes of sugar-sweetened foods, rather than increasing them. Those with higher education levels may be more aware of the negative relationship between health and carbohydrate-rich diets, especially sugar [49, 50], and hence attempt to lower their intakes [51].

Higher BMI was associated with tracking of high protein intake in females. In males BMI was positively associated with increasing margarine, and vitamin C, and with maintenance of n3 PUFA levels in the lowest baseline intake tertiles. High BMI is often associated with unhealthy dietary behaviours [52–54], however in the present study BMI does not seem to be a predominant predictor of unhealthy dietary change during adolescence. This could be due to common underreporting of unhealthy foods in overweight subjects [55, 56] (margarine may be regarded as healthy and hence not underreported, given its lower content of saturated fats compared to butter [57]). The lack of associations with BMI could also be explained by possible earlier influences of the exposure variable on food intake at baseline. Dietary behaviours already established before the baseline assessment could indicate an intake threshold was reached before puberty, impeding further change in that direction, e.g. higher BMI was associated with high starchy vegetable, meat, water and protein intakes at baseline (see Additional file 1: Table S1). Similarly, parental education level, child education and screen-time also showed significant differences in intakes of a number of food groups at baseline (e.g. higher parental education associated with higher intakes of grains, butter and oil and lower intakes of starchy vegetables and margarine; higher child education with higher fruit, wholegrain and butter intakes and lower intakes of starchy vegetables, meat and sugar-sweetened foods; and higher screen-time associated with lower fruit, vegetables, wholegrain and beta-carotene in both females and males). However earlier influences must be interpreted with caution, as these were cross-sectional associations and reverse causality cannot be excluded as there is no previous dietary data available for longitudinal analyses before the 10 year assessments. We hence highlight the importance of longitudinal analyses in investigating associations with dietary intake changes.

Strengths and limitations

The present study benefits from a large study population of males and females within two distinct German regions. The longitudinal nature of this study, covering a 5-year period from childhood into adolescence, is a key aspect which allows us to add to the limited knowledge regarding dietary behaviour changes during adolescence. The large amount of descriptive data, obtained from the GINIplus cohort, along with comprehensive dietary data from the food frequency questionnaires, provide a thorough overview of habitual dietary intake during two key stages, as well as possible determinants of changes in intakes during pubertal maturation.

Several possible shortcomings of the study must be considered. Even though study sampling was primarily population-based, our study population for analysis is, as in every cohort study, subject to selection bias, and thus the findings cannot be considered as representative for the study area. Owing to non-random loss-to-follow-up, the cohort on which the present analysis is based underrepresents children from lower social classes. The true social inequalities might therefore be even stronger than reported here. This would also explain the relatively few associations with parental education observed in our study despite the literature suggesting otherwise [11, 38].

The large number of food groups assessed, and the possibility that they may be correlated, increases the chance for type 1 error. We tried to account for this by using Bonferroni correction for multiple testing, lowering our two-sided alpha level to 0.0083. Furthermore, thorough analyses of interaction effects between independent variables were not possible. Despite our large sample size, analyses by baseline intake levels and sex already resulted in partly small groups, and hence the data could not provide enough power for further stratification.

The FFQ used in the present study was designed with a special focus on energy, antioxidant and fatty acid intake. Hence, the food item list may underestimate intakes of

other food items not included in the questionnaire. The same FFQ was administered at 10 and 15 years in order to use a consistent methodology to measure dietary changes over time. The FFQ was designed for measuring dietary intake in school-aged children, and validated using 24 h-dietary recalls. The test-retest performance of the questionnaire was not assessed, which is a limitation in the present study. Nevertheless, at 10 years it proved applicable and comprehensible, and produced highly plausible dietary estimates, justifying its use in future epidemiological studies [19]. A study testing the use of an FFQ in older children and adolescents aged 9-18 years, found it to be reproducible regardless of age [58]; and a review summarizing the validity and reliability of food frequency questionnaires in children and adolescents, reported mainly strong correlations in studies reporting test-retest reliability [59]. We hence believe that our results should not be majorly affected by this limitation. Furthermore, the FFQ was completed by a parent alongside the participant at baseline, and by the participants themselves with support of whoever cooked at home, at follow-up. It is generally believed that children before the age of 12 have difficulties recalling intakes and understanding portion sizes, and have a more limited knowledge of foods, all of which constrains their ability to self-report without parental assistance [60]. Furthermore, studies have reported that the parental indication of children's dietary intake appears to be moderately valid [28]. Therefore, a combined effort in the completion of the FFQ at baseline was considered appropriate to maximise response accuracy. Nevertheless, inter-reporter differences cannot be excluded, for example due to varying perceptions of quantification measures, or due to selective under- or over-reporting (in response to perceptions of social desirability). Therefore, the observed results could, to some extent represent reporting error at different time points, rather than actual dietary changes over time.

Finally, the possible role of secular trends shaping dietary intake over time cannot be excluded [61]. Nevertheless, in identifying possible determinants, intakes were categorised by tertiles and hence only changes large enough to produce a tertile shift over time (e.g. T1 to T2 or T3) were classified as changing. Therefore, while small changes which were common across the entire population could have indicated trends, our regression analyses most likely reveal individual associations with greater intake changes. Unfortunately, categorisation of data implies certain loss of information. However, using tertile categories rather than actual intakes, is commonly used to measure tracking [11, 16, 31, 32] and was preferred, in order to overcome the non-normal distribution of the dietary data, as well as possible problems of under- or over-reporting.

Conclusions

Average dietary intakes changed significantly from childhood to adolescence. Nevertheless a fair degree of tracking was observed, suggesting the presence of general, sex-specific trends in dietary behaviour during this period. Dietary intake changes were most frequently associated with socio-economic environment, where females with high SES tended towards healthier dietary behaviours. Associations with child education and BMI were also observed for some food groups and nutrients, while no effect was seen between intake changes and screen-time or pubertal onset. Our results support the rationale for dietary interventions targeting children in order to positively influence dietary changes during puberty. We suggest that sex-specific subpopulations, such as children with lower SES, or lower education levels, should be considered for further impact. We further highlight the need for longitudinal studies in this topic given its relevance in the development of public health nutrition strategies.

Additional files

Additional file 1: Associations between exposure variables and baseline food intake tertiles (PDF 628 kb)

Additional file 2: Comparison of lost-to-follow-up and not-lost-to-follow-up participants (PDF 264 kb)

Additional file 3: Associations with dietary intake changes stratified by baseline intake tertile (PDF 303 kb)

Abbreviations

BMI: Body mass index; FFQ: Food frequency questionnaire; %EI: Percentage contribution towards total energy intake; SES: Socio-economic status; SD: Standard deviation; OR: Odds ratio; CI: Confidence interval; T1: Lowest intake tertile; T2: Medium intake tertile; T3: Highest intake tertile.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CH and MS were involved in the conception and design of the study, DB, SK, CB and IB in the data acquisition, CH, MS, CF and ET in the statistical analyses, CH, MS, AB, BK and SK in the interpretation; CH drafted the manuscript and all authors revised it critically for important intellectual content and approved the final version to be published.

Acknowledgements

The authors thank all the families for their participation in the GINIplus study. Furthermore, we thank all members of the GINIplus Study Group for their excellent work. The GINIplus Study group consists of the following: Institute of Epidemiology I, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg (J. Heinrich, I. Brüske, H. Schulz, C. Flexeder, C. Zeller, M. Standl, M. Schnappinger, M. Sußmann, E. Thiering, C. Tiesler); Research Institute, Department of Pediatrics, Marien-Hospital, Wesel (D. Berdel, A. von Berg, B. Filipiak-Pittroff); Ludwig-Maximilians-University of Munich, Dr von Hauner Children's Hospital (S. Koletzko, K. Werkstetter); Department of pediatrics, Technische Universität München and Deutsche Rentenversicherung Bayern (C.P. Bauer, U. Hoffmann); IUF-Leibniz Institute for Environmental Research, Düsseldorf (B. Hoffmann, E. Link, C. Klümper, U. Krämer).

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Received: 21 May 2015 Accepted: 26 August 2015
Published online: 02 September 2015

References

1. Guidelines for school health programs to promote lifelong healthy eating. J Sch Health 1997, 67(1):9-26.
2. Katz DL, O'Connell M, Njike VY, Yeh MC, Nawaz H. Strategies for the prevention and control of obesity in the school setting: systematic review and meta-analysis. Int J Obes. 2008;32(12):1780–9.
3. Berenson GS, Srinivasan SR, Bao W, Newman 3rd WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med. 1998;338(23):1650–6.
4. Centers for Disease Control and Prevention (CDC). School health guidelines to promote healthy eating and physical activity. MMWR Recomm Rep. 2011;60(Rr-5):1–76.
5. Meredith CN, Dwyer JT. Nutrition and exercise: effects on adolescent health. Annu Rev Public Health. 1991;12:309–33.
6. Twisk JW, Kemper HC, Mellenbergh GJ. Mathematical and analytical aspects of tracking. Epidemiol Rev. 1994;16(2):165–83.
7. Madruga SW, Araujo CL, Bertoldi AD, Neutzling MB. Tracking of dietary patterns from childhood to adolescence. Rev Saude Publica. 2012;46(2):376–86.
8. Boulton TJ, Magarey AM, Cockington RA. Tracking of serum lipids and dietary energy, fat and calcium intake from 1 to 15 years. Acta paediatrica (Oslo, Norway : 1992). 1995;84(9):1050–5.
9. Resnicow K, Smith M, Baranowski T, Baranowski J, Vaughan R, Davis M. 2-year tracking of children's fruit and vegetable intake. J Am Diet Assoc. 1998;98(7):785–9.
10. Zive MM, Berry CC, Sallis JF, Frank GC, Nader PR. Tracking dietary intake in white and Mexican-American children from age 4 to 12 years. J Am Diet Assoc. 2002;102(5):683–9.
11. Wang Y, Bentley ME, Zhai F, Popkin BM. Tracking of dietary intake patterns of Chinese from childhood to adolescence over a six-year follow-up period. J Nutr. 2002;132(3):430–8.
12. Rasmussen M, Krolner R, Klepp K-I, Lytle L, Brug J, Bere E, et al. Determinants of fruit and vegetable consumption among children and adolescents: a review of the literature. Part I: quantitative studies. Int J Behav Nutr Phys Act. 2006;3(1):22.
13. Totland TH, Gebremariam MK, Lien N, Bjelland M, Grydeland M, Bergh IH, et al. Does tracking of dietary behaviours differ by parental education in children during the transition into adolescence? Public Health Nutr. 2013;16(4):673–82.
14. Gebremariam MK, Bergh IH, Andersen LF, Ommundsen Y, Totland TH, Bjelland M, et al. Are screen-based sedentary behaviors longitudinally associated with dietary behaviors and leisure-time physical activity in the transition into adolescence? Int J Behav Nutr Phys Act. 2013;10:9.
15. van Jaarsveld CH, Fidler JA, Simon AE, Wardle J. Persistent impact of pubertal timing on trends in smoking, food choice, activity, and stress in adolescence. Psychosom Med. 2007;69(8):798–806.
16. Li J, Wang Y. Tracking of dietary intake patterns is associated with baseline characteristics of urban low-income African-American adolescents. J Nutr. 2008;138(1):94–100.
17. Cusatis DC, Chinchilli VM, Johnson-Rollings N, Kieselhorst K, Stallings VA, Lloyd T. Longitudinal nutrient intake patterns of US adolescent women: the Penn State Young Women's Health Study. J Adolesc Health. 2000;26(3):194–204.
18. Berg A, Kramer U, Link E, Bollrath C, Heinrich J, Brockow I, et al. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. Clin Exp Allergy. 2010;40(4):627–36.
19. Stiegler P, Sausenthaler S, Buyken AE, Rzehak P, Czech D, Linseisen J, et al. A new FFQ designed to measure the intake of fatty acids and antioxidants in children. Public Health Nutr. 2010;13(1):38–46.
20. Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the German part of the EPIC project. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol. 1997;26 Suppl 1:S59–70.
21. Brandstetter BR, Korfmann A, Kroke A, Becker N, Schulze MB, Boeing H. Dietary habits in the German EPIC cohorts: food group intake estimated with the food frequency questionnaire. European Investigation into Cancer and Nutrition. Ann Nutr Metab. 1999;43(4):246–57.
22. McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011. Adv Nutr (Bethesda, Md). 2011;2(3):293–4.
23. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr. 2012;142(6):1009–18.
24. Shikany JM, Jacobs Jr DR, Lewis CE, Steffen LM, Sternfeld B, Carnethon MR, et al. Associations between food groups, dietary patterns, and cardiorespiratory fitness in the Coronary Artery Risk Development in Young Adults study. Am J Clin Nutr. 2013;98(6):1402–9.
25. Kennedy JF, Knill CJ. McCance and Widdowson's the Composition of Foods sixth summary edition: Food Standards Agency and Institute of Food Research, Royal Society of Chemistry, 2002, xv + 537 pages, ISBN 0-85404-428-0 (£45.00). Carbohydr Polym. 2003;54(3):393.
26. Preparation and use of food-based dietary guidelines. Report of a joint FAO/WHO consultation. FAO/WHO. World Health Organ Tech Rep Ser 1998, 880:i-vi, 1-108.
27. Sausenthaler S, Standl M, Buyken A, Rzehak P, Koletzko S, Bauer CP, et al. Regional and socio-economic differences in food, nutrient and supplement intake in school-age children in Germany: results from the GINIplus and the LISAplus studies. Public Health Nutr. 2011;14(10):1724–35.
28. Willett W. Nutritional Epidemiology. Oxford, USA: Oxford University Press; 1998.
29. Hartmann BM, Bell S, Vásquez-Caiquedo AL. Der Bundeslebensmittelschlüssel. German Nutrient DataBase. Karlsruhe: Federal Research Centre for Nutrition and Food (BfEL); 2005.
30. Carskadon MA, Acebo C. A self-administered rating scale for pubertal development. J Adolesc Health. 1993;14(3):190–5.
31. Patterson E, Warnberg J, Kearney J, Sjostrom M. The tracking of dietary intakes of children and adolescents in Sweden over six years: the European Youth Heart Study. Int J Behav Nutr Phys Act. 2009;6:91.
32. Robson PJ, Gallagher AM, Livingstone MB, Cran GW, Strain JJ, Savage JM, et al. Tracking of nutrient intakes in adolescence: the experiences of the Young Hearts Project, Northern Ireland. Br J Nutr. 2000;84(4):541–8.
33. Sim J, Wright CC. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. Phys Ther. 2005;85(3):257–68.
34. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159–74.
35. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.
36. Revelle W. Psych. In: Procedures for Personality and Psychological Research. 148th ed. Evanston, Illinois, USA: Northwestern University; 2014.
37. Venables W, Ripley B. Modern Applied Statistics with S. Fourthth ed. New York: Springer; 2002.
38. Von Post-Skagegard M, Samuelson G, Karlstrom B, Mohsen R, Berglund L, Bratteby LE. Changes in food habits in healthy Swedish adolescents during the transition from adolescence to adulthood. Eur J Clin Nutr. 2002;56(6):532–8.
39. Pearson N, Salmon J, Campbell K, Crawford D, Timperio A. Tracking of children's body-mass index, television viewing and dietary intake over five-years. Prev Med. 2011;53(4-5):268–70.
40. Libuda L, Alexy U, Sichert-Hellert W, Stehle P, Karaolis-Danckert N, Buyken AE, et al. Pattern of beverage consumption and long-term association with body-weight status in German adolescents—results from the DONALD study. Br J Nutr. 2008;99(6):1370–9.
41. Mikkila V, Rasanen L, Raitakari OT, Pietinen P, Viikari J. Consistent dietary patterns identified from childhood to adulthood: the cardiovascular risk in Young Finns Study. Br J Nutr. 2005;93(6):923–31.
42. EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies). Scientific opinion on dietary reference values for vitamin A. EFSA Journal. 2015;13(3):4028, 4084.
43. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary guidelines for Americans. Washington (DC): U.S. Government Printing Office; 2010.

44. EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies). Scientific opinion on dietary reference values for vitamin C. EFSA Journal. 2013;11(11):3418, 3468.
45. Drewnowski A, Darmon N. Food choices and diet costs: an economic analysis. J Nutr. 2005;135(4):900–4.
46. Weggemans RM, Zock PL, Katan MB. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. Am J Clin Nutr. 2001;73(5):885–91.
47. Hanson MD, Chen E. Socioeconomic status and health behaviors in adolescence: a review of the literature. J Behav Med. 2007;30(3):263–85.
48. Siriwardhana N, Kalupahana NS, Moustaid-Moussa N. Chapter 13 - Health Benefits of n-3 Polyunsaturated Fatty Acids: Eicosapentaenoic Acid and Docosahexaenoic Acid. In: Se-Kwon K, editor. Advances in Food and Nutrition Research, vol. 65. Waltham: Academic; 2012. p. 211–22.
49. Bray GA. Fructose: pure, white, and deadly? fructose, by any other name, is a health hazard. J Diabetes Sci Technol. 2010;4(4):1003–7.
50. Bray GA, Popkin BM. Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes?: health be damned! Pour on the sugar. Diabetes Care. 2014;37(4):950–6.
51. Lake AA, Hyland RM, Rugg-Gunn AJ, Wood CE, Mathers JC, Adamson AJ. Healthy eating: perceptions and practice (the ASH30 study). Appetite. 2007;48(2):176–82.
52. van der Sluis ME, Lien N, Twisk JW, Steenhuis IH, Bere E, Klepp KI, et al. Longitudinal associations of energy balance-related behaviours and cross-sectional associations of clusters and body mass index in Norwegian adolescents. Public Health Nutr. 2010;13(10a):1716–21.
53. Pan L, Li R, Park S, Galuska DA, Sherry B, Freedman DS. A longitudinal analysis of sugar-sweetened beverage intake in infancy and obesity at 6 years. Pediatrics. 2014;134 Suppl 1:S29–35.
54. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: WHO; 2009.
55. Pei Z, Flexeder C, Fuertes E, Standl M, Buyken A, Berdel D, et al. Food intake and overweight in school-aged children in Germany: Results of the GINIplus and LISAplus studies. Ann Nutr Metab. 2014;64(1):60–70.
56. Heitmann BL, Lissner L. Dietary underreporting by obese individuals–is it specific or non-specific. BMJ. 1995;311:986–9.
57. Centers for Disease Control and Prevention. Post-care: fats - saturated, unsaturated and trans fats [http://www.cdc.gov/diabetes/prevention/pdf/PostHandout_Session2.pdf]
58. Rockett HRH, Wolf AM, Colditz GA. Development and reproducibility of a food frequency questionnaire to assess diets of older children and adolescents. J Am Diet Assoc. 1995;95(3):336–40.
59. Kolodziejczyk JK, Merchant G, Norman GJ. Reliability and validity of child/adolescent food frequency questionnaires that assess foods and/or food groups. J Pediatr Gastroenterol Nutr. 2012;55(1):4–13.
60. Livingstone MBE, Robson PJ, Wallace JMW. Issues in dietary intake assessment of children and adolescents. Br J Nutr. 2004;92(SupplementS2):S213–22.
61. Müller-Nordhorn J, Rossnagel K, Mey W, Willich SN. Regional variation and time trends in mortality from ischaemic heart disease: East and West Germany 10 years after reunification. J Epidemiol Community Health. 2004;58(6):481–5.

Paper 4: Supplementary Material

Additional file 1. Associations between exposure variables and baseline food intake tertiles

Supplementary Table 1a Associations between exposure variables and baseline food intake tertiles in females

	Parental educ.[1]		Family income level[1]			School level[1]		Puberty[1]		BMI[2]	Screen time[1]	
	lower	higher	lower	med	higher	lower	higher	yes	no		lower	higher
N	178	445	168	229	195	210	386	294	339	589	578	53
Fruit												
T1	39.3	31.2	38.7	34.5	26.2	41.9	30.8	36.7	31.3	17.0 (2.4)	31.1	54.7
T2	31.5	34.4	31.0	32.8	37.4	34.3	31.1	29.9	36.9	16.6 (2.4)	33.9	30.2
T3	29.2	34.4	30.4	32.8	36.4	23.8	38.1	33.3	31.9	16.6 (2.3)	34.9	15.1
p-value	0.147		0.144			0.001*		0.153		0.285	0.001*	
Vegetable												
T1	41.0	30.8	33.9	36.2	31.3	38.1	31.1	33.0	33.6	16.7 (2.4)	31.3	52.8
T2	32.6	33.5	35.1	34.9	29.2	34.3	33.4	34.4	33.0	16.9 (2.3)	34.8	22.6
T3	26.4	35.7	31.0	28.8	39.5	27.6	35.5	32.7	33.3	16.6 (2.3)	33.9	24.5
p-value	0.026*		0.199			0.100		0.941		0.644	0.006*	
Starchy vegetables												
T1	23.6	37.5	29.8	35.4	36.4	25.2	36.8	34.0	33.0	16.8 (2.4)	34.3	28.3
T2	36.0	31.7	35.7	31.4	31.8	33.8	33.9	35.7	31.3	16.3 (1.8)	33.4	30.2
T3	40.4	30.8	34.5	33.2	31.8	41.0	29.3	30.3	35.7	17.1 (2.7)	32.4	41.5
p-value	0.003*		0.705			0.004*		0.305		0.008*	0.389	
Wholegrain												
T1	41.6	30.1	35.7	31.4	31.3	45.2	28.0	27.9	38.1	16.5 (2.4)	32.5	39.6
T2	30.3	35.3	31.5	37.1	33.3	29.5	35.2	36.7	30.7	16.8 (2.5)	33.6	35.8
T3	28.1	34.6	32.7	31.4	35.4	25.2	36.8	35.4	31.3	16.9 (2.2)	33.9	24.5
p-value	0.023*		0.707			<0.001*		0.025*		0.351	0.349	
Refined grain												
T1	41.6	29.7	37.5	28.4	33.8	37.6	32.4	33.7	32.4	16.5 (2.3)	32.7	37.7
T2	29.8	35.1	29.8	34.5	35.4	32.9	34.5	32.3	34.5	16.8 (2.4)	33.7	28.3
T3	28.7	35.3	32.7	37.1	30.8	29.5	33.2	34.0	33	16.9 (2.4)	33.6	34.0
p-value	0.017*		0.301			0.416		0.842		0.214	0.669	
Meat												
T1	26.4	35.7	29.2	35.4	33.8	27.6	35.5	31.6	34.8	16.4 (1.9)	33.6	34.0
T2	34.3	33.5	36.3	27.9	37.9	30.0	35.5	31.0	35.4	16.7 (2.3)	34.6	17.0
T3	39.3	30.8	34.5	36.7	28.2	42.4	29.0	37.4	29.8	17.1 (2.8)	31.8	49.1
p-value	0.046*		0.126			0.004*		0.125		0.013*	0.012*	
Fish												
T1	32.0	33.7	28.6	37.6	31.3	34.8	33.9	28.2	37.2	16.4 (2.2)	34.8	20.8
T2	33.7	33.7	35.7	30.6	33.8	29.5	35.5	33.0	33.6	16.7 (2.2)	32.2	43.4
T3	34.3	32.6	35.7	31.9	34.9	35.7	30.6	38.8	29.2	17.1 (2.6)	33.0	35.8
p-value	0.897		0.415			0.274		0.017*		0.019*	0.091	
Egg												
T1	30.9	33.7	31.0	36.2	32.8	36.2	31.9	33.7	33.0	16.7 (2.4)	32.4	41.5
T2	37.1	31.9	36.3	31.4	31.3	34.3	33.4	33.0	33.3	16.6 (2.3)	33.7	32.1
T3	32.0	34.4	32.7	32.3	35.9	29.5	34.7	33.3	33.6	16.9 (2.4)	33.9	26.4
p-value	0.465		0.696			0.385		0.986		0.446	0.352	
Nuts												
T1	38.2	31.7	31.0	34.1	34.9	33.8	33.7	33.0	33.9	16.9 (2.6)	32.5	45.3
T2	29.2	35.1	36.3	32.8	29.7	33.3	32.4	31.6	34.5	16.6 (2.2)	33.0	30.2
T3	32.6	33.3	32.7	33.2	35.4	32.9	33.9	35.4	31.6	16.7 (2.2)	34.4	24.5

p-value	0.231		0.760			0.958		0.571		0.297	0.143	
Butter												
T1	50.0	26.5	33.3	34.9	29.7	45.2	27.5	33.0	33.3	17.0 (2.7)	32.5	47.2
T2	25.3	36.9	33.9	29.7	39.5	27.6	36.0	31.3	35.7	16.7 (2.4)	33.6	30.2
T3	24.7	36.6	32.7	35.4	30.8	27.1	36.5	35.7	31.0	16.5 (2)	33.9	22.6
p-value	<0.001*		0.338			<0.001*		0.372		0.098	0.077	
Margarine												
T1	21.3	37.8	28.0	35.4	32.3	27.6	35.5	35.0	31.3	16.5 (2.1)	34.8	18.9
T2	35.4	32.4	36.9	29.3	36.9	32.9	33.4	33.0	33.6	16.7 (2.2)	33.0	34.0
T3	43.3	29.9	35.1	35.4	30.8	39.5	31.1	32.0	35.1	17.1 (2.7)	32.2	47.2
p-value	<0.001*		0.303			0.066		0.562		0.044*	0.031*	
Oil												
T1	39.3	31.2	31.5	32.8	35.4	37.1	31.3	31.0	35.4	17.0 (2.5)	33.6	35.8
T2	36.5	31.9	29.8	34.9	33.3	32.9	34.2	36.1	31.6	16.8 (2.5)	33.7	34.0
T3	24.2	36.9	38.7	32.3	31.3	30.0	34.5	33.0	33.0	16.5 (2.0)	32.7	30.2
p-value	0.009*		0.569			0.323		0.391		0.093	0.919	
Dairy												
T1	36.0	32.8	38.1	34.5	29.2	39.5	29.8	32.3	35.1	16.7 (2.5)	32.2	47.2
T2	34.3	33.3	30.4	32.8	35.9	30.0	35.0	32.3	33.3	16.8 (2.3)	33.4	34.0
T3	29.8	33.9	31.5	32.8	34.9	30.5	35.2	35.4	31.6	16.7 (2.2)	34.4	18.9
p-value	0.582		0.505			0.055		0.577		0.786	0.033*	
Sugar-sweetened food												
T1	30.3	34.2	31.5	31.4	38.5	27.6	35.0	35.7	31.3	16.7 (2.2)	34.1	18.9
T2	32.6	33.7	28.0	37.6	30.8	31.9	34.2	33.3	33.3	16.9 (2.4)	33.0	39.6
T3	37.1	32.1	40.5	31.0	30.8	40.5	30.8	31.0	35.4	16.6 (2.4)	32.9	41.5
p-value	0.464		0.085			0.045*		0.393		0.447	0.077	
Caloric drinks												
T1	32.0	34.2	33.9	37.6	29.7	34.3	31.9	32.7	34.2	17.0 (2.5)	34.3	22.6
T2	27.5	35.3	35.7	31.0	32.8	31.4	35.0	35.7	31.3	16.7 (2.2)	34.1	28.3
T3	40.4	30.6	30.4	31.4	37.4	34.3	33.2	31.6	34.5	16.6 (2.3)	31.7	49.1
p-value	0.045*		0.376			0.670		0.486		0.282	0.032*	
Tea [g/d]												
T1	39.3	31.2	27.4	39.3	30.8	38.6	30.6	33.3	33.6	16.8 (2.4)	33.2	32.1
T2	32.0	33.7	33.3	33.2	33.8	31.9	35.5	34.4	32.4	16.8 (2.5)	33.2	37.7
T3	28.7	35.1	39.3	27.5	35.4	29.5	33.9	32.3	33.9	16.7 (2.2)	33.6	30.2
p-value	0.125		0.061			0.140		0.861		0.846	0.787	
Water [g/d]												
T1	39.3	31.7	31.5	30.1	39.0	34.3	32.4	33.7	32.7	16.2 (2.0)	31.8	50.9
T2	28.1	35.7	35.1	32.3	32.3	31.9	33.4	30.3	36.6	16.8 (2.6)	34.8	20.8
T3	32.6	32.6	33.3	37.6	28.7	33.8	34.2	36.1	30.7	17.2 (2.3)	33.4	28.3
p-value	0.111		0.243			0.883		0.195		<0.001*	0.014*	
Protein												
T1	33.1	33.9	35.7	31.9	31.8	32.9	34.7	32.0	34.8	16.1 (1.9)	33.0	37.7
T2	30.3	34.6	32.7	33.2	34.4	30.5	35.2	31.3	34.8	16.7 (2.3)	34.4	22.6
T3	36.5	31.5	31.5	34.9	33.8	36.7	30.1	36.7	30.4	17.4 (2.7)	32.5	39.6
p-value	0.426		0.915			0.235		0.238		<0.001*	0.215	
Fat												
T1	33.7	33.5	31.0	32.3	34.9	30.0	33.7	32.3	33.9	16.6 (2.3)	33.7	28.3
T2	33.7	32.6	31.5	31.9	39.0	31.9	35.5	32.0	34.8	16.9 (2.2)	33.6	32.1
T3	32.6	33.9	37.5	35.8	26.2	38.1	30.8	35.7	31.3	16.7 (2.5)	32.7	39.6
p-value	0.942		0.148			0.199		0.488		0.504	0.559	
Carbohydrates												
T1	34.8	33.0	36.9	38.0	25.1	36.2	32.4	35.7	31.9	16.9 (2.6)	33.2	35.8

T2	30.9	33.9	31.5	28.4	42.1	32.9	33.9	31.6	34.5	16.8 (2.2)	33.0	37.7
T3	34.3	33.0	31.5	33.6	32.8	31.0	33.7	32.7	33.6	16.5 (2.3)	33.7	26.4
p-value	0.765		0.015*			0.625		0.567		0.282	0.547	
n3 PUFA												
T1	31.5	33.9	31.0	33.6	34.4	30.5	33.9	28.6	36.9	16.5 (2.2)	32.5	37.7
T2	34.3	32.6	32.7	32.3	35.4	35.2	33.7	35.7	31.3	16.7 (2.2)	34.3	26.4
T3	34.3	33.5	36.3	34.1	30.3	34.3	32.4	35.7	31.9	17.0 (2.6)	33.2	35.8
p-value	0.833		0.789			0.689		0.086		0.076	0.499	
n6 PUFA												
T1	32.0	33.9	29.2	34.5	36.4	29.5	35.2	29.3	36.6	16.5 (2)	34.8	20.8
T2	31.5	33.7	30.4	29.3	37.4	33.8	33.7	33.0	33.6	16.9 (2.5)	33.0	37.7
T3	36.5	32.4	40.5	36.2	26.2	36.7	31.1	37.8	29.8	16.8 (2.5)	32.2	41.5
p-value	0.610		0.043*			0.270		0.062		0.206	0.109	
Retinol [mg/d]												
T1	25.8	36.2	29.8	28.8	40.0	27.6	35.2	33.7	33.9	16.7 (2.4)	33.2	34.0
T2	34.3	32.6	36.9	34.5	29.2	34.3	33.9	32	33.3	16.7 (2.3)	33.2	37.7
T3	39.9	31.2	33.3	36.7	30.8	38.1	30.8	34.4	32.7	16.8 (2.3)	33.6	28.3
p-value	0.030*		0.113			0.101		0.899		0.977	0.702	
Beta Carotene [mg/d]												
T1	42.1	29.9	37.5	31.0	34.4	38.1	31.1	28.9	38.1	16.9 (2.5)	31.5	54.7
T2	37.1	32.6	30.4	34.9	29.7	33.8	32.6	35.0	31.6	16.6 (2.2)	33.2	32.1
T3	20.8	37.5	32.1	34.1	35.9	28.1	36.3	36.1	30.4	16.7 (2.3)	35.3	13.2
p-value	<0.001*		0.616			0.092		0.05		0.382	0.001*	
Vitamin C [mg/d]												
T1	32.0	33.5	34.5	34.5	32.8	34.8	33.2	30.6	36.3	16.8 (2.2)	32.2	49.1
T2	34.8	32.6	33.3	35.8	30.8	34.8	31.3	36.7	30.1	16.7 (2.5)	33.9	22.6
T3	33.1	33.9	32.1	29.7	36.4	30.5	35.5	32.7	33.6	16.6 (2.3)	33.9	28.3
p-value	0.861		0.666			0.448		0.162		0.713	0.040*	
alpha tocopherol [mg/d]												
T1	34.3	32.1	34.5	30.6	33.8	32.9	33.2	29.6	36.6	16.7 (2.3)	33.4	35.8
T2	29.2	35.3	28.0	35.8	35.4	30.5	35.5	33.7	33.3	16.8 (2.4)	32.7	39.6
T3	36.5	32.6	37.5	33.6	30.8	36.7	31.3	36.7	30.1	16.7 (2.3)	33.9	24.5
p-value	0.340		0.422			0.337		0.110		0.944	0.354	

[1] Presented as percentage and tested using Pearson's χ^2 test for count data; [2] Presented as mean (standard deviation) and tested using one-way analysis of variance; *p-value <0·05

Supplementary Table 1b Associations between exposure variables and baseline food intake tertiles in males

	Parental educ.[1]		Family income level[1]			School level[1]		Puberty[1]		BMI[2]	Screen time[1]	
	lower	higher	lower	med	higher	lower	higher	yes	no		lower	higher
N	211	357	166	196	174	215	337	63	516	527	523	61
Fruit												
T1	39.3	30.8	41.0	33.2	27.6	38.6	29.4	36.5	33.1	16.8 (2.3)	31.0	52.5
T2	33.6	33.3	28.9	33.2	34.5	30.2	34.4	27.0	34.1	16.9 (2.3)	34.0	27.9
T3	27.0	35.9	30.1	33.7	37.9	31.2	36.2	36.5	32.8	16.7 (2.3)	35.0	19.7
p-value	0.049*		0.138			0.079		0.526		0.726	0.002*	
Vegetable												
T1	33.6	34.5	38.6	34.7	28.2	38.1	30.0	28.6	34.1	16.7 (2.3)	31.4	49.2
T2	38.4	30.3	33.7	31.6	32.2	33.5	33.5	36.5	32.8	16.9 (2.1)	33.8	27.9
T3	28.0	35.3	27.7	33.7	39.7	28.4	36.5	34.9	33.1	16.9 (2.5)	34.8	23.0
p-value	0.088		0.164			0.073		0.668		0.579	0.017*	
Starchy vegetables												
T1	27.0	37.5	28.9	34.2	35.6	30.2	35.9	49.2	31.4	16.4 (2.1)	34.0	29.5
T2	31.3	34.5	34.9	32.1	33.9	28.4	35.6	23.8	34.7	16.8 (2.1)	33.3	32.8
T3	41.7	28.0	36.1	33.7	30.5	41.4	28.5	27.0	33.9	17.4 (2.6)	32.7	37.7
p-value	0.002*		0.675			0.007*		0.017*		<0.001*	0.687	
Wholegrain												
T1	44.1	27.7	37.3	31.1	33.3	42.8	28.2	27.0	34.7	16.9 (2.3)	31.2	52.5
T2	33.2	33.3	31.3	33.2	33.9	31.6	34.1	31.7	32.9	16.8 (2.4)	34.6	24.6
T3	22.7	38.9	31.3	35.7	32.8	25.6	37.7	41.3	32.4	16.7 (2.1)	34.2	23.0
p-value	<0.001*		0.776			0.001*		0.310		0.742	0.004*	
Refined grain												
T1	33.2	33.6	30.1	33.2	32.8	34.4	32.6	28.6	34.1	16.7 (2.4)	33.8	31.1
T2	36.5	31.7	37.3	30.1	33.9	40.0	29.7	31.7	33.3	16.8 (2.2)	32.9	36.1
T3	30.3	34.7	32.5	36.7	33.3	25.6	37.7	39.7	32.6	17.0 (2.3)	33.3	32.8
p-value	0.425		0.691			0.006*		0.494		0.542	0.866	
Meat												
T1	27.5	36.4	33.7	35.2	31.0	27.0	37.1	30.2	33.3	16.3 (2.0)	34.0	29.5
T2	35.1	31.7	32.5	31.6	37.4	33.5	33.2	33.3	33.5	16.9 (2.4)	32.9	34.4
T3	37.4	31.9	33.7	33.2	31.6	39.5	29.7	36.5	33.1	17.3 (2.3)	33.1	36.1
p-value	0.089		0.805			0.020*		0.834		<0.001*	0.772	
Fish												
T1	32.7	33.6	33.1	31.1	33.9	35.3	32.0	34.9	32.8	16.6 (2.1)	33.3	31.1
T2	32.2	34.5	33.7	35.2	32.8	31.2	35.9	25.4	34.7	17.1 (2.4)	32.7	41.0
T3	35.1	31.9	33.1	33.7	33.3	33.5	32.0	39.7	32.6	16.8 (2.4)	34.0	27.9
p-value	0.732		0.982			0.502		0.305		0.086	0.404	
Egg												
T1	35.5	31.9	30.7	35.2	32.2	36.7	31.5	34.9	33.3	17.0 (2.4)	32.5	41.0
T2	30.8	35.3	31.9	34.2	32.8	33.0	33.2	30.2	33.3	16.6 (2.1)	33.8	27.9
T3	33.6	32.8	37.3	30.6	35.1	30.2	35.3	34.9	33.3	16.9 (2.4)	33.7	31.1
p-value	0.510		0.738			0.348		0.880		0.187	0.393	
Nuts												
T1	37.0	31.1	34.3	31.1	32.2	39.5	29.4	27.0	34.1	16.9 (2.4)	32.1	44.3
T2	31.3	33.9	30.1	35.2	33.3	32.1	34.1	38.1	33.1	16.9 (2.4)	33.8	27.9
T3	31.8	35.0	35.5	33.7	34.5	28.4	36.5	34.9	32.8	16.7 (2.1)	34.0	27.9
p-value	0.356		0.895			0.033*		0.511		0.822	0.164	
Butter												
T1	48.8	24.9	39.8	31.6	32.2	44.7	26.1	27.0	34.1	17.0 (2.4)	31.9	47.5
T2	29.4	35.6	31.9	32.7	32.2	31.6	33.8	44.4	32.0	16.9 (2.4)	33.3	31.1

T3	21.8	39.5	28.3	35.7	35.6	23.7	40.1	28.6	33.9	16.5 (2.0)	34.8	21.3
p-value	<0.001*		0.413			<0.001*		0.138		0.097	0.030*	
Margarine												
T1	23.2	38.7	27.7	39.3	35.1	23.3	40.4	38.1	32.8	16.7 (2.1)	34.4	23.0
T2	33.6	32.8	31.9	29.6	35.6	36.3	31.5	33.3	33.1	16.7 (2.2)	32.9	37.7
T3	43.1	28.6	40.4	31.1	29.3	40.5	28.2	28.6	34.1	17.1 (2.5)	32.7	39.3
p-value	<0.001*		0.085			<0.001*		0.608		0.189	0.195	
Oil												
T1	41.7	29.1	39.8	34.7	24.1	40.5	29.4	22.2	34.7	16.7 (2.3)	32.7	42.6
T2	33.6	33.1	33.1	30.6	38.5	34.0	32.6	38.1	32.9	17.0 (2.4)	32.7	37.7
T3	24.6	37.8	27.1	34.7	37.4	25.6	38.0	39.7	32.4	16.8 (2.2)	34.6	19.7
p-value	0.001*		0.024*			0.004*		0.137		0.539	0.058	
Dairy												
T1	37.0	31.9	34.9	33.2	31.6	34.0	32.6	41.3	32.9	16.9 (2.4)	32.3	42.6
T2	33.2	31.9	34.9	31.6	34.5	31.6	34.7	27.0	33.9	16.7 (2.3)	33.3	32.8
T3	29.9	36.1	30.1	35.2	33.9	34.4	32.6	31.7	33.1	16.9 (2.2)	34.4	24.6
p-value	0.272		0.848			0.753		0.369		0.617	0.190	
Sugar-sweetened food												
T1	26.1	37.3	25.9	35.2	41.4	30.2	36.2	41.3	32.6	17.1 (2.3)	34.2	27.9
T2	33.6	33.1	33.1	33.7	33.3	30.7	34.7	30.2	33.3	16.9 (2.1)	34.2	26.2
T3	40.3	29.7	41.0	31.1	25.3	39.1	29.1	28.6	34.1	16.5 (2.4)	31.5	45.9
p-value	0.009*		0.013*			0.050		0.376		0.057	0.078	
Caloric drinks												
T1	32.2	34.5	30.7	34.2	31.0	32.1	34.1	33.3	33.5	16.8 (2.3)	34.2	24.6
T2	32.7	33.1	37.3	31.6	32.8	31.6	34.4	38.1	32.8	16.9 (2.3)	32.5	39.3
T3	35.1	32.5	31.9	34.2	36.2	36.3	31.5	28.6	33.7	16.8 (2.3)	33.3	36.1
p-value	0.793		0.767			0.500		0.628		0.867	0.298	
Tea [g/d]												
T1	36.5	30.8	28.9	34.7	35.1	39.5	30.9	30.2	33.9	16.9 (2.4)	32.7	39.3
T2	34.6	33.6	37.3	28.6	32.8	33.5	32.3	30.2	33.7	16.9 (2.4)	33.1	36.1
T3	28.9	35.6	33.7	36.7	32.2	27.0	36.8	39.7	32.4	16.7 (2.1)	34.2	24.6
p-value	0.210		0.415			0.034*		0.507		0.595	0.302	
Water [g/d]												
T1	39.3	30.0	41.6	28.1	33.9	37.7	30.6	28.6	34.5	16.5 (2.3)	33.5	37.7
T2	30.3	35.0	28.9	38.3	33.3	28.8	36.8	34.9	32.9	16.8 (2.2)	33.1	34.4
T3	30.3	35.0	29.5	33.7	32.8	33.5	32.6	36.5	32.6	17.2 (2.4)	33.5	27.9
p-value	0.073		0.105			0.106		0.633		0.035*	0.657	
Protein												
T1	30.3	34.7	36.7	32.7	32.2	30.2	35.3	25.4	34.3	16.4 (2.1)	33.3	34.4
T2	34.1	33.3	33.7	33.7	33.3	33.0	33.8	30.2	33.9	16.7 (2.2)	32.5	41.0
T3	35.5	31.9	29.5	33.7	34.5	36.7	30.9	44.4	31.8	17.4 (2.5)	34.2	24.6
p-value	0.517		0.847			0.299		0.117		<0.001*	0.256	
Fat												
T1	34.1	32.2	29.5	38.8	31.6	29.8	34.7	28.6	33.7	16.6 (2.3)	33.3	34.4
T2	33.2	33.3	36.1	31.1	36.8	32.1	35.0	36.5	33.1	17.1 (2.4)	33.5	32.8
T3	32.7	34.5	34.3	30.1	31.6	38.1	30.3	34.9	33.1	16.8 (2.1)	33.3	32.8
p-value	0.874		0.387			0.153		0.707		0.083	0.984	
Carbohydrates												
T1	34.6	33.6	31.9	27.6	38.5	36.3	31.8	42.9	32.4	17.1 (2.3)	33.7	31.1
T2	33.2	33.6	34.9	38.3	28.7	35.8	32.0	33.3	33.5	16.9 (2.3)	33.3	34.4
T3	32.2	32.8	33.1	34.2	32.8	27.9	36.2	23.8	34.1	16.4 (2.2)	33.1	34.4
p-value	0.972		0.200			0.129		0.161		0.019*	0.926	
n3 PUFA												

T1	31.8	34.5	33.1	35.7	31.6	30.2	36.2	31.7	33.5	16.4 (1.9)	32.3	42.6
T2	30.3	35.0	35.5	31.6	33.3	31.6	32.3	34.9	33.1	16.9 (2.4)	34.0	27.9
T3	37.9	30.5	31.3	32.7	35.1	38.1	31.5	33.3	33.3	17.3 (2.4)	33.7	29.5
p-value	0.189		0.868			0.210		0.948		0.001*	0.267	
n6 PUFA												
T1	28.9	35.9	27.7	36.2	36.8	30.2	36.2	28.6	33.7	16.4 (2.1)	33.3	36.1
T2	36.5	31.7	35.5	32.1	28.7	33.0	32.0	31.7	33.7	16.8 (2.3)	32.9	36.1
T3	34.6	32.5	36.7	31.6	34.5	36.7	31.8	39.7	32.6	17.2 (2.4)	33.8	27.9
p-value	0.220		0.337			0.303		0.504		0.005*	0.644	
Retinol [mg/d]												
T1	32.7	34.2	33.1	32.7	35.1	34.0	33.2	31.7	33.1	16.5 (2.1)	32.9	37.7
T2	31.8	33.1	31.3	32.7	35.1	30.2	36.5	28.6	33.9	16.8 (2.4)	34.2	26.2
T3	35.5	32.8	35.5	34.7	29.9	35.8	30.3	39.7	32.9	17.1 (2.4)	32.9	36.1
p-value	0.796		0.824			0.249		0.530		0.045*	0.451	
Beta Carotene [mg/d]												
T1	39.3	30.3	34.3	30.6	34.5	43.7	26.7	27.0	33.7	16.7 (2.3)	31.5	47.5
T2	35.5	32.8	36.1	34.7	28.7	33.0	34.1	41.3	32.4	17.2 (2.2)	32.9	36.1
T3	25.1	37.0	29.5	34.7	36.8	23.3	39.2	31.7	33.9	16.6 (2.3)	35.6	16.4
p-value	0.010*		0.469			<0.001*		0.335		0.057	0.006*	
Vitamin C [mg/d]												
T1	37.0	31.7	36.1	37.2	28.2	37.7	31.2	30.2	33.9	16.6 (2.2)	32.7	36.1
T2	31.8	34.7	30.7	32.1	37.4	30.7	35.3	23.8	34.7	16.8 (2.1)	33.1	36.1
T3	31.3	33.6	33.1	30.6	34.5	31.6	33.5	46.0	31.4	17.1 (2.5)	34.2	27.9
p-value	0.431		0.369			0.268		0.053		0.073	0.609	
alpha tocopherol [mg/d]												
T1	36.5	31.4	31.3	38.8	31.6	35.8	33.2	31.7	33.5	16.6 (2.2)	32.7	37.7
T2	34.1	33.9	36.1	31.6	32.2	33.5	32.3	19.0	34.9	16.8 (2.3)	33.8	29.5
T3	29.4	34.7	32.5	29.6	36.2	30.7	34.4	49.2	31.6	17.1 (2.3)	33.5	32.8
p-value	0.332		0.432			0.651		0.009*		0.136	0.695	

[1] Presented as percentage and tested using Pearson's χ^2 test for count data; [2] Presented as mean (standard deviation) and tested using one-way analysis of variance; *p-value <0·05

Additional file 2. Comparison of lost-to-follow-up and not-lost-to-follow-up participants

Table 2 Comparison of lost-to-follow-up and not-lost-to-follow-up participants

	LTF	NLTF	p-value
N	680	1232	
Sex[1]			
Boys	55.3	47.8	0.002*
Girls	44.7	52.2	
Parental education level[1,3]			
Low (≤ 10 years)	40.1	32.7	0.002*
High (> 10 years)	59.9	67.3	
Family income level[1,4]			
Lower	36.0	29.6	0.020*
Middle	32.8	37.7	
Higher	31.2	32.7	
Child education level[1,3]			
Low (≤ 10 years)	47.1	37.0	<0.001*
High (> 10 years)	52.9	63.0	
Pubertal onset at BL[1]			
Yes	27.0	29.5	0.287
No	73.0	70.5	
Pubertal stage at FU[1]			
Pre-pubertal	0.3	0.6	0.736
Early puberty	2.6	1.9	
Mid puberty	21.2	18.8	
Late puberty	67.2	70.6	
Post-pubertal	8.7	8.1	
BMI [kg/m][2]	17.0 (2.4)	16.8 (2.3)	0.071
Screen-time at BL[1,5]			
Low (≤ 2h)	85.1	90.6	0.002*
High (> 2h)	14.9	9.4	
Age at BL [y][2]	11.0 (0.5)	11.0 (0.5)	0.877
Energy intake at BL [kcal/day][2]	1975.1 (581.1)	1962.4 (542.2)	0.638
Study center[1]			
Munich	40.1	52.5	<0.001*
Wesel	59.9	47.5	
Study arm[1]			
Control group	54.9	55.0	1.000
Infant intervention	45.1	45.0	

LTF=lost-to-follow-up (data at baseline only); NLTF=not-lost-to-follow-up (data at baseline and follow-up); BL=baseline; FU=follow-up; [1]Presented as percentages, tested by Fisher's exact test (variables with 2 levels), or by Pearson's Chi^2 test (variables with > 2 levels) ; [2]Presented as mean (standard deviation), tested by t-test; [3]Highest level achieved by mother or father or achievable in the case of child education ;[4]Tertiles stratified by study centre and merged; [5] Hours spent on screen-behaviours; *p-value<0.05.

Additional file 3. Associations with dietary intake changes stratified by baseline intake tertile

Supplementary Table 3a Associations[1] with dietary intake changes stratified by baseline tertile in females

Reference	**Tracking in T1[2]**	**Tracking in T2[3]**		**Tracking in T3[4]**
Change	**Increase**	**Increase**	**Decrease**	**Decrease**
Fruit				
ParEdu high	0.6 (0.3;1.5)	1.1 (0.4;3.0)	1.0 (0.4;2.5)	0.5 (0.2;1.3)
Income med	2.0 (0.9;4.7)	1.0 (0.3;3.2)	1.1 (0.4;3.3)	1.2 (0.5;2.9)
Income high	1.9 (0.7;5.2)	0.8 (0.2;2.5)	1.0 (0.3;3.1)	1.0 (0.4;2.6)
ChildEdu high	1.9 (0.9;4.2)	0.9 (0.4;2.5)	0.8 (0.3;1.9)	1.7 (0.7;4.1)
Puberty yes	1.3 (0.7;2.7)	0.8 (0.3;2.0)	1.5 (0.7;3.4)	0.8 (0.4;1.5)
BMI	1.0 (0.8;1.1)	1.1 (0.9;1.3)	1.0 (0.8;1.2)	0.9 (0.8;1.0)
Sed high	1.2 (0.5;3.1)	0.8 (0.1;5.3)	1.6 (0.4;7.2)	2.6 (0.4;16.3)
Vegetables				
ParEdu high	1.3 (0.6;3.0)	0.5 (0.2;1.3)	0.3 (0.1;0.8)	0.8 (0.3;2.0)
Income med	0.4 (0.2;1.0)	2.2 (0.7;6.8)	1.2 (0.4;4.3)	0.6 (0.2;1.4)
Income high	0.4 (0.1;0.9)	1.7 (0.5;6.2)	2.0 (0.5;7.6)	0.5 (0.2;1.2)
ChildEdu high	0.8 (0.4;1.7)	1.1 (0.4;3.1)	1.3 (0.4;3.8)	0.5 (0.2;1.1)
Puberty yes	1.2 (0.6;2.4)	1.0 (0.4;2.2)	3.0 (1.3;7.4)	0.8 (0.4;1.5)
BMI	1.0 (0.9;1.2)	1.1 (0.9;1.3)	0.8 (0.7;1.1)	0.9 (0.8;1.1)
Sed high	0.5 (0.2;1.4)	1.5 (0.3;7.1)	1.4 (0.3;7.9)	0.4 (0.1;2.1)
Starchy vegetables				
ParEdu high	0.5 (0.2;1.4)	1.9 (0.6;5.7)	0.7 (0.3;2.0)	0.4 (0.1;0.9)
Income med	2.2 (0.9;5.7)	1.4 (0.4;4.4)	1.1 (0.4;3.1)	0.6 (0.2;1.6)
Income high	2.4 (0.9;7.0)	1.3 (0.4;4.2)	1.1 (0.4;3.0)	1.4 (0.5;4.1)
ChildEdu high	0.7 (0.3;1.6)	4.2 (1.4;12.0)	2.6 (1.0;6.5)	1.8 (0.8;4.1)
Puberty yes	0.7 (0.4;1.4)	0.8 (0.3;1.9)	0.4 (0.2;0.9)	1.0 (0.4;2.1)
BMI	1.0 (0.9;1.2)	1.2 (0.9;1.5)	1.0 (0.8;1.3)	1.0 (0.8;1.1)
Sed high	1.5 (0.4;5.3)	0.8 (0.2;4.2)	0.9 (0.2;4.2)	0.9 (0.3;3.0)
Refined grain				
ParEdu high	1.7 (0.7;4.0)	1.5 (0.5;4.2)	1.3 (0.5;3.5)	0.9 (0.4;2.1)
Income med	0.8 (0.3;2.1)	1.3 (0.4;4.3)	0.5 (0.2;1.4)	1.4 (0.6;3.4)
Income high	0.5 (0.2;1.4)	0.6 (0.2;2.2)	0.4 (0.1;1.4)	1.0 (0.4;2.6)
ChildEdu high	2.3 (1.0;5.1)	0.9 (0.3;2.3)	0.5 (0.2;1.2)	0.9 (0.4;1.9)
Puberty yes	1.0 (0.5;2.1)	1.2 (0.5;2.9)	1.2 (0.5;2.7)	0.7 (0.4;1.5)
BMI	1.0 (0.8;1.1)	1.1 (0.9;1.3)	1.0 (0.9;1.2)	1.0 (0.8;1.1)
Sed high	2.6 (0.7;9.6)	2.1 (0.4;11.1)	2.1 (0.5;9.3)	1.9 (0.6;6.1)
Meat				
ParEdu high	1.0 (0.4;2.5)	1.7 (0.6;4.2)	1.3 (0.5;3.3)	0.9 (0.4;1.9)
Income med	1.1 (0.4;2.7)	0.8 (0.3;2.2)	1.6 (0.5;4.8)	0.4 (0.2;1.0)
Income high	1.4 (0.5;3.7)	0.7 (0.2;2.0)	1.5 (0.5;4.6)	0.4 (0.1;1.1)
ChildEdu high	0.4 (0.2;0.9)	0.5 (0.2;1.2)	0.5 (0.2;1.4)	1.4 (0.6;3.1)
Puberty yes	1.7 (0.8;3.5)	1.1 (0.5;2.4)	1.4 (0.6;3.2)	1.4 (0.7;3.0)
BMI	1.0 (0.8;1.2)	1.1 (0.9;1.3)	1.0 (0.8;1.2)	0.9 (0.8;1.0)
Sed high	2.3 (0.6;8.3)	0.2 (0.0;1.7)	0.6 (0.1;3.0)	0.4 (0.1;1.3)
Fish				
ParEdu high	1.2 (0.5;2.8)	0.7 (0.3;1.9)	0.8 (0.3;2.2)	0.4 (0.2;1.0)
Income med	0.6 (0.3;1.5)	2.1 (0.7;6.2)	1.2 (0.4;3.4)	1.9 (0.7;5.0)
Income high	0.5 (0.2;1.3)	1.8 (0.6;5.2)	1.2 (0.4;3.4)	1.8 (0.6;4.9)
ChildEdu high	1.2 (0.6;2.7)	0.7 (0.3;1.8)	0.8 (0.3;2.0)	1.2 (0.5;2.7)
Puberty yes	0.8 (0.4;1.5)	0.8 (0.3;1.8)	0.9 (0.4;1.9)	0.6 (0.3;1.2)
BMI	1.1 (0.9;1.3)	1.1 (0.9;1.3)	1.2 (0.9;1.4)	1.1 (0.9;1.2)
Sed high	1.6 (0.4;6.5)	1.6 (0.4;5.9)	0.5 (0.1;2.2)	2.2 (0.7;7.2)

Egg				
ParEdu high	0.8 (0.4;1.9)	1.5 (0.5;4.2)	1.7 (0.6;4.5)	0.5 (0.2;1.3)
Income med	0.6 (0.3;1.6)	2.2 (0.8;6.0)	2.0 (0.7;5.5)	1.2 (0.5;2.9)
Income high	0.8 (0.3;2.0)	3.2 (1.0;10.4)	2.6 (0.8;8.4)	0.6 (0.2;1.6)
ChildEdu high	1.1 (0.5;2.3)	0.3 (0.1;0.8)	0.5 (0.2;1.3)	1.7 (0.7;3.9)
Puberty yes	1.6 (0.8;3.2)	1.1 (0.5;2.7)	0.6 (0.3;1.5)	1.7 (0.8;3.4)
BMI	1.1 (0.9;1.3)	1.2 (1.0;1.5)	1.1 (0.9;1.4)	1.0 (0.9;1.2)
Sed high	0.8 (0.3;2.3)	2.2 (0.5;10.3)	1.8 (0.4;8.7)	0.4 (0.1;1.8)
Butter				
ParEdu high	0.9 (0.4;2.0)	0.7 (0.2;2.0)	0.8 (0.3;2.6)	0.9 (0.3;2.2)
Income med	3.5 (1.3;9.2)	0.5 (0.1;1.4)	1.3 (0.4;3.9)	0.7 (0.3;1.7)
Income high	1.3 (0.4;4.1)	0.9 (0.3;2.8)	1.6 (0.5;5.5)	1.3 (0.5;3.3)
ChildEdu high	0.6 (0.3;1.3)	2.4 (0.8;7.4)	1.0 (0.4;2.5)	0.6 (0.3;1.4)
Puberty yes	1.9 (0.9;4.1)	1.0 (0.4;2.4)	0.8 (0.3;1.8)	1.0 (0.5;2.1)
BMI	1.0 (0.8;1.1)	0.9 (0.7;1.1)	1.0 (0.9;1.3)	1.0 (0.9;1.3)
Sed high	0.8 (0.2;2.7)	1.0 (0.2;4.9)	0.9 (0.2;3.9)	2.0 (0.5;7.4)
Margarine				
ParEdu high	0.3 (0.1;0.7)	0.9 (0.3;2.2)	1.7 (0.5;5.6)	1.0 (0.5;2.1)
Income med	1.4 (0.5;3.8)	0.8 (0.3;2.3)	0.3 (0.1;1.1)	0.7 (0.3;1.6)
Income high	1.1 (0.3;3.4)	0.2 (0.1;0.8)	0.3 (0.1;0.8)	1.1 (0.4;2.9)
ChildEdu high	0.8 (0.3;2.0)	1.6 (0.6;4.3)	1.2 (0.4;3.5)	0.8 (0.3;1.6)
Puberty yes	0.9 (0.4;1.9)	1.1 (0.5;2.4)	1.5 (0.6;3.7)	1.9 (1.0;3.9)
BMI	1.0 (0.8;1.2)	1.1 (0.9;1.3)	1.0 (0.8;1.3)	0.9 (0.8;1.0)
Sed high	0.6 (0.1;3.5)	1.9 (0.5;7.2)	0.3 (0.0;2.5)	0.9 (0.3;2.7)
Oil				
ParEdu high	1.3 (0.6;2.9)	0.9 (0.3;2.3)	0.8 (0.3;2.0)	1.3 (0.5;3.2)
Income med	1.8 (0.7;4.5)	0.9 (0.3;2.8)	2.1 (0.6;7.0)	0.9 (0.4;2.0)
Income high	1.3 (0.5;3.6)	0.8 (0.2;2.8)	2.0 (0.5;7.6)	0.8 (0.3;1.9)
ChildEdu high	1.4 (0.6;2.8)	1.3 (0.5;3.5)	0.8 (0.3;2.2)	0.8 (0.3;1.8)
Puberty yes	1.7 (0.8;3.5)	0.9 (0.4;2.2)	0.9 (0.4;2.1)	1.3 (0.6;2.5)
BMI	1.0 (0.9;1.2)	1.1 (0.9;1.3)	1.0 (0.8;1.2)	1.1 (0.9;1.3)
Sed high	0.6 (0.2;2.0)	3.6 (0.8;16.6)	0.8 (0.1;5.3)	1.0 (0.3;3.0)
Dairy				
ParEdu high	1.1 (0.5;2.4)	1.0 (0.4;2.7)	0.9 (0.3;2.5)	0.4 (0.2;1.0)
Income med	0.9 (0.4;2.0)	0.7 (0.2;2.0)	0.8 (0.2;2.4)	0.6 (0.2;1.4)
Income high	0.4 (0.1;0.9)	0.3 (0.1;0.9)	0.5 (0.1;1.7)	0.5 (0.2;1.4)
ChildEdu high	1.4 (0.6;2.9)	1.8 (0.6;5.2)	1.1 (0.4;3.3)	0.9 (0.4;2.0)
Puberty yes	1.4 (0.7;2.7)	0.9 (0.4;2.0)	0.5 (0.2;1.3)	1.1 (0.5;2.3)
BMI	1.0 (0.9;1.1)	1.1 (0.9;1.3)	1.1 (0.9;1.3)	0.8 (0.7;1.0)
Sed high	0.9 (0.3;2.4)	0.8 (0.1;4.3)	2.9 (0.7;12.0)	1.8 (0.3;10.4)
Sugar-sweetened food				
ParEdu high	0.8 (0.4;2.0)	0.3 (0.1;0.9)	1.2 (0.4;3.7)	0.8 (0.3;1.7)
Income med	1.3 (0.5;3.3)	2.9 (0.9;9.1)	1.1 (0.3;3.5)	3.1 (1.3;7.5)
Income high	1.0 (0.4;2.7)	2.5 (0.7;9.2)	1.7 (0.5;6.0)	1.3 (0.5;3.2)
ChildEdu high	0.9 (0.4;2.1)	0.6 (0.2;1.6)	0.4 (0.1;1.1)	0.6 (0.3;1.3)
Puberty yes	1.7 (0.8;3.3)	0.6 (0.2;1.4)	0.7 (0.3;1.8)	1.8 (0.9;3.5)
BMI	1.0 (0.8;1.2)	1.0 (0.8;1.2)	1.0 (0.8;1.2)	1.1 (1.0;1.3)
Sed high	0.7 (0.1;3.7)	1.3 (0.3;5.5)	1.7 (0.4;6.7)	0.6 (0.2;1.9)
Caloric drinks				
ParEdu high	0.7 (0.3;1.7)	1.1 (0.4;2.8)	2.3 (0.7;7.5)	0.4 (0.2;0.9)
Income med	1.1 (0.4;2.7)	0.9 (0.3;2.6)	1.1 (0.4;3.4)	2.3 (0.9;5.9)
Income high	1.9 (0.7;5.6)	0.9 (0.3;2.6)	1.4 (0.4;4.3)	0.9 (0.3;2.4)
ChildEdu high	0.6 (0.2;1.3)	0.3 (0.1;0.8)	0.6 (0.2;1.8)	2.3 (1.0;5.4)
Puberty yes	1.3 (0.7;2.7)	0.8 (0.4;1.9)	1.3 (0.5;2.9)	0.9 (0.4;1.8)
BMI	1.0 (0.9;1.2)	1.0 (0.9;1.3)	1.0 (0.8;1.2)	1.1 (0.9;1.2)
Sed high	0.6 (0.1;2.7)	1.3 (0.2;7.8)	2.0 (0.3;12.3)	1.1 (0.4;3.3)

Tea [ml/d]				
ParEdu high	1.8 (0.8;4.3)	1.0 (0.3;2.8)	0.6 (0.2;1.6)	0.8 (0.3;2.1)
Income med	0.6 (0.2;1.7)	1.3 (0.4;3.8)	0.6 (0.2;1.9)	0.7 (0.3;1.7)
Income high	0.7 (0.2;2.2)	0.9 (0.3;2.9)	0.5 (0.1;1.7)	0.7 (0.3;1.8)
ChildEdu high	1.5 (0.7;3.4)	0.9 (0.3;2.5)	1.0 (0.4;2.7)	0.8 (0.3;1.8)
Puberty yes	1.7 (0.8;3.5)	2.2 (1.0;5.0)	1.0 (0.4;2.4)	1.1 (0.5;2.2)
BMI	1.0 (0.8;1.1)	0.8 (0.7;1.0)	1.0 (0.9;1.2)	1.0 (0.9;1.2)
Sed high	0.9 (0.3;3.3)	1.0 (0.3;3.6)	0.3 (0.1;1.8)	3.5 (0.8;15.4)
Water [ml/d]				
ParEdu high	0.8 (0.3;1.7)	1.0 (0.3;2.8)	1.0 (0.4;3.1)	1.6 (0.6;3.9)
Income med	1.3 (0.5;3.1)	1.0 (0.3;2.8)	0.5 (0.2;1.4)	0.8 (0.3;2.0)
Income high	0.9 (0.3;2.2)	0.9 (0.3;3.0)	1.3 (0.5;4.0)	0.8 (0.3;2.4)
ChildEdu high	1.7 (0.8;3.9)	1.1 (0.4;2.9)	0.5 (0.2;1.3)	0.4 (0.2;1.0)
Puberty yes	1.2 (0.6;2.4)	1.3 (0.5;3.1)	1.7 (0.7;4.1)	0.9 (0.4;1.8)
BMI	1.1 (0.9;1.3)	1.1 (0.9;1.3)	1.1 (0.9;1.3)	0.9 (0.8;1.1)
Sed high	0.7 (0.3;2.0)	3.6 (0.3;41.4)	2.7 (0.3;29.8)	0.6 (0.2;2.5)
Fat				
ParEdu high	0.7 (0.3;1.4)	0.5 (0.2;1.5)	0.6 (0.2;1.8)	0.7 (0.3;1.7)
Income med	1.1 (0.5;2.6)	2.2 (0.7;6.5)	1.9 (0.6;6.3)	0.8 (0.4;1.8)
Income high	0.8 (0.3;2.1)	2.6 (0.8;8.4)	2.0 (0.5;7.2)	1.1 (0.4;3.0)
ChildEdu high	0.9 (0.4;2.0)	1.2 (0.5;2.9)	1.1 (0.4;3.1)	0.8 (0.4;1.6)
Puberty yes	0.9 (0.5;1.8)	0.8 (0.3;1.7)	0.4 (0.2;1.0)	1.0 (0.5;2.0)
BMI	0.9 (0.8;1.1)	1.1 (0.9;1.3)	1.1 (0.9;1.4)	1.0 (0.9;1.2)
Sed high	0.7 (0.2;2.4)	2.3 (0.5;10.7)	2.6 (0.5;13.2)	1.6 (0.5;5.4)
Carbohydrate				
ParEdu high	0.8 (0.3;1.9)	0.4 (0.1;1.2)	0.6 (0.2;1.6)	1.2 (0.5;2.6)
Income med	0.7 (0.3;1.7)	2.6 (0.8;8.3)	1.1 (0.3;3.4)	1.5 (0.6;3.5)
Income high	0.8 (0.3;2.0)	2.1 (0.6;7.2)	1.3 (0.4;4.0)	0.7 (0.3;1.9)
ChildEdu high	1.6 (0.7;3.5)	1.0 (0.4;2.5)	2.5 (0.9;6.4)	0.6 (0.3;1.4)
Puberty yes	1.6 (0.8;3.2)	1.1 (0.5;2.6)	1.5 (0.7;3.4)	1.1 (0.5;2.2)
BMI	1.0 (0.8;1.1)	1.0 (0.8;1.3)	1.2 (1.0;1.4)	1.0 (0.9;1.2)
Sed high	1.1 (0.3;3.8)	2.6 (0.5;12.7)	2.2 (0.5;9.8)	0.4 (0.1;1.6)
n3PUFA				
ParEdu high	0.8 (0.4;1.8)	0.9 (0.3;2.5)	0.5 (0.2;1.3)	0.4 (0.2;0.9)
Income med	0.8 (0.3;2.0)	1.0 (0.4;3.0)	2.4 (0.8;7.1)	0.6 (0.3;1.4)
Income high	1.0 (0.4;2.7)	1.1 (0.4;3.2)	1.3 (0.4;4.1)	0.7 (0.3;1.7)
ChildEdu high	1.3 (0.6;2.7)	1.0 (0.4;2.8)	0.5 (0.2;1.2)	1.8 (0.8;4.0)
Puberty yes	1.1 (0.6;2.3)	1.9 (0.8;4.4)	0.7 (0.3;1.6)	0.5 (0.2;1.0)
BMI	1.0 (0.8;1.1)	0.8 (0.7;1.0)	0.9 (0.8;1.1)	1.0 (0.9;1.2)
Sed high	1.0 (0.3;2.8)	0.3 (0.0;2.3)	0.3 (0.1;2.1)	0.7 (0.2;2.2)
n6PUFA				
ParEdu high	1.0 (0.4;2.3)	2.0 (0.7;5.7)	0.6 (0.2;1.7)	1.1 (0.5;2.6)
Income med	1.5 (0.6;3.6)	1.1 (0.4;3.3)	2.2 (0.7;7.2)	0.6 (0.3;1.4)
Income high	1.0 (0.4;2.6)	0.4 (0.1;1.3)	1.4 (0.4;4.5)	0.8 (0.3;2.2)
ChildEdu high	0.6 (0.2;1.3)	0.6 (0.2;1.5)	0.7 (0.3;2.1)	2.4 (1.0;5.3)
Puberty yes	1.0 (0.5;2.0)	0.9 (0.4;2.0)	0.8 (0.3;1.8)	1.1 (0.5;2.2)
BMI	1.1 (0.9;1.4)	0.9 (0.8;1.1)	1.0 (0.8;1.1)	1.1 (1.0;1.3)
Sed high	0.3 (0.1;1.5)	1.3 (0.4;4.6)	1.1 (0.2;5.1)	0.5 (0.2;1.7)
Beta-Carotene [ml/d]				
ParEdu high	1.8 (0.8;4.0)	1.3 (0.5;3.3)	0.8 (0.3;2.0)	1.5 (0.6;4.0)
Income med	1.1 (0.5;2.5)	0.4 (0.2;1.3)	1.4 (0.4;4.8)	0.4 (0.2;1.0)
Income high	1.0 (0.4;2.4)	0.3 (0.1;0.9)	1.8 (0.5;6.5)	0.4 (0.1;1.0)
ChildEdu high	0.8 (0.4;1.7)	0.8 (0.3;2.1)	0.6 (0.2;1.6)	0.7 (0.3;1.5)
Puberty yes	1.1 (0.5;2.1)	1.2 (0.5;2.7)	1.4 (0.6;3.2)	1.1 (0.6;2.2)
BMI	0.9 (0.8;1.1)	1.0 (0.8;1.2)	0.9 (0.8;1.2)	1.0 (0.9;1.2)
Sed high	0.9 (0.3;2.2)	1.2 (0.2;6.4)	3.3 (0.7;14.2)	4.5 (0.4;45.6)

Alpha-Tocopherol [ml/d]				
ParEdu high	1.9 (0.8;4.3)	1.9 (0.6;5.7)	0.3 (0.1;0.9)	1.3 (0.6;2.8)
Income med	0.9 (0.4;2.3)	1.9 (0.6;5.9)	1.0 (0.4;2.9)	0.6 (0.3;1.4)
Income high	0.6 (0.2;1.5)	1.4 (0.4;4.6)	0.5 (0.1;1.6)	0.6 (0.3;1.5)
ChildEdu high	0.7 (0.3;1.6)	1.1 (0.4;2.8)	1.1 (0.4;2.9)	1.3 (0.6;2.7)
Puberty yes	0.8 (0.4;1.6)	1.5 (0.7;3.4)	1.4 (0.6;3.3)	0.9 (0.4;1.6)
BMI	0.9 (0.8;1.1)	0.9 (0.7;1.0)	0.9 (0.8;1.1)	1.0 (0.9;1.1)
Sed high	0.4 (0.1;1.4)	0.6 (0.1;2.2)	1.0 (0.3;3.4)	1.3 (0.3;4.6)

[1]Odds ratio (95% CI); [2] Logistic regression (increase vs. tracking in lowest tertile). [3]Multinomial logistic regression (increase or decrease vs. tracking in medium tertile), [4]Logsitic regression (decrease vs. tracking in highest tertile); ParEdu high: parental education (high vs. low); Income med/high: family income (medium/high vs. low); ChildEdu high: child education (high vs. low); Puberty yes: pubertal onset at baseline (yes vs. no); Screen high: screen-time at baseline (high vs. low).*p-value < 0.0083 (Bonferroni correction for multiple testing: 0.05/6)

Supplementary Table 3b Associations[1] with dietary intake changes stratified by baseline tertile in males

Reference	**Tracking in T1[2]**	**Tracking in T2[3]**		**Tracking in T3[4]**
Change	**Increase**	**Increase**	**Decrease**	**Decrease**
Fruit				
ParEdu High	0.8 (0.4;1.9)	1.3 (0.4;4.3)	1.1 (0.3;3.8)	0.8 (0.3;2.1)
Income med	0.9 (0.4;2.2)	0.6 (0.2;1.8)	0.7 (0.2;2.3)	1.2 (0.5;3.2)
Income high	0.6 (0.2;1.5)	0.7 (0.2;2.7)	0.8 (0.2;3.1)	1.1 (0.4;2.9)
School high	2.0 (0.9;4.7)	0.3 (0.1;0.9)	0.8 (0.2;2.5)	0.8 (0.3;2.0)
Puberty yes	0.4 (0.1;1.4)	0.5 (0.1;2.8)	1.6 (0.3;8.9)	1.3 (0.4;3.9)
BMI	1.0 (0.9;1.2)	1.0 (0.8;1.3)	0.9 (0.7;1.1)	0.8 (0.7;1.0)
Sed high	0.6 (0.2;1.7)	3.7 (0.9;16.1)	0.7 (0.1;4.6)	2.3 (0.6;8.9)
Vegetables[5]				
ParEdu High	1.0 (0.4;2.6)	1.3 (0.5;4.0)	2.5 (0.8;8.2)	2.0 (0.8;5.4)
Income med	2.0 (0.8;5.4)	0.8 (0.3;2.4)	0.8 (0.3;2.8)	1.4 (0.5;3.5)
Income high	3.2 (1.1;9.4)	0.4 (0.1;1.5)	0.6 (0.2;2.2)	0.7 (0.3;1.9)
School high	1.0 (0.4;2.5)	1.9 (0.6;5.4)	2.0 (0.6;6.5)	0.9 (0.3;2.1)
Puberty yes	0.4 (0.1;1.4)	0.5 (0.1;1.9)	1.7 (0.4;7.1)	1.3 (0.4;4.2)
BMI	1.0 (0.9;1.2)	1.2 (1.0;1.5)	0.9 (0.7;1.1)	0.9 (0.8;1.1)
Sed high	0.3 (0.1;0.9)	1.9 (0.3;11.7)	8.3 (1.4;50.0)	2.5 (0.6;10.7)
Starchy vegetables[5,6]				
ParEdu High	1.3 (0.5;3.5)	0.3 (0.1;1.0)	0.4 (0.1;1.6)	0.7 (0.3;1.6)
Income med	0.6 (0.3;1.7)	1.4 (0.5;4.1)	2.9 (0.8;10.2)	1.7 (0.7;4.1)
Income high	0.4 (0.2;1.2)	2.1 (0.6;7.0)	3.3 (0.8;13.1)	2.4 (0.9;6.7)
School high	1.0 (0.4;2.5)	0.8 (0.3;2.4)	0.8 (0.2;3.0)	2.0 (0.9;4.6)
Puberty yes	0.5 (0.2;1.7)	-	-	2.9 (0.8;10.8)
BMI	1.0 (0.8;1.2)	0.8 (0.7;1.0)	0.8 (0.6;1.0)	1.0 (0.8;1.1)
Sed high	0.5 (0.2;1.9)	0.4 (0.1;1.9)	0.7 (0.1;3.1)	1.3 (0.5;4.0)
Wholegrain				
ParEdu High	1.3 (0.6;3.0)	2.5 (0.8;8.0)	1.4 (0.5;4.4)	1.7 (0.6;4.6)
Income med	1.3 (0.5;3.1)	0.6 (0.2;1.8)	0.6 (0.2;2.0)	0.6 (0.2;1.5)
Income high	1.3 (0.5;3.3)	1.2 (0.3;4.1)	0.7 (0.2;2.4)	0.9 (0.3;2.7)
School high	1.4 (0.6;3.4)	1.1 (0.4;3.4)	0.7 (0.2;1.9)	0.3 (0.1;0.9)
Puberty yes	0.9 (0.3;3.0)	5.6 (0.5;60.3)	18.8 (1.9;183.0)	1.1 (0.4;3.3)
BMI	1.1 (1.0;1.3)	1.1 (0.9;1.3)	0.8 (0.6;1.0)	1.0 (0.8;1.2)
Sed high	1.0 (0.4;2.5)	1.3 (0.2;7.3)	0.9 (0.2;4.7)	10.5 (1.6;67.3)
Refined grain[5]				
ParEdu High	1.2 (0.4;3.4)	1.0 (0.4;2.7)	1.2 (0.5;3.2)	0.8 (0.3;1.9)
Income med	2.2 (0.8;6.1)	1.3 (0.5;3.8)	0.7 (0.3;2.0)	0.9 (0.4;2.2)
Income high	4.4 (1.3;14.8)	0.9 (0.3;2.8)	0.3 (0.1;1.0)	1.6 (0.6;4.1)
School high	1.3 (0.5;3.6)	0.9 (0.3;2.2)	0.9 (0.3;2.2)	1.4 (0.6;3.4)
Puberty yes	2.9 (0.7;12.7)	1.0 (0.2;4.3)	0.6 (0.1;2.6)	1.6 (0.6;4.5)
BMI	0.9 (0.7;1.0)	1.0 (0.9;1.2)	1.0 (0.9;1.2)	1.1 (0.9;1.2)
Sed high	1.4 (0.4;4.3)	1.0 (0.2;4.2)	1.5 (0.4;5.4)	1.1 (0.3;3.6)
Meat[5]				
ParEdu High	0.2 (0.1;0.7)	1.9 (0.7;5.3)	2.5 (0.9;7.5)	1.3 (0.5;3.1)
Income med	2.4 (0.9;6.4)	1.3 (0.4;4.2)	1.1 (0.3;3.9)	1.9 (0.8;4.7)
Income high	0.7 (0.2;2.3)	0.5 (0.2;1.7)	0.6 (0.2;2.0)	1.2 (0.4;3.2)
School high	2.9 (1.0;8.2)	0.7 (0.3;2.0)	0.8 (0.3;2.3)	1.3 (0.5;3.1)
Puberty yes	1.2 (0.3;4.5)	0.6 (0.1;2.3)	0.6 (0.1;2.5)	0.7 (0.2;2.0)
BMI	1.1 (0.9;1.4)	1.1 (0.9;1.3)	1.0 (0.8;1.2)	1.1 (1.0;1.4)
Sed high	2.2 (0.6;8.6)	2.7 (0.6;12.1)	4.3 (0.9;19.2)	0.2 (0.1;0.7)
Fish				
ParEdu High	0.8 (0.3;2.0)	1.6 (0.6;4.6)	2.3 (0.8;7.0)	0.7 (0.3;1.8)
Income med	0.6 (0.2;1.6)	0.4 (0.1;1.3)	0.5 (0.2;1.5)	0.8 (0.3;2.0)
Income high	0.6 (0.2;1.7)	0.6 (0.2;1.8)	0.2 (0.1;0.8)	1.2 (0.4;3.2)
School high	1.4 (0.5;3.8)	0.8 (0.3;2.1)	0.8 (0.3;2.4)	1.7 (0.7;4.3)

Puberty yes	1.2 (0.3;4.3)	1.8 (0.5;7.2)	0.7 (0.1;3.5)	0.9 (0.3;2.6)
BMI	1.1 (0.9;1.4)	1.0 (0.8;1.1)	1.0 (0.8;1.2)	1.3 (1.0;1.6)
Sed high	1.1 (0.3;3.6)	0.9 (0.3;3.1)	0.9 (0.3;3.0)	0.9 (0.2;3.7)
Nuts[5]				
ParEdu High	1.8 (0.7;4.4)	0.7 (0.2;2.1)	1.3 (0.4;4.0)	1.1 (0.4;2.8)
Income med	0.5 (0.2;1.3)	1.0 (0.3;2.7)	1.3 (0.4;4.1)	0.6 (0.2;1.5)
Income high	0.6 (0.2;1.7)	0.9 (0.3;2.7)	1.2 (0.4;4.1)	1.1 (0.4;2.8)
School high	0.6 (0.2;1.4)	2.5 (0.9;6.9)	1.2 (0.4;3.5)	1.3 (0.5;3.3)
Puberty yes	0.3 (0.1;1.5)	1.3 (0.4;4.5)	1.6 (0.5;5.7)	1.5 (0.5;4.5)
BMI	1.1 (0.9;1.3)	1.1 (0.9;1.3)	1.1 (0.9;1.3)	0.8 (0.7;1.0)
Sed high	0.5 (0.2;1.5)	1.0 (0.3;4.3)	1.8 (0.4;7.2)	1.0 (0.3;3.8)
Butter[5]				
ParEdu High	1.1 (0.5;2.9)	1.3 (0.4;3.9)	0.5 (0.2;1.6)	0.9 (0.3;2.7)
Income med	1.2 (0.4;3.1)	2.2 (0.7;7.3)	0.5 (0.1;1.6)	1.4 (0.5;4.0)
Income high	1.1 (0.4;3.3)	2.0 (0.6;7.0)	0.6 (0.2;2.4)	0.5 (0.2;1.4)
School high	1.9 (0.8;4.7)	1.4 (0.4;4.3)	4.2 (1.2;15.4)	0.5 (0.2;1.5)
Puberty yes	1.7 (0.5;5.9)	0.6 (0.2;2.3)	0.6 (0.1;3.1)	0.8 (0.2;2.8)
BMI	1.2 (1.0;1.4)	0.9 (0.8;1.1)	0.9 (0.8;1.2)	1.0 (0.8;1.2)
Sed high	0.8 (0.3;2.3)	1.0 (0.2;4.4)	1.2 (0.3;5.4)	0.3 (0.1;1.8)
Oil				
ParEdu High	2.0 (0.8;5.0)	0.6 (0.2;1.9)	0.4 (0.1;1.4)	2.1 (0.8;5.6)
Income med	2.7 (1.1;6.6)	1.7 (0.5;5.5)	1.0 (0.3;3.1)	3.7 (1.3;10.2)
Income high	2.2 (0.8;6.3)	1.1 (0.3;3.3)	0.4 (0.1;1.5)	1.6 (0.6;4.5)
School high	0.7 (0.3;1.6)	1.5 (0.5;4.7)	3.1 (0.9;10.0)	0.5 (0.2;1.3)
Puberty yes	1.5 (0.4;6.1)	3.0 (0.6;14.7)	6.8 (1.2;37.2)	1.0 (0.3;3.3)
BMI	1.0 (0.9;1.2)	1.0 (0.8;1.2)	1.1 (0.9;1.3)	1.2 (1.0;1.5)
Sed high	2.2 (0.7;6.7)	0.2 (0.1;0.9)	0.8 (0.2;2.5)	1.1 (0.2;6.1)
Dairy				
ParEdu High	1.4 (0.6;3.5)	1.7 (0.5;5.7)	1.0 (0.3;3.2)	0.6 (0.2;1.5)
Income med	1.3 (0.5;3.2)	0.9 (0.3;2.8)	0.5 (0.1;1.5)	1.7 (0.6;4.8)
Income high	1.1 (0.4;3.0)	0.4 (0.1;1.4)	0.3 (0.1;1.0)	1.5 (0.5;4.4)
School high	0.5 (0.2;1.2)	1.2 (0.4;3.4)	2.4 (0.8;7.0)	1.2 (0.5;3.0)
Puberty yes	2.8 (0.9;8.5)	4.3 (0.7;27.7)	4.7 (0.7;31.4)	0.7 (0.2;2.2)
BMI	0.9 (0.8;1.1)	0.9 (0.8;1.1)	0.9 (0.8;1.1)	1.0 (0.9;1.2)
Sed high	0.4 (0.1;1.1)	2.8 (0.6;13.5)	2.5 (0.5;13.8)	2.5 (0.7;9.1)
Caloric drinks				
ParEdu High	1.4 (0.5;3.8)	4.5 (1.3;15.2)	0.7 (0.2;2.0)	2.2 (0.9;5.6)
Income med	0.3 (0.1;0.8)	0.8 (0.3;2.5)	0.8 (0.3;2.4)	0.8 (0.3;2.1)
Income high	1.0 (0.3;2.8)	0.6 (0.2;2.1)	1.2 (0.4;4.0)	1.3 (0.5;3.6)
School high	0.8 (0.3;2.2)	0.8 (0.3;2.3)	1.3 (0.5;3.7)	0.5 (0.2;1.1)
Puberty yes	1.7 (0.5;5.5)	2.4 (0.6;9.9)	2.6 (0.6;10.8)	1.0 (0.3;3.6)
BMI	1.0 (0.9;1.2)	1.0 (0.8;1.2)	0.9 (0.7;1.1)	1.0 (0.9;1.2)
Sed high	2.0 (0.5;8.0)	3.0 (0.7;12.3)	1.1 (0.3;4.3)	1.7 (0.6;5.0)
Tea [ml/d]				
ParEdu High	1.0 (0.4;2.8)	1.5 (0.4;5.0)	1.3 (0.4;4.5)	1.6 (0.7;3.9)
Income med	0.6 (0.2;1.8)	1.1 (0.4;3.4)	1.2 (0.4;3.8)	0.9 (0.4;2.3)
Income high	1.1 (0.4;3.2)	1.1 (0.3;3.4)	1.0 (0.3;3.4)	0.5 (0.2;1.5)
School high	3.0 (1.2;7.5)	0.5 (0.1;1.6)	0.5 (0.1;1.5)	0.5 (0.2;1.1)
Puberty yes	0.3 (0.0;2.8)	1.3 (0.3;5.4)	0.7 (0.1;3.7)	0.7 (0.3;2.1)
BMI	0.9 (0.8;1.1)	1.0 (0.8;1.2)	0.8 (0.7;1.0)	0.9 (0.8;1.1)
Sed high	1.2 (0.4;4.1)	0.8 (0.2;3.8)	1.3 (0.4;4.9)	1.0 (0.3;3.4)
Water [ml/d]				
ParEdu High	1.4 (0.5;3.6)	1.0 (0.4;2.7)	1.1 (0.4;3.4)	1.0 (0.4;2.6)
Income med	0.4 (0.2;1.2)	2.4 (0.8;7.2)	1.6 (0.5;5.1)	1.2 (0.4;3.3)
Income high	1.0 (0.4;2.6)	2.1 (0.6;7.4)	1.1 (0.3;4.3)	1.1 (0.4;3.3)
School high	2.7 (1.0;7.4)	0.8 (0.3;2.0)	1.1 (0.4;3.0)	0.7 (0.3;1.8)

Puberty yes	0.4 (0.1;2.0)	1.6 (0.5;5.3)	1.1 (0.3;4.7)	1.1 (0.3;3.8)
BMI	1.2 (1.0;1.4)	1.1 (0.9;1.4)	1.2 (1.0;1.5)	0.8 (0.7;1.0)
Sed high	0.5 (0.1;1.9)	1.2 (0.3;4.0)	1.1 (0.3;4.0)	0.9 (0.2;3.8)
Protein[5]				
ParEdu High	0.8 (0.3;1.9)	1.0 (0.4;2.5)	0.5 (0.2;1.6)	1.7 (0.7;4.2)
Income med	0.9 (0.4;2.4)	0.8 (0.3;2.2)	2.2 (0.7;7.5)	0.9 (0.3;2.3)
Income high	0.8 (0.3;2.0)	0.7 (0.2;2.1)	2.2 (0.6;8.4)	0.6 (0.2;1.7)
School high	0.8 (0.3;2.0)	2.4 (0.9;6.1)	2.4 (0.8;7.1)	0.5 (0.2;1.2)
Puberty yes	1.8 (0.5;7.0)	1.5 (0.4;6.5)	1.9 (0.4;9.3)	0.8 (0.3;2.2)
BMI	1.1 (0.9;1.3)	1.0 (0.8;1.2)	1.0 (0.8;1.2)	1.0 (0.9;1.2)
Sed high	1.0 (0.3;3.3)	1.9 (0.5;6.9)	2.6 (0.7;10.5)	1.1 (0.3;3.8)
Fat				
ParEdu High	0.6 (0.2;1.5)	0.4 (0.1;1.3)	0.8 (0.3;2.2)	0.9 (0.3;2.2)
Income med	1.9 (0.7;4.8)	0.8 (0.2;2.3)	1.3 (0.5;3.6)	1.0 (0.4;2.7)
Income high	2.9 (1.0;8.5)	0.8 (0.3;2.7)	1.1 (0.4;3.2)	1.6 (0.6;4.7)
School high	0.8 (0.3;2.1)	1.6 (0.5;4.6)	1.1 (0.4;2.9)	1.1 (0.5;2.7)
Puberty yes	0.7 (0.2;2.8)	2.3 (0.6;8.6)	1.3 (0.4;4.8)	0.7 (0.2;2.3)
BMI	0.9 (0.7;1.1)	0.8 (0.7;1.0)	0.9 (0.8;1.1)	1.1 (0.9;1.3)
Sed high	0.8 (0.2;2.9)	8.1 (1.3;48.6)	7.5 (1.3;42.0)	1.5 (0.5;4.7)
Carbohydrate				
ParEdu High	1.9 (0.7;4.8)	1.6 (0.6;4.6)	0.9 (0.3;2.7)	0.5 (0.2;1.4)
Income med	0.8 (0.3;2.1)	1.9 (0.6;5.6)	0.6 (0.2;1.8)	2.2 (0.8;6.0)
Income high	1.1 (0.4;3.0)	1.9 (0.6;6.4)	0.6 (0.2;2.2)	2.4 (0.9;6.6)
School high	0.9 (0.4;2.3)	1.6 (0.5;4.4)	1.6 (0.5;5.0)	0.6 (0.2;1.6)
Puberty yes	0.9 (0.3;2.5)	1.6 (0.4;6.4)	1.7 (0.4;7.3)	0.4 (0.1;1.8)
BMI	1.1 (0.9;1.3)	0.9 (0.8;1.1)	0.9 (0.7;1.1)	0.9 (0.8;1.1)
Sed high	4.3 (1.0;17.7)	7.0 (1.2;41.0)	9.4 (1.7;50.9)	0.5 (0.2;1.8)
n6PUFA				
ParEdu High	0.8 (0.3;2.2)	1.1 (0.4;3.1)	1.9 (0.6;5.9)	2.3 (1.0;5.5)
Income med	1.2 (0.4;3.5)	1.1 (0.4;3.1)	1.4 (0.4;4.3)	0.8 (0.3;2.1)
Income high	1.4 (0.5;4.4)	0.6 (0.2;2.3)	2.0 (0.6;7.1)	1.1 (0.4;2.9)
School high	1.7 (0.7;4.5)	0.6 (0.2;1.7)	1.0 (0.3;3.0)	0.9 (0.4;2.1)
Puberty yes	2.2 (0.6;8.5)	0.6 (0.1;2.5)	0.4 (0.1;2.1)	0.6 (0.2;2.0)
BMI	1.1 (0.9;1.3)	0.8 (0.6;1.0)	0.8 (0.7;1.0)	1.1 (0.9;1.3)
Sed high	1.8 (0.6;5.8)	0.4 (0.1;1.8)	0.7 (0.2;2.6)	0.9 (0.2;3.4)
Retinol [ml/d]				
ParEdu High	1.3 (0.5;3.5)	2.3 (0.8;6.4)	1.5 (0.5;4.1)	1.0 (0.4;2.4)
Income med	0.7 (0.3;1.8)	2.5 (0.8;7.7)	1.0 (0.4;3.0)	0.7 (0.3;1.8)
Income high	0.5 (0.2;1.2)	5.1 (1.5;17.2)	1.1 (0.3;3.8)	0.7 (0.2;2.4)
School high	0.7 (0.2;1.7)	0.5 (0.2;1.4)	1.6 (0.6;4.3)	1.0 (0.4;2.7)
Puberty yes	1.0 (0.3;3.2)	8.3 (1.3;52.2)	5.0 (0.8;30.4)	0.7 (0.2;2.0)
BMI	1.0 (0.8;1.2)	1.0 (0.9;1.2)	1.1 (1.0;1.4)	1.1 (0.9;1.3)
Sed high	1.0 (0.4;2.8)	1.4 (0.3;6.9)	0.8 (0.2;4.0)	0.5 (0.1;1.6)
Beta-Carotene [ml/d]				
ParEdu High	0.7 (0.3;1.8)	0.5 (0.2;1.4)	0.6 (0.2;2.0)	1.9 (0.7;5.0)
Income med	0.9 (0.3;2.4)	0.8 (0.3;2.1)	0.5 (0.2;1.5)	0.9 (0.3;2.4)
Income high	1.2 (0.4;3.2)	0.9 (0.3;3.1)	0.7 (0.2;2.4)	0.6 (0.2;1.6)
School high	1.7 (0.7;4.2)	1.5 (0.5;4.1)	1.8 (0.6;5.2)	1.0 (0.4;2.7)
Puberty yes	0.7 (0.2;2.7)	0.6 (0.2;1.9)	0.8 (0.2;2.7)	2.1 (0.6;7.8)
BMI	1.0 (0.9;1.2)	1.2 (1.0;1.5)	1.2 (0.9;1.4)	0.8 (0.6;1.0)
Sed high	0.5 (0.2;1.4)	0.6 (0.1;2.5)	1.3 (0.4;4.9)	2.1 (0.4;10.4)
Alpha tocopherol [ml/d]				
ParEdu High	1.4 (0.6;3.5)	1.3 (0.4;3.9)	0.8 (0.3;2.5)	1.8 (0.7;4.7)
Income med	1.1 (0.4;2.8)	2.1 (0.7;6.8)	1.0 (0.3;3.1)	0.6 (0.2;1.6)
Income high	1.3 (0.5;3.6)	1.4 (0.4;4.9)	1.5 (0.4;5.1)	0.6 (0.2;1.6)
School high	0.8 (0.3;1.8)	0.6 (0.2;1.7)	0.6 (0.2;1.8)	1.2 (0.4;3.2)

Puberty yes	1.0 (0.3;3.0)	0.6 (0.1;4.1)	1.0 (0.2;6.0)	1.9 (0.6;5.8)
BMI	0.9 (0.8;1.1)	1.0 (0.8;1.2)	0.9 (0.7;1.1)	1.1 (0.9;1.3)
Sed high	2.3 (0.7;7.8)	0.1 (0.0;1.3)	1.5 (0.4;5.2)	6.4 (1.2;33.4)

[1]Odds ratio (95% CI); [2] Logistic regression (increase vs. tracking in lowest tertile). [3]Multinomial logistic regression (increase or decrease vs. tracking in medium tertile), [4]Logsitic regression (decrease vs. tracking in highest tertile); [5]Multinomial regression not adjusted for diet change; [6]Multinomial regression not adjusted for pubertal onset; ParEdu high: parental education (high vs. low); Income med/high: family income (medium/high vs. low); ChildEdu high: child education (high vs. low); Puberty yes: pubertal onset at baseline (yes vs. no); Screen high: screen-time at baseline (high vs. low).*p-value < 0.0083 (Bonferroni correction for multiple testing: 0.05/6)

Acknowledgements

First, I would like to thank Prof. Prof. Dr. Dr. Berthold Koletzko, from the Dr. von Hauner Children's Hospital at the Ludwig-Maximilians-University of Munich, for his professional and attentive supervision of this thesis.

I am grateful to Dr. Joachim Heinrich, former director of the Institute of Epidemiology I at the Helmholtz Zentrum München, German Research Centre for Environmental Health, for offering me the opportunity to join his research group as a doctoral student, for his excellent advice, and much appreciated encouragement.

I also thank Prof. Dr. Holger Schulz, acting director of the Institute of Epidemiology I at the Helmholtz Zentrum München, German Research Centre for Environmental Health, for his persistent support, which has allowed the timely completion of this work.

Special thanks go to Dr. Marie Standl, who has been an admirable leader and role model, offering valuable knowledge, and continuous guidance and reassurance.

I thank the GINIplus and LISAplus study groups and all families and participants of these studies. In addition, I highly acknowledge and am thankful for the generous help and contribution of Prof. Dr. Anette Buyken.

I would like to express my appreciation to my colleagues, for their company and kindness since my first arrival, as well as their time, patience and constructive comments during the realisation of this work.

Finally, a very special thank you to my family and friends for their unconditional love and support.

Publications

Publications included in this thesis

C Harris, A Buyken, A von Berg, D Berdel, I Lehmann, B Hoffmann, *et al*. Prospective associations of meat consumption during childhood with measures of body composition during adolescence: results from the GINIplus and LISAplus birth cohorts. *Nutrition Journal*, 2016, 15, 101.

C Harris, A Buyken, S Koletzko, A von Berg, D Berdel, T Schikowski, *et al*. Dietary fatty acids and changes in blood lipids during adolescence: the role of substituting nutrient intakes. *Nutrients*, 2017, 9, 127.

C Harris, H Demmelmair, A von Berg, I Lehmann, C Flexeder, B Koletzko, *et al*. Associations between fatty acids and low-grade inflammation in children from the LISAplus birth cohort study. *Eur J Clin Nutr*, 2017, 71, 1303–1311.

C Harris, C Flexeder, E Thiering, A Buyken, D Berdel, S Koletzko, *et al*. Changes in dietary intake during puberty and their determinants: results from the GINIplus birth cohort study. *BMC Public Health*, 2015, 15, 841.

Further publications

I Markevych, M Standl, D Sugiri, C Harris, W Maier, D Berdel, *et al*. Residential greenness and blood lipids in children: A longitudinal analysis in GINI plus and LISAplus. *Environmental Research*, 2016, 151, 168–173.

Eidesstattliche Versicherung

Ich, Carla Harris, erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema "Dietary intake, body composition and biomarkers in children and adolescents" selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.
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München, 24. 10. 2018

Carla Harris