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Sugar intake, sugar sources and associated factors in European children

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1. INTRODUCTION

About 80 % of deaths worldwide are caused by noncommunicable diseases (NCDs), predominantly cardiovascular diseases, cancer and chronic respiratory diseases (1). Four widespread risk factors are strongly associated with most NCDs: smoking, lack of exercise, unhealthy diet and alcohol misuse. Four key metabolic changes often result from these life style factors: raised blood pressure, overweight or obesity, hyperglycemia and hyperlipidemia (2). In recent years, the prevalence of these diseases has continued to increase, and their impact on health care costs has grown as a result. Nutritional prevention is an important factor to reduce the incidence and accordingly the costs of these diseases (3).

Carbohydrates contribute an important component of human nutrition (> 50 % of total energy intake), especially in children(4). Correspondingly, they could have a substantial influence on the prevention of nutrition-related diseases. Carbohydrates consist of energy delivering mono-, di- and polysaccharides and non-digestible dietary fibres. All mono- and disaccharides are sweet tasting and considered to be sugars. Monosaccharides include glucose, fructose and galactose. The table sugar most customarily used as food is sucrose, a disaccharide composed of glucose and fructose. Other disaccharides are maltose and lactose (milk sugar).

Total sugar consumption in Europe is high, with higher levels in northern countries: e.g. 180.1 g/day for boys in Belgium versus 89.7 g/day for boys in Spain (5). Children and males consume more total sugar than adults and females (5). Sugar consumption as a percentage of energy decreases from 20 - 38.4 % in children (< 4 years) to 13.5 -24.6 % in adults (6).

Factors associated with intake of sugar and sweet foods

Sugar intake seems to be influenced by genetic, environmental and socio-demographic factors. A British study from 2005 investigated food patterns in 4 year old and 7 year old children. A junk food pattern, based on foods with a high fat and sugar content, processed, and convenience foods, was significantly more likely in white children who had more siblings and whose mothers had less education (7). Kranz and Siega-Riz investigated sociodemographic determinants of added sugar

intake in preschoolers (2 to 5 years) and found strong associations with age, ethnicity, family income, day care/school attendance, region of residence, and educational level of the female householder (8). In older children and adolescents, apart from parental permissiveness, higher consumption of sugar is correlated with high availability of snacks and soft drinks at home, in school-canteen and in shops in children's living environment (9; 10).

Aside from sociodemographic factors, food choices and eating behaviour are significantly influenced by taste preferences. Perception of taste is individually different and depends on genetic variation in certain taste receptor genes. Genetically determined variation in taste sensitivity in humans has been reported for four of the basic tastes: sweet (11), bitter (12), sour (13) and umami (the taste of monosodium glutamate) (14). Children generally prefer sweet and umami tastes, what is plausible from an evolutionary point of view because sugar and protein are easily digested energy providers(15). Bitter taste is mostly declined, because it is often associated with a warning against toxin ingestion (15).

Natural and added sugars

Dietary sugar can be found in two forms: natural sugar, which occurs in fruits, vegetables and milk, and added sugar, which is added to foods or beverages during processing or during preparation at home(16). The term "free sugars" was used by the 2002 Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases and includes all mono- and disaccharides added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and fruit juices (17). In our western diet, added sugars provide a large contribution to energy intake. In the What We Eat in America (WWEIA) study and the American National Health and Nutrition Examination Survey (NHANES), 12.0 % of total energy intake in 2 to 5 year old children is derived from added sugars (18). Similar amounts of added sugars were found in the German DONALD study, with 12.4 E% in children with a mean age between 6.7 and 9.6 years derived from added sugar (19). Although the body handles naturally occurring and added sugar in the same way, foods high in added sugar normally have a lower nutrient density, while foods with naturally occurring sugar are high in valuable nutrients(16). For this reason, it is interesting to investigate sources of sugar intake.

In recent years particularly, there has been intensive and controversial discussion about the role of carbohydrates and sugar in the development of overweight, obesity and nutrition-related diseases. Also, there is no consistent recommendation on the intake of added or free sugars. A recent review of guidelines on dietary sugar showed that recommendations for dietary sugar do not meet criteria for trustworthy recommendations and are based on low-quality evidence. Nevertheless, they all suggested a reduction of non-intrinsic sugars. Nutrient displacement, dental caries, weight gain and obesity risk served as rationale for most of the reviewed recommendations (20).

Sugar intake, obesity and related diseases

Obesity is a complex condition with biological, genetic, behavioral, social, cultural, and environmental influence factors. The current high rates of overweight and obesity among children and adults in high-income countries are primarily a result of individual behaviors and environmental factors that lead to excess caloric intake and inadequate amounts of physical activity(21).

According to the WHO, over 50% of both men and women in Europe are overweight (BMI ≥ 25 kg/m²), and roughly 23% of women and 20% of men obese (BMI ≥ 30 kg/m²) (2). Globally, 40 million (or 6%) preschool children in 2008 were overweight based on WHO criteria (21). Over 60% of children who are overweight before puberty will be overweight in early adulthood. Obesity in childhood is strongly associated with a higher risk for cardiovascular diseases, type 2 diabetes, and orthopaedic problems, and it often leads to lower self-confidence and school performance (22).

The main factors contributing to excess caloric intake are high consumption of sugar-sweetened beverages (23) (24) (25), a typical western diet with a high proportion of energy dense food (26), large portion sizes (27; 28), and increased snacking (29).

Te Morenga et al. concluded in a 2013 review on the role of dietary sugar intake on body weight that in people with ad libitum diets, intake of free sugars or sugar sweetened beverages has an impact on body weight. As the exchange of other carbohydrates by sugars and consistent energy intake is not associated with weight change, the difference in body fatness with modifying sugar intake seems to be the result of higher energy intake in a high-sugar diet (30). The German guideline for

carbohydrate intake states that there is possible evidence that higher consumption of sugar sweetened beverages increases obesity risk (31).

While studies on the association between mono- and disaccharide intake and risk for type 2 diabetes show conflicting or no results, the majority of studies on the association between the consumption of sugar-sweetened beverages and type 2 diabetes indicate an increased risk of type 2 diabetes mellitus due to regular consumption of sugar-sweetened beverages (3).

A recent meta-analysis on the effect of sugar intake on blood lipids showed that higher sugar intake significantly increases plasma triglyceride concentrations, total cholesterol, low density lipoprotein and high density lipoprotein cholesterol (30). It is discussed whether fructose has a greater lipogenic effect than glucose and other carbohydrates. There is convincing evidence that fasting plasma triglyceride concentration increases with higher fructose intake of up to 350 g/day (3; 32), but fructose intake of up to 100 g/day does not influence triglyceride concentration (31).

Te Morenga's meta-analysis (30) also examined the effect of higher sugar intake on blood pressure and found significant effects on increased systolic and diastolic blood pressure. The effect was greater in studies with longer durations (≥ 8 weeks) (30).

The German guideline for carbohydrates states that there is possible evidence that an increased intake of fructose, sucrose or sugar sweetened beverages increases the risk for hypertension (31).

There is no clear association between sugar intake and metabolic syndrome or coronary heart disease. However, the German nutritional guideline summarizes that there is possible evidence that an increased intake of sugar sweetened beverages increases the risk for metabolic syndrome (31).

Sugar intake and nutrient deficiencies

High sugar intake might be associated with low nutrient density. In a German study on children aged 2-18 years, added sugars were related to a reduction of vitamin and mineral intake and to intake of several food groups, with fruits and vegetables affected most (19). In a review from 2007, it was stated that there is insufficient data and inconsistency between studies investigating associations between the intake of added sugars and micronutrients (33). Possibly negative associations between

added sugar intake and micronutrient density are rather related to food patterns with high levels of added sugars than to the intake of added sugars *per se* (34).

Sugar intake and dental caries

According to the EFSA scientific opinion from 2010 available data do not allow to set a firm upper limit for sugar intake based on risk reduction of dental caries, because caries development does not depend predominantly on the amount of sugar consumed but is influenced by various other factors such as oral hygiene, fluoride prophylaxis, meal frequency, heredity, and flow and composition of saliva (34). In contrast, a recently released review about the relationship between sugar intake and caries underlines the important role of sugar in the development of caries (32). The WHO recommendation on free sugars is apart from associations with overweight and obesity supported by the evidence, that higher rates of dental caries are found when the intake of free sugars is above 10 E% compared with an intake of free sugars below 10 E% (35).

In summary of the current evidence, the WHO recommends a reduced intake of free sugars throughout the lifecourse (strong recommendation). Furthermore, in both adults and children, WHO recommends reducing the intake of free sugars to less than 10% of total energy intake (strong recommendation). As a conditional recommendation, the WHO suggests a further reduction of the intake of free sugars to below 5% of total energy (35). Sheiham et al. suggested to even reduce sugar intake to 2 - 3 % of total energy intake per day as new data show the strong relation between sugar intake and dental caries (32).

CHildhood Obesity Project

Study design

The dissertation was conducted within the framework of the CHildhood Obesity Project CHOP. The main objective of CHOP was to test the hypothesis that a higher early protein intake leads to more rapid growth in the first 2 years of life and to a higher prevalence of overweight and obesity at 6 years of age (36; 37).

The CHOP is a double-blind, randomized, controlled intervention trial. The study was conducted in 5 European countries (Belgium, Germany, Italy, Poland, and Spain) and compares two groups of children each fed two types (standard and follow-on) of cow

milk-based formula for the first year of life. Children, whose mothers decided against breast feeding were grouped randomly in either the low or high protein content formula milk. The isoenergetic infant formulas differed in the protein and compensatory fat content, while there was no difference in the composition of protein and fat or in the content of all other ingredients. The composition followed European legislation at the time (European Commission. Commission Directive 91/321/EEC of 14 May 1991). Breastfed infants were included as a non randomized reference group if the mother intended to exclusively breastfeed for at least 3 months (36).

The following criteria for exclusion were defined: maternal hormonal or metabolic disease, illicit drug consumption during pregnancy, multiple pregnancy, complications during pregnancy, preterm delivery and obvious diseases or deformities of the newborn (36).

Recruitment was carried out during the first 8 weeks of life between 1st October 2002 and 31st July 2004 (36). Every study center obtained an approval by the responsible ethics committee and the principles of the Declaration of Helsinki were met (36). Written informed parental consent was obtained for each infant(36).

Introduction and composition of complementary feeding was not influenced by the study. Children in the intervention and the breastfed group were followed up identically. After the first two years, all families not already dropped out were invited to participate in the follow-up study.

Anthropometric measures

Anthropometric measures, except birth weight and length which were taken from hospital, were obtained at visits to the study centers. The measurements were obtained at enrolment, at the ages of 3, 6, 12, 24 months of age and thereafter every 6 months until the age of 72 months and again at 96 months. Study personnel were trained several times following the standard operating procedures based on the WHO Multicentre Growth Reference Study (38). All study centers used the same equipment and all anthropometric measurements were done twofold with the mean used for analysis.

Questionnaires at enrollment and several times during study collected medical and socioeconomic data concerning the development of pregnancy, maternal weight before pregnancy, the child's medical history, and the family's socioeconomic

status. Children were dropped out when parents could no longer be contacted or refused further participation.

Dietary intake

A prospective 3-day weighed dietary record was used to collect detailed data on food and beverage intake as well as time and site of meal. Food intake was recorded on 3 consecutive days, including 1 weekend day and 2 weekdays, monthly for the first 9 months of infant's life and at 12, 18, 24, 36, 48, 60, 72 and 96 months of age. From the age of 36 months onwards, parents had the possibility to complete an alternative dietary record if food was consumed out of the home where weighing was not feasible. Parents were provided with a food album, where standard dishes and amounts of food were depicted so food amount could be estimated(39).

A dedicated software programme called Nutrcalc was developed as part of this project to enable data entry and calculation of nutrient intakes from dietary records (39). The German Bundeslebensmittelschlüssel (BLS) 3.01 provided the basis for the final nutrient calculation.

Data input of records into the database was done locally by trained dieticians who validated the protocols, clarified open issues with the parents and transferred the dietary records into the database in each study centre. Standard operating procedures (SOPs) were established for food data entry and food categorization to unify calculations. After data entry, categorization of custom food items was carried out according to SOPs developed on the basis of the BLS categorization.

Foodstuffs and recipes not included in the BLS were added during data entry by the local dietician evaluating the dietary records (so called custom food items). For custom food items, energy, fat, carbohydrate and protein content had to be entered, and optionally, if available, data on saturated, mono- and polyunsaturated fatty acids, oleic acid, n-6 long-chain fatty acids, n-3 long-chain fatty acids, cholesterol, vegetable and animal protein, lactose, sucrose, fructose, mono- and disaccharides, starches, dietary fiber, and selected vitamins and minerals (39).

Mono- and disaccharides were considered as sugars. Nutritional contents for custom food items were derived from either producers or national food databases. In some cases, data on sugar content were not available and were therefore estimated by comparison with similar products.

Dietary protocols with energy, fat, carbohydrate or protein intake 3 SD above or under the mean intake for each respective country by time point, and those deemed incomplete and inaccurate or with reported concurrent illness, were excluded.

The key objectives of this work are:

- Describe total sugar intake and sugar sources and their associated factors in European children
- Investigate the genetic and environmental factors associated with the intake of sweet tasting food in European children

This dissertation is based on the following published articles:

1. Research article

Pawellek I*, Grote V*, Theurich M , Closa-Monasterolo R, Stolarczyk A, Verduci E, Xhonneux A, Koletzko B; European Childhood Obesity Trial Study Group. Factors associated with sugar intake and sugar sources in European children from 1 to 8 years of age. Eur J Clin Nutr. 2017 Jan;71(1):25-32.

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2. Research article

Pawellek I, Grote V, Rzehak P, Xhonneux A, Verduci E, Stolarczyk A, Closa-Monasterolo R, Reischl E, Koletzko B; European Childhood Obesity Trial Study Group. Association of TAS2R38 variants with sweet food intake in children aged 1 to 6 years. Appetite. 2016 Jul 28; 107: 126-134

Objectives

Obesity and its secondary disorders are called a global burden, an epidemic or one of the greatest public health challenges in industrial countries. Hidden dietary fat and added sugars increase the energy density of foods and are by this major parameters of high energy intake. In addition to impacting overweight and its health consequences, high sugar consumption affects the intake of other food groups, and by this, possibly nutrient intake (19), dental health (32) and plasma triglyceride concentration (30).

The first publication aimed to determine the total sugar intake of children aged 1 to 8 years in five European countries. Further aims were the determination of main sugar sources as well as factors associated with total sugar intake and sugar intake from main sugar sources.

In the second publication the association of genetic variants of the bitter receptor gene TAS2R38 with the intake of (energy dense) sweet tasting foods was investigated. Intake of energy, macronutrients, sugar and energy from single food groups as well as BMI were further variables possibly associated with genotype of TAS2R38.

Methods

The dissertation was conducted in the framework of the CHildhood Obesity Project CHOP, a double-blind, randomized, controlled trial conducted in five European countries (Germany, Belgium, Italy, Poland and Spain).

Anthropometric measures were obtained at visits to study centers at enrolement, at the ages of 3, 6, 12, 24 months of age and thereafter every 6 months until the age of 72 months and at 96 months.

Dietary intake was recorded using monthly 3-day dietary records for the first 9 months of the infant’s life, as well as at months 12, 18, 24, 36, 48, 60, 72 and 96. Recorded food was classified by its composition into subgroups, with further classification generated into sweet/not sweet. The German BLS (Bundeslebensmittelschlüssel) 3.01 served as a data base and was expanded by custom food items (local food items not contained in the data base) in each study center. Mono- and disaccharides were captured as sugar. Further factors possibly

associated with sugar or sweet food intake (gender, birth order, mother's education, age, marital status and BMI pre-pregnancy) were gathered at study entry.

Buccal cells were collected at any time point of the study on filter cards. Genotyping was performed with a MALDI TOF (matrix assisted laser desorption/ionisation time of flight mass spectrometry), which is dedicated to analysis of single nucleotide polymorphisms. Individuals were grouped by variants of the first SNP of the bitter receptor gene TAS2R38 (rs713598), with PA and PP representing the taster group and AA representing the nontaster group.

Results

Total sugar intake increased from 65 g/d (30.0 % of energy intake (E%)) at 12 months of age to 83 g/d (20.9 E%) at 96 months of age, while the energy contribution of sugar decreased. Around 80% of children's sugar intake came from the following sugar sources: milk and dairy products, fruits and fruit products, confectionary and sugar sweetened beverages (SSB). Total sugar intake and dietary sugar sources varied significantly with country of residence. Also gender was associated with total sugar consumption: boys had a significantly (p=0.003) higher total sugar consumption than girls. Next to country of residence, sugar sources were associated with maternal age and education and with children's birth order: SSB consumption was significantly higher in children from young mothers while sugar intake from fruit products was lower in children from mothers with lower educational status and in children with higher birth order (40).

TAS2R38 genotype was not related to sugar intake but to energy intake from sweet tasting foods: children with PP and PA genotypes consumed an average of 83 kJ/d (95% CI 21 to 146; p=0.009) more energy from sweet tasting foods than children with AA genotype and a mean 56 kJ/d (95% CI 15 to 98; p=0.007) more energy from energy dense sweet products. Additionally, consumption of sweet tasting foods was lower in girls than boys and differed between countries. TAS2R38 genotype was not associated with the intake of energy, macronutrients, single food groups and BMI z-score (41).

Conclusions

The results of the two studies on intake of sugar and sweet tasting food showed that several factors are associated with sugar and sweet food consumption: gender and study country could be determined as main factors. Intake of sweet food is also associated with genotype of the bitter receptor gene TAS2R38 while sugar sources vary with maternal age and education as well as with birth order.

These results illustrate once more that the reasons for inappropriate eating behaviour and its consequences are complex. Eating culture has great influence on personal eating behaviour, while the genetic influence is not that great. To improve food choices of children from young and low educated mothers it is necessary to offer low-threshold services to reach this population group.

2. PUBLICATION 1:

"FACTORS ASSOCIATED WITH SUGAR INTAKE AND SUGAR SOURCES IN EUROPEAN CHILDREN FROM 1 TO 8 YEARS OF AGE"

European Journal of Clinical Nutrition (2017) **71,** 25–32

www.nature.com/ejcn

ORIGINAL ARTICLE

Factors associated with sugar intake and sugar sources in European children from 1 to 8 years of age

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BACKGROUND/OBJECTIVES: The World Health Organization recommends to limit intake of free sugars to 5% of total energy per day because of the great impact of high sugar intake on body fat deposition, adiposity and dental caries. However, little data exist about total intake and sources of sugar in European children. Therefore, this paper aims to describe sugar intake and dietary sugar sources and associated factors.

SUBJECTS/METHODS: Three-day weighed dietary records were obtained at eight time points from children 1 to 8 years of age ($n = 995$) in five European countries. Food items were classified into subgroups according to food composition. Linear mixed models were used to examine associated factors.

RESULTS: Total sugar intake increased from 65 g/day (30.0% of energy intake (E%)) at 12 months of age to 83 g/day (20.9 E%) at 96 months of age. Around 80% of children's sugar intake was derived from the following sources: milk and dairy products, fruits and fruit products, confectionary and sugar sweetened beverages (SSB). Total sugar intake and dietary sugar sources varied significantly by country of residence. Boys had a significantly ($P = 0.003$) higher total sugar consumption than girls. SSB consumption was significantly higher in children from young mothers while sugar intake from fruit products was lower in children from mothers with lower educational status and those with higher birth order.

CONCLUSIONS: Sugar intake in our population was lower than in other studies. Total sugar intake was associated with country of residence and gender, while dietary sugar sources varied by country of residence, maternal age, education and birth order.

European Journal of Clinical Nutrition (2017) **71,** 25–32; doi:10.1038/ejcn.2016.206; published online 9 November 2016

INTRODUCTION

Obesity is one of the greatest current challenges for public health. A recent European survey showed a prevalence of overweight children aged 4–7 years in the range of 7.6% (girls in Germany) to 29.8% (girls in Spain), based on the World Health Organization criteria.[1]

Changes in sources and the total intake of carbohydrates in the last century have been linked to an increase in the prevalence of obesity and diabetes.[2] Several studies in children confirmed significant associations between the intake of sugar sweetened beverages (SSBs) and the risk of becoming overweight.[3–6] SSBs lead to short-term satiation that oftentimes results in an increase in the frequency of their consumption.[7] No associations with body weight, however, have been found between consumption of 100% fruit juice and risk for being overweight in 2–11 years old children.[8] High added sugar intake disturbs energy balance since frequent consumption of energy-dense foods and liquids is associated with excessive weight gain.[7] Te Morenga *et al.*[7] concluded in their review about the effect of dietary sugars on body weight that a higher intake of dietary sugars is associated with higher body weight, but not as a physiological or metabolic consequence of the sugar intake but as a consequence of an energy imbalance.

Simple sugars include monosaccharides (galactose, fructose and glucose) and disaccharides (lactose and sucrose). In general, simple carbohydrates taste sweet and are the building blocks of more complex carbohydrates like starches. Complex carbohydrates are generally considered as healthy, satiating and are often accompanied by valuable vitamins and minerals. Total sugar content consists of both naturally occurring sugars like fructose in fruits and vegetables and lactose in milk products as well as added sugars. Processed foods often have added sugars, which are simple carbohydrates added to food during industrial processing, production or food preparation. Added sugars are linked to high energy density and considered to be 'empty calories'.[9] Free sugars imply all monosaccharides and disaccharides added to foods during food production or processing, and sugars naturally present in honey, syrups and fruit juices.[10]

While there is no common recommendation for total sugar intake, the intake of added sugars has been recommended to be below 25% of the daily energy intake by the Institute of Medicine;[11] several European countries advise to restrict added sugars to below 10% of the daily energy intake.[12] The World Health Organization proposed in its recently released guidelines to reduce intake of free sugars to less than 5% of total energy per day because of the impact of free sugars on body fat deposition, adiposity and, especially, dental caries.[10] Although

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caries development does not depend only on the amount of sugar consumed but also on many other factors,[13] a recently released review about the relationship between sugars and caries advised to reduce the recommendation for total sugar intake to even 2–3% of energy intake.[14]

This paper aims to determine the total sugar intakes and dietary sources of sugar in a longitudinal study of European children between 1 and 8 years of age. Furthermore, this paper investigates which factors are associated with dietary intake and sources of sugar in childhood.

SUBJECTS AND METHODS

Our subjects participated in the European CHildhood Obesity Project (CHOP), a double-blind randomized multicenter intervention trial in Germany, Belgium, Italy, Poland and Spain on the effect of the intake of higher or lower protein formula milk on growth in the first two years of life and adiposity at 6 years of age.[15] Children were randomized to receive either a higher or lower protein formula during the first year of life. A group of breastfed children was followed as a control group. All participants were apparently healthy and full-term infants who were born from uncomplicated, singleton pregnancies. Informed consent was obtained from the parents at enrollment and again for further follow-up at 24 months. Additionally consent of children was obtained at 8 years of age. The study was approved by the ethics committees of all study centers. Trial registration number at www.clinicaltrials.gov is NCT00338689.[15]

Nutritional assessment

Breast milk intake was not quantified, so children with any breast milk intake past 12 months of age were excluded from this analysis. In total, 995 children were included. Three-day weighed dietary records on three consecutive days (1 weekend day and 2 week days) were obtained at the ages of 12 ($n=827$), 18 ($n=717$), 24 ($n=747$), 36 ($n=531$), 48 ($n=503$), 60 ($n=445$), 72 ($n=468$) and 96 ($n=399$) months. Parents were provided with food scales and explained how to weigh offered foods and leftovers. From 36 months on parents had the possibility to fill out an alternative dietary record by comparing consumed food with pictures of standardized and weighed portion sizes. Food intake at nursery school, school or anywhere else outside home that was not prepared by parents was recorded with the help of other proxy reporters (i.e. instructed educators). Standard operating procedures were applied for dietary data entry and plausibility checks. A trained dietician validated the protocols, clarified open issues with the parents and transferred the dietary records into the database in each study center. The German food composition database, BLS 3.01 (Bundeslebensmittelschlüssel Version 3.01), was used to calculate nutrient intakes from the food intake data in all participating countries. So-called 'custom food items' were items not found in BLS and added by study dieticians. For custom food items, information on nutrient content was either provided from the food manufacturer or estimated through recipe simulation.[16]

For the purpose of this study we investigated the joint intake of natural and added sugars in form of all mono- and disaccharides. In some cases, data on sugar content were not available and estimated by comparison with similar products. Food items were categorized into subgroups according to food composition.

A total of 4850 different food items were reported by the subjects in the 3-day dietary records and classified into food groups. Table 1 shows the food groups containing sources of sugar. Important sugar sources were selected on basis of their contribution to total sugar intake (>2% of total sugar intake) and relevance: milk and dairy products, fruit and fruit products, bread and cereals, confectionary and SSBs.

Explanatory variables

Weight and height were measured at the age of 12, 24, 36, 48, 60, 72 and 96 months by trained study nurses. In all study centers the same scales and stadiometers were used (Seca 336 at $\leqslant$24 months and Seca 702 at $\geqslant$24 months; Stadiometer 242; Seca, Hamburg, Germany).

Maternal education was assessed according to the International Standard Classification of Education and categorized in high, middle and low. Maternal age at birth was categorized in younger (<28 years), middle (28–33 years) and older ($\geqslant$34 years). Pre-pregnancy weight was reported by the mother and maternal body mass index was classified as normal (<25 kg/m^2), overweight (25–30 kg/m^2) and obese (>30 kg/m^2). Subjects' birth order was reported at study entry.

Statistical analysis

Our primary end point was the total daily sugar intake (g/day) and energy intake from sugar in four main sugar sources (E%). Secondly, we investigated the association of study country, age, gender, maternal age at birth, maternal education level, early feeding type and birth order with these outcomes.

Linear mixed models with random intercept and random linear (age) slope and fixed cubic age terms were used to describe group differences in sugar and energy intake from sugar in four main sugar sources from 12 to 96 months of age. Country–age interaction terms were included together with main fixed effects of countries, gender, early feeding type

Table 1. Classification of sugar containing food items reported in the 3-day dietary records

Name of subgroup	*Milk and dairy products*	*Fruit and fruit products*	*Confectionary*	*Bread and cereals*	*Sugar sweetened beverages*
Infant formula milk, breast milk	X				
Milk and dairy products without added sugars	X				
Milk and dairy products with added sugars	X				
Fresh fruits		X			
Fruit mash or compote		X			
Fruit jam		X			
Fruit concentrate		X			
Dried fruits		X			
Fruit juice		X			
Pastry			X		
Desserts			X		
Sugar, honey			X		
Sweets			X		
Infant cereals, muesli without added sugars				X	
Cereals with added sugars				X	
Bread and bread products				X	
Sugared infusions					X
Fruit drinks					X
Soft drinks					X
Sugar sweetened instant tea					X

(breastfeeding versus formula feeding), birth order, maternal age and education level.

A fixed effect can be interpreted as the population average effect and a random effect as the individual deviation of that average effect. In this longitudinal model the intercept was set to age 12 months by subtracting age by 12.

Dietary records with energy, fat, carbohydrate or protein intake 3 s.d. above or under the mean intake by time point and country were excluded.

Data management and statistical analyses were carried out with the software packages SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) and Stata version 13.1 (StataCorp LP, College Station, TX, USA).

RESULTS

Table 2 shows the characteristics of the study population with completed 3-day dietary records at any time point ($n = 995$). The number of available dietary records ranged from 827 at 12 months to 399 at 96 months of age. About 70% ($n = 692$) of infants were originally in the intervention group that received study formula during the first year of life and 30% ($n = 303$) of the infants were exclusively breastfed for at least the first 3 months of life. More than half of the mothers had a middle educational level. The mean age at birth was 30.7 (5.0) years; mean maternal pre-pregnancy body mass index was 23.4 kg/m^2, with 7.3% of the mothers being obese and 20.7% overweight.

Table 2. Characteristics of study population with completed 3-day dietary protocol at least one time point between 12 and 96 months ($n = 995$)

	n *(%)*
Country	
Germany	159 (16.0)
Belgium	120 (12.1)
Italy	276 (27.7)
Poland	183 (18.4)
Spain	257 (25.8)
Gender	
Boys	477 (47.9)
Girls	518 (52.1)
Early feeding type	
FF	692 (69.5)
BF	303 (30.5)
Maternal education level	
Low	220 (22.2)
Middle	504 (50.8)
High	269 (27.1)
Unknown	2 (0.2)
Maternal age at birth (years)	
< 28	280 (28.2)
28–34	391 (39.3)
> 34	323 (32.5)
Unknown	1 (0.1)
Maternal pre-pregnancy BMI	
< 20	176 (18.4)
20 to < 25	513 (53.6)
25 to < 30	198 (20.7)
⩾ 30	70 (7.3)
Unknown	38 (3.8)
Birth order	
1st child	579 (58.2)
2nd child	317 (31.9)
> 2nd child	98 (9.9)

Abbreviation: BMI, body mass index.

Total intake of macronutrients and sugar

Mean energy intake increased from 3658 kJ/day at 12 months to 6662 kJ/day at 96 months (Table 3) while mean energy intake per kg body weight decreased from 377 to 240 kJ/kg/day. While the carbohydrate intake increased from age 12 to 96 months, the contribution of sugar declined from 57 to 43%. The average contribution of carbohydrates to total energy intake varied between 52 and 48% with a slight decrease over time. The observed decline was generally most pronounced between 12 and 36 months of age (data not shown). Total sugar intake increased from 65 g/day (30% of energy intake (E%)) at the age of 12 months to 83 g/day (21 E%) at the age of 96 months.

Factors associated with total sugar intake

Table 4 shows the factors associated with total sugar intake and dietary sugar sources. Greatest differences were found between countries. Italian children had the lowest and Polish children the highest daily sugar intakes over the whole-study period, ranging from 51 to 73 and 76 to 93 g/day, respectively, from 12 to 96 months of age. Boys had a significantly ($P = 0.003$) higher total sugar consumption than girls. Total sugar intake was not associated with any of the other variables.

Dietary sugar sources

Around 80% of children's sugar intake derived from the following sugar sources at all time points: milk and dairy products, fruits and fruit products, confectionary products, bread and cereals, and SSBs. Sugar intake from milk and dairy products decreased from 47 to 19% of total sugar intake from 12 to 96 months of age; sugar intake from fruit and fruit products and bread and cereals was relatively stable around 30 and 5%, respectively, over the whole-study period while the proportional sugar intake from the other food groups increased: for confectionary from 5 to 24% of total sugar intake and for SSBs from 2.0 to 6.7%. The overall energy contribution of SSBs was negligible (< 1%). While sweetened infant teas contributed most sugar up to 2 years of age, carbonated drinks followed by fruit juices were the major source at later ages (data not shown).

While the overall contribution of sugar to energy intake decreased from 1 to 8 years of age, the E% from sugars from confectionaries and sweetened beverages increased from 2 to 5 E % and 0.6 E% to 1.4 E%, respectively. The overall decline was mainly accounted by the reduction in the sugar intake from milk and dairy products: 14 E% at 1 year and 4 E% at 8 years of age (Figure 1).

Comparison among European countries

Country of residence had the strongest association with dietary sources of sugars with differing trends over the study period (Table 4 and Figure 1). Spanish children had the highest E% from sugar in dairy products throughout the whole-study period while Italian and German children had the lowest. E% from sugar intake in fruits was highest in German children and lowest in Spanish and Belgian children at almost every time point. SSB consumption was low in all participating countries with the lowest intake levels in Spain and the highest in Italy.

Other factors associated with E% by sugar sources

SSB consumption was significantly ($P < 0.001$) higher in children from younger mothers while sugar intake from fruit products was significantly lower in children with lower educated mothers and in children with higher birth order. Furthermore, formerly breastfed

Table 3. Mean daily intake of energy (kJ/day) and macronutrients (g/day) total and per kg body weight[a] from 12 to 96 months

	Age (months)							
	12 (n = 827)	*18 (n = 717)*	*24 (n = 747)*	*36 (n = 531)*	*48 (n = 503)*	*60 (n = 445)*	*72 (n = 468)*	*96 (n = 399)*
Energy								
kJ/day (s.d.)	3658 (721)	4275 (835)	4622 (960)	5077 (976)	5487 (990)	5795 (1081)	6144 (1022)	6662 (1191)
kJ/kg/day (s.d.)	377 (79)	Not available	376 (83)	349 (73)	328 (67)	304 (67)	284 (58)	240 (57)
Fat								
g/day (s.d.)	31.8 (8.2)	37.7 (9.7)	42.0 (11.7)	46.7 (12.5)	51.5 (13.5)	54.2 (14.1)	57.5 (14.0)	64.8 (16.9)
g/kg/day (s.d.)	3.3 (0.9)	Not available	3.4 (1.0)	3.2 (0.9)	3.1 (0.9)	2.9 (0.8)	2.7 (0.7)	2.3 (0.7)
Protein								
g/day (s.d.)	32.1 (9.6)	41.7 (11.4)	44.9 (12.8)	46.7 (11.8)	49.7 (12.6)	51.7 (13.3)	54.7 (12.4)	60.7 (15.0)
g/kg/day (s.d.)	3.3 (1.0)	Not available	3.6 (1.1)	3.2 (0.8)	3.0 (0.8)	2.7 (0.8)	2.5 (0.6)	2.2 (0.6)
Carbohydrates								
g/day (s.d.)	114.4 (26.9)	128.5 (30.6)	136.6 (32.6)	151.7 (35.4)	163.0 (33.9)	173.4 (39.6)	183.8 (38.4)	192.3 (40.7)
g/kg/day (s.d.)	11.8 (3.0)	Not available	11.1 (2.8)	10.4 (2.6)	9.8 (2.3)	9.1 (2.3)	8.5 (2.1)	6.9 (1.8)
Sugar								
g/day (s.d.)	65.2 (20.3)	65.9 (21.8)	69.7 (23.2)	75.3 (24.3)	78.5 (24.1)	81.6 (26.7)	83.6 (25.1)	82.9 (27.2)
g/kg/day (s.d.)	6.7 (2.1)	Not available	5.7 (2.0)	5.2 (1.8)	4.7 (1.6)	4.3 (1.5)	3.9 (1.3)	3.0 (1.1)
	n = 815		*n* = 726	*n* = 508	*n* = 490	*n* = 438	*n* = 461	*n* = 395
Weight kg (s.d.)	9.8 (1.1)	Not available	12.4 (1.4)	14.7 (1.8)	16.9 (2.1)	19.4 (2.9)	22.1 (3.8)	28.6 (6.1)
BMI-for-age *z*-score (s.d.)	0.35 (1.01)	Not available	0.26 (0.94)	0.29 (0.98)	0.36 (0.96)	0.36 (1.04)	0.34 (1.18)	0.46 (1.21)

Abbreviation: BMI, body mass index. [a]Mean per kg body weight for children with available body weight.

children had a significantly lower consumption of sugars from confectionary (Table 4).

DISCUSSION

Total sugar intake in our study population increased over the investigated time period, but E% from sugar intake decreased. Around 80% of total sugar intake was from five main food sources over all time points: milk and dairy products, fruit and fruit products, confectionary, bread and cereals and SSBs. Total sugar intake was significantly associated with country of residence and gender, dietary sources of sugars varied considerably by country of residence, mother's age and education and birth order.

Total sugar intake

In a Canadian study with data from 2004 total sugar intake was about 110 g/day (27 E%) in 1- to 3-year-old children and about 120 g/day (26 E%) in 4- to 8-year-old children,[17] a US study from 1981 reported a total sugar consumption of 134.3 g/day in 5- to 12-year-old children.[18] The European-wide IDEFICS study investigated total sugar intake in children aged 2–9 years: total sugar intake ranged from 77 g/day (19 E%) in Estonia to 114 g/day (30 E%) in Germany.[19] The European Nutrition and Health report of 2009 reports a mean E% of sucrose of 15.5 in European children aged 4–14 years.[20] A recently released review on sugar consumption in dietary surveys worldwide showed a range of mean total sugar intake in 4- to 10-year-old children from 83.6 (4-year-old Irish children) to 167 g/day (12-year-old German boys). E% from total sugar ranged from 17% in 3- to 10-year-old Italian children to 34.8% in Dutch 4- to 6-year-olds.[21] Total sugar intake is much lower in our population while energy contribution is comparable to the Canadian and the IDEFICS study and to data in 4- to 10-year-olds from the worldwide review.[21] These differences probably derive from different methods for dietary assessment and different populations.

Sugar intake also varied between study countries. Whereas some countries have recommendations on sugar intake, there is no common recommendation on sugar intake in Europe.[13] The World Health Organization recently released guidelines on free sugar intake, proposing to reduce the intake of free sugars to 5% of total energy per day.[10] We did not distinguish between natural occurring and added sugar or free sugar, so we cannot directly compare actual intake to recommended intake. However, at 96 months of age about 6.5% of total energy intake was taken from sugar in confectionary and SSBs, which represent 100% free sugar. Thus, together with free sugar from dairy products, fruit products or other sources most of our 8-year-old children consumed more than 5% of total energy intake from free sugars.

Dietary sources of sugar

Negative health impacts of sugar function mainly via an energy imbalance than via sugar itself.[7] By this it is important to investigate from which sources sugar is taken, as in some sources sugar is accompanied by fat leading to high energy density (example, confectionary) and in some not (example, fruits).

In Canada as well as in the United States, milk contributed greatly to sugar intakes; 19.9% of total sugar intakes in the Canadian study[17] and 20.4% of sugar intakes in the 5- to 12-year-old US children.[18] In our population, milk and dairy products were the top source of sugars at 12 months of age, but at 96 months of age, more sugar was taken from fruit and fruit products. At 12 months of age formula milk and milk-based baby food has a high energy contribution and many of these baby foods contain next to lactose glucose or sucrose which can explain the high sugar intake from milk and dairy products in this age.[22]

Fruits generally played a greater role in the diets of the children in our study population, compared with the US and Canadian

Table 4. Effects of country, gender, maternal age at birth, maternal education level, early feeding type and birth order on total sugar intake and E% from sugar in four main sugar sources based on a linear mixed model (significant interactions between age and country not displayed) (4445 observations of $n = 955$ participants at 7 time points)

	Total sugar intake (g/day)	*E% from sugar in milk and dairy products*	*E% from sugar in fruit and fruit products*	*E% from sugar in confectionary*	*E% from sugar in SSB*$_3$
Country[a]					
GE			Reference group		
BE	4.01 (−0.85, 8.86)	5.42** (1.84, 9.00)	−3.92* (−7.71, −0.12)	6.03*** (3.37, 8.69)	−2.62** (−4.21, −1.03)
IT	−10.34*** (−14.23, −6.39)	−0.00 (−2.92, 2.91)	1.82 (−1.27, 4.91)	2.11 (−0.05, 4.26)	1.87** (0.58, 3.16)
PL	13.95*** (9.63, 18.27)	8.41*** (5.22, 11.60)	−5.07** (−8.45, −1.69)	1.64 (−0.73, 4.01)	0.35 (−1.06, 1.77)
ES	13.12*** (9.11, 17.12)	9.22*** (6.26, 12.18)	−12.34*** (−15.49, −9.20)	−0.98 (−3.18, 1.22)	−2.92*** (−4.24, −1.61)
Age					
Age	0.19 (−0.20, 0.58)	−1.26*** (−1.55, −0.97)	0.25 (−0.05, 0.56)	1.37*** (1.14, 1.60)	0.04 (−0.10, 0.17)
Age[b]	0.00915 (−0.00312, 0.02141)	0.02349*** (0.01431, 0.03267)	−0.00609 (−0.01572, 0.00354)	−0.03127*** (−0.03855, −0.02400)	−0.00002 (−0.03855, −0.02400)
Age[c]	−0.00009 (−0.00019, 0.00001)	−0.00014*** (−0.00022, −0.00007)	0.00003 (−0.00005, 0.00011)	0.00022*** (0.00016, 0.00028)	0 (−0.00004, 0.00003)
Gender					
Boys			Reference group		
Girls	−2.95** (−4.84, −1.07)	0.63 (−0.63, 1.89)	0.25 (−1.13, 1.64)	0.35 (−0.58, 1.28)	−0.02 (−0.60, 0.57)
Maternal age at birth					
< 28			Reference group		
28 to < 33	−1.85 (−4.34, 0.65)	2.01* (0.34, 3.68)	0.30 (−1.53, 2.13)	0.11 (−1.12, 1.34)	−1.31*** (−2.09, −0.54)
33–44	−1.17 (−4.02, 1.68)	1.80 (−0.10, 3.70)	1.27 (−0.82, 3.36)	−0.04 (−1.44, 1.37)	−1.53*** (−2.41, −0.64)
Maternal education level					
No/low			Reference group		
Middle	0.83 (−1.63, 3.29)	−0.83 (−2.49, 0.82)	1.98* (0.17, 3.80)	−0.35 (−1.57, 0.87)	−0.55 (−1.32, 0.22)
High	−0.13 (−3.08, 2.82)	−1.56 (−3.52, 0.41)	3.62*** (1.47, 5.78)	−0.23 (−1.68, 1.22)	−0.56 (−1.47, 0.36)
Early feeding type					
FF			Reference group		
BF	0.34 (−1.84, 2.52)	0.25 (−1.19, 1.68)	0.80 (−0.78, 2.39)	−1.18* (−2.25, −0.11)	−0.12 (−0.80, 0.55)
Birth order					
1st child			Reference group		
2nd child	0.45 (−1.71, 2.61)	−1.21 (−2.64, 0.22)	−0.36 (−1.94, 1.22)	0.78 (−0.28, 1.84)	0.75* (0.08, 1.42)
> 2nd child	3.23 (−0.26, 6.71)	−0.33 (−2.67, 2.00)	−3.81** (−6.37, −1.25)	1.63 (−0.09, 3.36)	−0.07 (−1.15, 1.02)
Total sugar intake		−0.08*** (−0.10, −0.06)	0.11*** (0.09, 0.13)	−0.01 (−0.03, 0.01)	0.05*** (0.04, 0.06)
Constant term[d]	69.94*** (62.76, 77.13)	42.97*** (37.86, 48.07)	25.63*** (20.07, 31.18)	5.06** (1.25, 8.86)	−0.01 (−2.37, 2.36)

95% confidence intervals in brackets; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. [a]GE, Germany; BE, Belgium; IT, Italy; PL, Poland; ES, Spain. [b]FF, formula-fed; BF, breastfed. [c]SSB, sugar sweetened beverages. [d]Total sugar intake or %E from respective sugar source at 12 months of age in the baseline group (= Germany, boy, maternal age < 28, maternal education low, FF and 1st child).

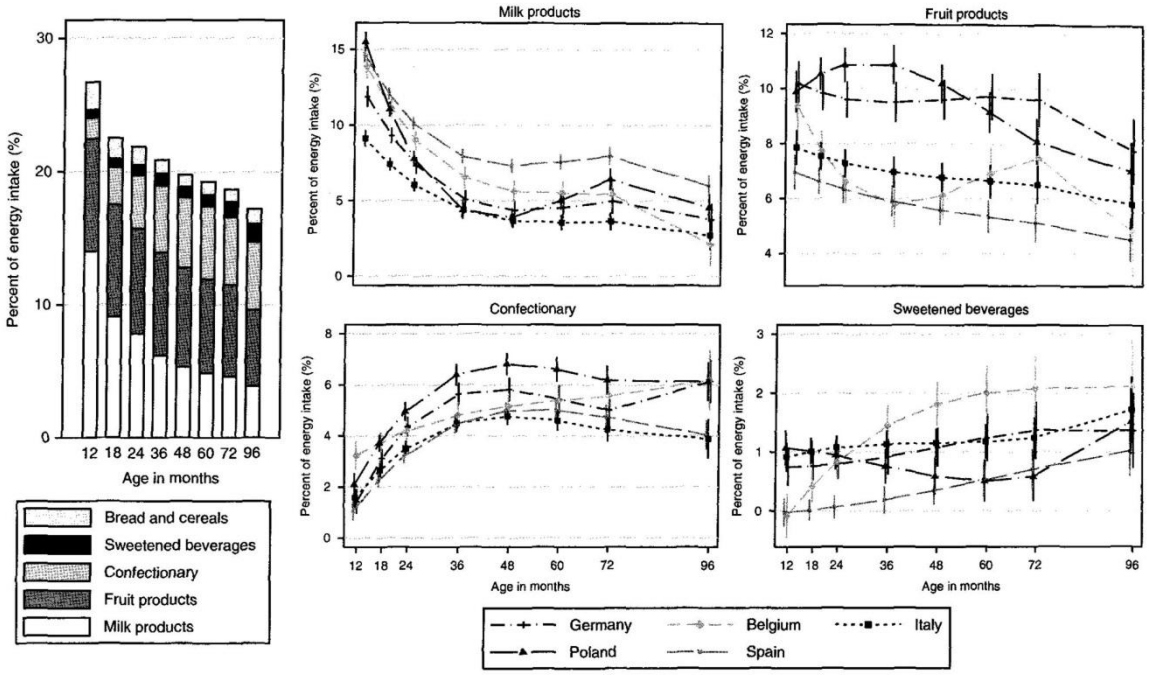

Figure 1. Percentage of total energy intake from sugar in main sugar sources from 12 to 96 months of age.

studies. Canadian children reported 14.9% of sugars from fruits and US children reported only 11.5%.[17,18] As sugars are almost the only energy source in fruits, high sugar intake from fruits and fruit products means high intake of fruit and fruit products, which might positively affect children's health. The slightly higher contribution of fruits and fruit products to total sugar consumption at 12 months might have two reasons: on the one hand baby fruit jars with added sugars are common in many European countries;[22] on the other hand at 96 months of age other sources of added sugars like confectionary or sweetened beverages replace sugar intake from fruits and fruit products in parts.

Sugar intake from SSB was much lower in our study (6% of total sugar intake at 96 months): in the Canadian study 9.8% of total sugar intake was taken from regular soft drinks and fruit drinks[17] and in the US study 13.8% from SSB.[18] As sugar in beverages lead to high energy intake without satiating effects, a low sugar intake from beverages might have positive health impacts.[7]

Canadian children 1–8 years take 19.4% of total sugar intake from confectionary, sugar and syrups,[17] which is approximately comparable to our classification 'confectionary'. We could show that sugar intake from that food group strongly increases during the investigated period and delivers at the age of 8 years almost a quarter of consumed sugars. The German DONALD study shows that consumption of confectionary rich in sugar and fat increases until the age of 14 years and decreases after that age.[23]

Variables associated with dietary sugar sources

Dietary sources of sugars seemed to be associated mainly with country of residence. Further associated factors were maternal age at birth, maternal education level and the child's birth order.

Country. To our knowledge there is no other study that compares the sources of sugars in different European countries. The European-wide IDEFICS study found wide ranges of total sugar intakes in different European countries (ranging from 77 to 114 g/d), suggesting also differences in the sugar sources.[19] Comparing our results with sugar sources in the US, Swedish or Canadian studies, we observed great differences. The selection of sugar sources seems to be strongly influenced by eating culture in the participating countries. Therefore, it is important to consider this in the specific countries before determining recommendations for intake of sugar or food groups.

Maternal age and education. Children with mothers in the older age category had a significantly lower sugar intake from SSB than children with younger mothers. In the American Feeding Infants and Toddlers Study, higher maternal age was associated with multiple positive feeding practices in infant and toddlers aged 4–24 months.[24] Karp and Lutenbacher[25] investigated infant feeding practices of young American mothers and found high rates for inappropriate feeding practices like putting additional cereals in the bottle or inappropriate food selection.

Sugar intakes from fruits and fruit products were significantly lower in children of mothers with low educational status compared with children from mothers in the higher or middle educational category. This result is not surprising as several studies confirmed the healthier lifestyle in children with higher educated parents as, for example, the Feeding Infants and Toddlers Study, in which a mother's college education was associated with the largest number of positive feeding behaviors.[24] This is an important observation, since parental education is also associated with fruits and vegetable intake in adolescents, making it a consistently influential variable throughout childhood.[26] It should be investigated to what extent parents read and understand food labels. Improvement in the quality of children's diet involves nutrition education especially for parents with a low educational status.[27] This fact leads to the conclusion that nutritional recommendations should especially focus to young and lower educated parents.

Birth order. Increasing birth order posed a significant dietary risk, as children with higher birth orders had a lower sugar intake

from fruits and fruit products. On the one hand, offering children fresh fruit may be more time consuming than offering convenience food. On the other hand, time and motivation to encourage children to eat healthy food is maybe less in parents with more children. A review on the family environment and fruit and vegetable intake showed that there were positive associations between parental encouragement to try and eat fruits and vegetables and actual intake of these foods in children.[26]

Strengths and limitations

Over a span of 7 years, the number of participants decreased from 827 at the age of 12 months to 399 at the age of 96 months. Attrition was thought to be partly due to the time consuming and demanding task of completing the 3-day dietary records, in addition to the request to complete multiple other questionnaires. Except for the first year of life, when formula milk was provided free of charge, the participants had no benefit from study participation. In long-term follow-up studies of healthy infants, high participation rates are generally difficult to achieve. It was proposed that follow-up rates of 50–80% are acceptable in longitudinal epidemiological cohort studies.[28]

Families with higher education and former breastfeeders were more inclined to stay in our study. On the other hand children from lower educated families and formula-fed children are more likely to have a poorer diet.[24,29,30] So we would expect that the shift towards unhealthy food choices would be even greater with a higher participation rate of children from lower educated families and formula-fed children. Furthermore, there was differential loss to follow-up seen between countries with higher attrition rates in Italy and Belgium due to logistical problems at 96 months only. However, any major effect resulting from differences in follow-up rates between countries should have been captured by adjusting for country–age interactions in the longitudinal model. Nevertheless, part of the observed effects might be due to attrition bias.

In our study it was not possible to distinguish between natural and added sugars, which limits the information available on the dietary sources of added or natural sugars in this cohort. A distinction of added and natural sugar is easier in a dietary assessment via food frequency questionnaires with limited number of food items and predefined values for added or natural sugars but not in our method with an incomparable higher number of food items actually consumed. So on the one hand, conclusions about the relation of added and natural sugars could improve explanatory power and show more approaches to improvement, but on the other hand a dietary assessment via detailed 3-day dietary records is considered to be the most precise method of dietary intake assessment in young children.[16] We used a standardized approach with quality assurance to data collection and evaluation that is expected to reduce bias and errors.

One strength of our study is the longitudinal assessment with sound methodology allowing for direct comparison between study countries. Furthermore, we could see that there are great differences in total sugar intakes, depending on sociocultural factors and country of residence.

CONCLUSION

Children from the five European countries in this study had a lower total sugar intake than children in the US, Canada or the population investigated in the IDEFICS study. Eating habits in the participating European countries were very different during the complementary feeding period and in later childhood. Total sugar intake was associated with the country of residence and gender, while dietary sugar sources varied by country of residence, maternal age and education and birth order. Our results show that it is important to address European-wide nutrition-related recommendations especially in the complementary feeding period in consideration of different country-specific eating cultures. Further efforts have to be done to reach parents of children with nutritional risks, namely young and lower educated parents.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

This manuscript does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area.

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3. PUBLICATION 2

"ASSOCIATION OF TAS2R38 VARIANTS WITH SWEET FOOD INTAKE IN CHILDREN AGED 1-6 YEARS"

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Association of TAS2R38 variants with sweet food intake in children aged 1–6 years

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ABSTRACT

We aimed at studying whether genetic variants of the TAS2R38 gene are associated with energy intake from sweet tasting foods, total energy and macronutrient intake and body weight in children.

Children (n = 691) from five European countries were genotyped for the first variant site rs713598 of the TAS2R38 bitter receptor gene. Three-day dietary records were obtained yearly from one to six years of age. Foods were categorized in sweet and non-sweet-tasting. Mixed models were used to describe group differences in food and nutrient intake and BMI z-score over time.

TAS2R38 genotype was related to energy intake from sweet tasting foods: Children with PP and PA genotype consumed an average 83 kJ/d (95% CI 21 to 146; p = 0.009) more sweet tasting foods than children with AA genotype and a mean 56 kJ/d (95% CI 15 to 98; p = 0.007) more energy from energy dense sweet products. Intake of sweet tasting foods was lower in girls than boys and differed between countries. TAS2R38 genotype was not associated with the intake of energy, macronutrients, sugar, single food groups and BMI z-score.

Despite many other factors influencing food preference and intake in children, actual intake of sweet food items is associated with TAS2R38 genotype. Children with PP or PA genotype consume more (energy dense) sweet tasting foods.

1. Introduction

Obesity and the metabolic syndrome has become one of the greatest challenges for health care systems, with high ensuing costs in industrialised countries. A recent study showed a prevalence of overweight preschool children aged 4–7 years in the range of 7.6% (girls in Germany) to 29.8% (girls in Spain), based on the WHO criteria (van Stralen et al., 2012). Obesity is the result of an impaired energy balance with too high energy intake relative to energy expenditure. 7 to 14 year-old US children consume 46% of their total energy intake via discretionary dietary fat and added sugar (Brady, Lindquist, Herd, & Goran, 2000); thus these dietary components have a high contribution to energy intake without delivering valuable nutrients. Children's food preferences are influenced by genetic, environmental and educational factors (Scaglioni, Arrizza, Vecchi, & Tedeschi, 2011). Numerous studies confirmed that obesity is strongly influenced by a genetic predisposition (Keller, Pietrobelli, Must, & Faith, 2002) and genetics may also play a role in the development of food preferences and dietary habits (Scaglioni et al., 2011). The WHO proposed in their recently released guidelines to reduce intake of free sugars to 5% of total energy per day because of the effects of high sugar intake on body fat deposition, adiposity and dental caries (World Health

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Organisation [WHO], 2015). The TAS2R38 gene encodes a seven-transmembrane G protein-coupled receptor for the perception of glucosinolates, bitter-tasting phytochemicals in Brassica vegetables (Kim & Drayna, 2005). Individual variation in the sensitivity to taste the two bitter compounds phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP), chemical compounds related to the glucosinolates can partially be explained by the genetic variation of the TAS2R38 receptor (Kim & Drayna, 2005). Outside of Africa mainly two allele forms can be found that differ at three nucleotide positions in the gene and in three amino acids in the receptor protein (A49P, A262V, and V296I) (Kim & Drayna, 2005). The amino acid combination PAV represents the taster variant (49%) and AVI (47%) the non-taster variant (Kim & Drayna, 2005).

In numerous investigations, tasters showed lower acceptance of cruciferous, green and raw vegetables (Drewnowski, Henderson, Hann, Berg, & Ruffin, 2000; Kaminski, Henderson, & Drewnowski, 2000; Yackinous & Guinard, 2002), whereas a recent study found no association between genetic variations of the TAS2R38 gene and the intake of brassica vegetables (Gorovic et al., 2011). Intake of sweet tasting food or sugar intake was also discussed to be associated with bitter taste sensitivity. Some studies reported that taster children had a higher intake of sugar and sweet tasting food (Mennella, Pepino, & Reed, 2005; Keller & Tepper, 2004; Joseph, Reed, & Mennella, 2016), while others did not find relationships (Keller et al., 2010, 2014).

Furthermore some studies hypothesized that PROP taste sensitivity is associated with sensitivity to other bitter tastes, sweet taste, the pungency of chili peppers, the astringency of alcohol, and the texture of fats (Tepper, White, Koelliker, & Lanzara, 2009). We hypothesized that children with the TAS2R38 PP or PA taster genotype have a higher actual intake of sugar and sweet tasting food to compensate for the bitter taste and other taste sensitivities. The aim of our study was to examine whether TAS2R38 genotype is associated with intake of total energy, energy from sweet tasting foods, sugar, carbohydrate, fat, protein, single food groups and BMI z-score in European children.

2. Material and methods

The analysis was performed in the framework of a double-blind, randomized, multicentre intervention trial in Germany, Belgium, Italy, Poland, and Spain on early protein intake and early growth (Koletzko et al., 2009) and adiposity at six years of age (Weber et al., 2014). All participants were apparently healthy and term infants who were born from uncomplicated, singleton pregnancies (Koletzko et al., 2009).

2.1. Bitter receptor

Buccal cells were collected on filter cards (IsoCode Cards of Schleicher and Schüll, Dassel, Germany) or on FTA Indicator Cards (Whatman, Middlesex USA) during study visits. DNA extraction from the cards was accomplished by a washing procedure according to protocols provided by the manufacturers.

The polymerase chain reaction (PCR) and genotyping analysis of the TAS2R38 gene was performed in the Helmholtz Zentrum München using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Individuals were grouped by the first SNP rs713598 resulting in an allele change on base pair 145 from G(uanine) to C(ytosine) which causes an amino acid change on codon 49 from A(lanine) to P(roline). TAS2R38 genotypes PP and PA are grouped together, as they represent the taster variant, while AA represent the non-taster variant (Kim & Drayna, 2005).

2.2. Dietary intake assessment

Parents completed three-day, weighed dietary records on three days (1 weekend day and 2 week days) at the ages of 12, 24, 36, 48, 60 and 72 months. For evaluation a trained dietician validated the protocols, clarified open issues with the parents and transferred the dietary records in the database in each study centre via a dedicated software program (Verwied-Jorky et al., 2011).

The German BLS 3.01 (Bundeslebensmittelschlüssel Version 3.01) formed the basis for calculation of nutrient intakes from food intake data in all participating countries. Food items not found in BLS were added by the dieticians evaluating the dietary records (custom food items). Standard operating procedures were developed and implemented for the introduction of the dietary records in the program to harmonize procedures (Verwied-Jorky et al., 2011). For custom food items, information on nutrient content was provided from manufacturers. Mono- and disaccharides were considered as sugars. If data on sugar content was not available it was estimated based on comparison with similar products. Food items were categorized into subgroups according to food composition and taste and divided into sweet and non-sweet tasting. In a first step this was done by a qualified dietician, in further steps the categorization was reworked by other dieticians, especially for custom food items in each study centre. In case there was no consensus, the food item was categorized in 'not sweet'. Sweet tasting foods comprise pastry products, sweet tasting beverages (fruit juice, soft drinks, fruit drinks), sweetened cereals (sugar >10 g/100 g), desserts, fruit and fruit products, sweetened dairy products, sweets, instant cacao powder, sugar and honey and sweet main dishes.

Fruit and fruit products contain fresh and dried fruits as well as fruit mash or preserves and jam. For further analysis sweet tasting food items were divided into sweet products with high energy density (energy density $\geq$ 200 kcal/100 g) and sweet products with low energy density (energy density < 200 kcal/100 g).

2.3. Anthropometrics and other explanatory variables

Body weight and other anthropometric variables were evaluated yearly from 12 to 72 months of age. All study centres used the same equipment for measuring body weight (Seca 336 scales at $\leq$24 months and Seca 702 scales at $\geq$ 24 months; Seca, Hamburg, Germany).

Mother's education, mother's age at child's birth and child's birth order was reported at study entry. Mother's height and weight was measured during study visits. Mother's education was categorized in high, middle and low by the level of graduation. Children's feeding type was categorized into three groups: higher or lower protein formula group (randomized groups) and breastfed children.

2.4. Statistical analysis

Our primary endpoint was the total daily energy intake from sweet tasting foods (kJ/d). Secondly, we investigated the association of TAS2R38 genotype with the average daily intake of energy from sweet products with high energy density (kJ/d), energy from sweet products with low energy density (kJ/d), carbohydrates (g/d), sugar (g/d), fat (g/d), protein (g/d), total energy (kJ/d), energy intake from single food groups (kJ/d) and BMI z-score.

Differences between TAS2R38 genotypes in the distribution of categorical variables were tested by a chi-square test or Kruskall-Wallis test as appropriate. Mixed linear (growth) models with random intercept and random linear slope (age) and fixed quadratic and cubic age terms were used to describe group

differences in food and nutrient intake as well as BMI z-score from 12 to 72 months of age. We adjusted for gender and country. Additionally all models looking at the effects on macronutrient and sugar intake were adjusted for total energy intake; models with BMI as the outcome were also adjusted for early feeding type (formula/breastfeeding). Effects of potential confounders like single mother status, mother's education attainment, marital status, maternal age at birth, birth order, and maternal smoking were assessed. In a second step we additionally adjusted for current BMI and energy misreporting status in a subset in which weight and height at the specific time point was available. We calculated misreporting based on the ratio of mean energy intake to energy requirements. Energy requirements were based on Butte (12 months) and Torun (≥24 months) using regression functions including weight (Butte, 2005; Torun, 2005); energy needed for tissue disposition of growth was added. Individual normal ranges of the ratio were defined according to the method described by Black and Cole (2000); ratios below and above the normal range were defined as under- and over-reporters, respectively, while all others were defined as normal reporters.

The following equation specifies the estimated mixed model formally:

$$Y_{it} = \beta_{0i} + \beta_{1i} \times AGE_{it} + \beta_2 \times AGE_t^2 + \beta_3 \times AGE_t^3 + \beta_3 \times genotype + \beta_{4i} \times (AGE_{it} \times genotype) + \beta_5 \times gender + \beta_6 \times DE + \beta_7 \times PL + \beta_8 \times IT + \beta_9 \times BE + e_{it}$$

where Y_{it} is intake (kJ) for subject i at age t.$ß_{0i} = \beta_0 + u_i$ is fixed effects intercept plus individual deviation u_i for person i.$ß_{1i} = \beta_1 + v_i$ is fixed effects linear slope plus individual deviation v_i for person i.β_2 is fixed effects quadratic slope for all children.β_3 is fixed effects cubic slope for all children.$ß_{4i}$ is interaction effect of linear age slope and genotype plus individual deviation for this interaction effect.β_5 is fixed effects of gender for all children.β_6 to β_9 are fixed effects of study countries (GE = Germany, BE=Belgium, IT=Italy, PL=Poland; reference group are study children from Spain).e_{it} is individual error for child i at age t.

A fixed effect can be interpreted as the population average effect and a random effect as the individual deviation of that average effect. In this longitudinal model β_0 gives the average outcome at age 12 months and the u_i are the individual deviations from that outcome at that age (e.g. Individual different energy intake from sweet tasting foods). The estimate β_1 gives the average linear change in the outcome and v_i is the individual deviation from this average change for the outcome (e.g consuming more energy from sweet tasting foods at a given age). The other age related effects are specified as fixed effects and that means: Although the outcome changes individually with a different velocity (linear random slope) resulting in individually different outcome trajectories the quadratic or cubic change is not allowed to individually vary.

Missing data in modern methods like multilevel modelling are assumed to be missing at random (MAR). Multilevel models are maximum likelihood based methods and thus evaluates the entire joint distribution of the responses (the repeated outcomes over time) and thus yield valid estimated if missing data are MAR (Fitzmaurice, Laird, & Ware, 2004). In contrast, analysis based only on complete data are assumed to be invalid (Twisk, 2003).

Dietary records with energy, fat, carbohydrate or protein intake 3 SD above or under the mean intake for each respective country by time point were excluded. Accordingly, we excluded for the effect estimates of TAS2R38 genotype on energy intake from sweet tasting foods 29 values of 24 subjects with >3 SD above or below the mean of energy intake from sweet tasting foods.

Overall, there were 3156 observations from 691 children with TAS2R38 genotype and nutritional information; there were 2603 observations from 684 children with BMI measurement and 2548 observations from 675 children with additional misreporting status.

Data management and statistical analyses were carried out with the software packages SPSS Statistics 21 (SPSS Inc, Chicago, IL) and Stata version 13.1 (StataCorp LP, College Station, TX).

3. Results

TAS2R38 genotype and dietary data for at least one time point between 12 and 72 months was available in 691 children (444 or 64.3% genotype PP or PA, 247 or 35.7% genotype AA). There were slight but non significant differences in the distribution of genotype between countries (CHI square test, p = 0.586): Spain (67.7%) and Poland (60.1%). The number of children with a food protocol decreased from 574 (64.6% tasters) at 12 months of age to 382 (64.4% tasters) at 72 months.

3.1. Study population

Table 1 shows characteristics of our study population with at least one valid three-day dietary record between 12 and 72 months of age. Distribution of TAS2R38 genotype did not differ by any of the displayed characteristics except single mother status: There were significant more single mothers in AA genotype group than in the PP/PA group (p = 0.042).

3.2. Food categorization

In total 4850 different food items were consumed. 1653 (34.1%) were categorized as sweet tasting food items, 927 (19.1%) with low energy density and 726 (15.0%) with high energy density. In Table 2 the main food groups containing sweet food items are displayed. While fruit and fruit products, sweetened dairy products, desserts and beverages are mainly in the low energy density group, pastries, sweetened cereals and sweets mainly have a high energy density.

3.3. Intake of energy, macronutrients, sugar and sweet food

Table 3 shows the average daily intake of total energy, macronutrients, sugar and energy from sweet tasting foods (with high or low energy density) as well as BMI z-scores. Total sugar intake increased from 67 g/d (30% of total energy intake [E%]) to 85 g/d (24 E%) and total intakes of sweet tasting foods increased from an average of 1110 kJ/d (30 E%) at 12 months to 2054 kJ/d (33 E%) at 72 months. Total energy, macronutrient and sugar intake, energy intake from single food groups as well as BMI z-score was differed not according to TAS2R38 genotype, but energy intake from sweet tasting foods was significantly higher in children with PP/PA genotype (p = 0.009).

The composition of consumed sweet tasting foods differed between genotypes: While children with PP/PA genotype have a significant higher energy intake from sweet products with high energy density (on average 56 kJ/d; 95% CI 15–98 kJ/d; p = 0.007), energy intake from sweet products with low energy density varies not significantly by TAS2R38 genotype (p = 0.200).

Energy and sugar intake varied considerably between countries over the whole study period. While Spain and Poland had the highest energy intakes, sugar intake was much higher in Poland and much lower in Italy compared to all other countries. Energy intake was highest in Spain (4001 kJ/d) at 12 months and in Poland (6408 kJ/d) at 72 months of age. Sugar intake at 12 months of age ranged from 52 g/d in Italy to 76 g/d in Spain and from 76 g/d in Italy to 95 g/d in Poland at 72 months of age.

Table 1
Characteristics of study population with TAS2R38 genotype and nutritional data at any time point between 12 and 72 months by TAS2R38 genotype (n = 691).

		PP/PA	AA	Total
Country	Germany n (%)	76 (17.1)	47 (19.0)	123 (17.8)
	Belgium n (%)	55 (12.4)	31 (12.6)	86 (12.4)
	Italy n (%)	86 (19.4)	43 (17.4)	129 (18.7)
	Poland n (%)	95 (21.4)	63 (25.5)	158 (22.9)
	Spain n (%)	132 (29.7)	63 (25.5)	195 (28.2)
Sex	Boys n (%)	226 (50.9)	114 (46.2)	340 (49.2)
	Girls n (%)	218 (49.1)	133 (53.8)	351 (50.8)
Maternal educational level	Low n (%)	99 (22.3)	49 (19.8)	148 (21.4)
	Intermediate n (%)	220 (49.5)	121 (49.0)	341 (49.3)
	High n (%)	125 (28.2)	75 (30.4)	200 (28.9)
	Unknown (%)	0 (0.0)	2 (0.8)	2 (0.3)
Maternal age at child's birth (y)	<28 n (%)	127 (28.6)	74 (30.0)	201 (29.1)
	28 - < 33 n (%)	175 (39.4)	98 (39.7)	273 (39.5)
	33 - 44 n (%)	142 (32.0)	74 (30.0)	216 (31.3)
	Unknown n (%)	0 (0.0)	1 (0.4)	1 (0.1)
Type of formula	Low protein n (%)	155 (34.9)	96 (38.9)	251 (36.3)
	High protein n (%)	166 (37.4)	76 (30.8)	242 (35.0)
	Breastfed n (%)	123 (27.7)	75 (30.4)	198 (28.7)
Mother's BMI pre-pregnancy (kg/m^2)	<25 n (%)	303 (68.2)	173 (70.0)	476 (68.9)
	25 - <30 n (%)	102 (23.0)	49 (19.8)	151 (21.9)
	≥30 n (%)	30 (6.8)	19 (7.7)	49 (7.1)
	Unknown n (%)	9 (2.0)	6 (2.4)	15 (2.2)
Single mother	Yes n (%)	13 (2.9)	15 (6.1)	28 (4.1)
	No n (%)	431 (97.1)	230 (93.1)	661 (95.7)
	Unknown n (%)	0 (0.0)	2 (0.8)	2 (0.3)
Child's birth order	1st child n (%)	242 (54.5)	150 (60.7)	392 (56.7)
	2nd child n (%)	160 (36.0)	74 (30.0)	234 (33.9)
	≥3rd child n (%)	41 (9.2)	23 (9.3)	64 (9.3)
	Unknown n (%)	1 (0.2)	0 (0.0)	1 (0.1)
Total		**444 (64.3)**	**247 (35.7)**	**691 (100)**

Table 2
Number of food items from relevant food groups contained in sweet food items with either low (<200 kcal/100 g) or high (≥200 kcal/100 g) energy density n (% of total sweet food items).

	Food items n	Sweet food items n	Sweet food items with low energy density n (%)	Sweet food items with high energy density n (%)
Fruit and fruit products	448	433	382 (88.2)	51 (11.8)
Pastry	298	298	15 (5.0)	283 (95.0)
Sweetened cereals	223	223	67 (30.0)	156 (70.0)
Beverages	241	186	182 (97.8)	4 (2.2)
Sweets	185	185	32 (17.3)	153 (82.7)
Sweet dairy products	133	133	132 (99.2)	1 (0.8)
Desserts	99	96	81 (84.4)	15 (15.6)
Sweet main dishes	59	57	34 (59.6)	23 (40.4)
Instant cacao powder	19	19	1 (5.2)	18 (94.8)
Sugar, honey, syrup	16	16	0 (0.0)	16 (100)
Others	3129	7	6 (85.7)	1 (14.3)

3.4. Energy intake from sweet tasting food

Fixed effects of TAS2R38 genotype on energy intake from sweet tasting foods adjusted for country and gender from a mixed model are displayed in Table 4. Children with PP or PA genotype had a significantly higher energy intake from sweet tasting foods than children with AA genotype (difference on average 83 kJ/d; 95% CI 21 to 146; p = 0.009). Individual energy intake from sweet tasting food varied considerably (Fig. 1, Tables 3 and 4).

Additional adjustment for single mother status, mother's education attainment, marital status, maternal age at birth, birth order or maternal smoking during pregnancy did not attenuate any genotype effect estimates. In the subgroup analysis with further adjustment for BMI and energy misreporting the association of TAS2R38 genotype with energy intake from sweet tasting food was somewhat strengthened, especially after including the misreporting status (+99 kJ/d; 95% CI 35 to 163; p = 0.002) in comparison to results in the same subgroup without adjustment (+91 kJ/d; 95% CI 24 to 157; p = 0.008).

Girls had a lower energy intake from sweet tasting foods between 12 and 72 month (−128 kJ/d; 95% CI -188 to −68; p < 0.001). Intake of sweet tasting food also differed between the participating study countries. Polish children had the highest energy intake per day from sweet tasting foods (+387 kJ/d compared to baseline Spain; 95% CI 302 to 472; p < 0.001), while the intake was lowest in Italy (−511 kJ/d compared to baseline Spain; 95% CI -597 to −424; p < 0.001). The estimated mean energy intake from sweet food items at 12 months of age after adjustment (=constant term in mixed model) was 1333 kJ/d (95% CI 1212 to 1455; p < 0.001). The estimation of the influence of age on energy intake from sweet food items includes linear terms ('age'; per month +53 kJ/d), quadratic terms ('age^2'; per month −1 kJ/d) and cubic terms ('age^3'; per month 0 kJ/d).

4. Discussion

The PP/PA genotype of the bitter taste receptor gene TAS2R38 was associated with a higher intake of (energy dense) sweet tasting

Table 3
Means (SD) of intake of energy (kJ), macronutrients (g/d), sugar (g/d and [E%]) and energy intake from relevant food groups (kJ/d and [E%]) and BMI z-score by TAS2R38 genotype over time.

Nutrient intake[a]	TAS2R38 genotype	Age (months)						
		Number of participants						
		12 n = 574	24 n = 525	36 n = 415	48 n = 405	60 n = 355	72 n = 382	p-value[c]
Energy	PP/PA	3703 (750)	4616 (965)	5121 (992)	5537 (1016)	5840 (1064)	6155 (1042)	0.902
	AA	3671 (701)	4736 (1034)	5150 (989)	5497 (995)	5724 (1070)	6120 (960)	
	Total	3696 (732)	4658 (991)	5138 (990)	5526 (1007)	5808 (1065)	6146 (1010)	
Carbohydrates	PP/PA	116 (27)	137 (34)	152 (36)	164 (34)	174 (41)	184 (39)	0.309
	AA	116 (26)	139 (34)	153 (34)	162 (34)	174 (38)	182 (35)	
	Total	116 (27)	138 (34)	153 (35)	164 (34)	175 (40)	184 (38)	
Protein	PP/PA	33 (10)	44 (12)	47 (12)	50 (13)	52 (13)	54 (13)	0.086
	AA	32 (10)	46 (13)	48 (11)	50 (12)	50 (13)	55 (12)	
	Total	32 (10)	45 (13)	47 (12)	50 (12)	52 (13)	55 (13)	
Fat	PP/PA	32 (8)	42 (12)	48 (13)	52 (14)	55 (14)	58 (14)	0.704
	AA	32 (8)	43 (13)	47 (12)	52 (14)	53 (13)	57 (14)	
	Total	32 (8)	42 (12)	48 (13)	52 (14)	54 (13)	58 (14)	
Sugar[b]	PP/PA	67 (20) [30]	72 (24) [26]	77 (25) [25]	80 (24) [24]	83 (27) [24]	85 (26) [23]	0.878
	AA	68 (20) [31]	73 (23) [26]	79 (23) [26]	79 (23) [24]	83 (26) [24]	85 (26) [23]	
	Total	67 (20) [30]	72 (28) [26]	78 (24) [26]	80 (24) [25]	84 (27) [24]	85 (26) [24]	
Energy from sweet tasting foods (E%)	PP/PA	1134 (560) [31]	1680 (833) [36]	1788 (745) [35]	1907 (749) [34]	2016 (824) [35]	2047 (819) [33]	0.009
	AA	1069 (524) [29]	1605 (733) [34]	1783 (775) [35]	1816 (756) [33]	1995 (796) [35]	2061 (790) [34]	
	Total	1110 (548) [30]	1653 (799) [35]	1785 (755) [35]	1875 (752) [34]	2009 (813) [34]	2054 (808) [33]	
Energy from sweet products with low energy density (E%)	PP/PA	712 (433) [19]	974 (667) [21]	945 (548) [18]	942 (482) [18]	1011 (580) [17]	1009 (541) [16]	0.200
	AA	701 (406) [19]	966 (640) [20]	995 (528) [19]	906 (470) [16]	970 (543) [17]	981 (496) [16]	
	Total	708 (423) [19]	971 (657) [21]	962 (541) [19]	929 (477) [17]	997 (567) [17]	999 (525) [16]	
Energy from sweet products with high energy density (E%)	PP/PA	422 (362) [11]	707 (485) [15]	843 (514) [16]	966 (576) [17]	1005 (546) [17]	1039 (572) [17]	0.007
	AA	369 (320) [10]	639 (404) [13]	788 (516) [15]	910 (518) [17]	1024 (550) [18]	1081 (575) [18]	
	Total	403 (348) [11]	682 (458) [15]	824 (515) [16]	946 (556) [17]	1012 (547) [18]	1054 (572) [17]	
		12 n = 568	**24 n = 515**	**36 n = 400**	**48 n = 394**	**60 n = 349**	**72 n = 377**	
BMI for age z-score	PP/PA	0.38 (0.95)	0.28 (0.95)	0.37 (0.92)	0.37 (0.98)	0.41 (1.06)	0.42 (1.23)	0.170
	AA	0.30 (1.05)	0.24 (0.96)	0.24 (1.11)	0.32 (0.98)	0.24 (1.02)	0.22 (1.2)	
	Total	0.35 (0.99)	0.26 (0.96)	0.32 (0.99)	0.35 (0.98)	0.35 (1.05)	0.35 (1.21)	

All values are Means (SD).
[a] Dietary intakes measured via three-day dietary records.
[b] Mono- and disaccharides (g/d).
[c] Differences in values were assessed via mixed models with random intercept and slope including quadratic and cubic age terms all adjusted for country and gender; additionally adjusted for energy intake in models of macronutrients and sugar and for type of formula in model of BMI for age z-score.

foods in children from five European countries. Further associated factors of sweet food intake were gender and country. The intake of macronutrients and sugar as well as BMI z-score and single food groups was not significantly associated with the TAS2R38 genotype.

4.1. Association of TAS2R38 genotype with intake of (energy dense) sweet food

In former studies the taste receptor TAS2R38 was reported to influence the preference for sweet taste and intake of sweet tasting food in children's food (Mennella et al., 2005; Keller & Tepper, 2004; Joseph et al., 2016). We could confirm that the consumption of sweet foods is associated with TAS2R38 genotype. Several studies linked sugar intake or sweet liking with PROP phenotype, which is explained for 55–85% by TAS2R38 genotype: Keller and Tepper (2004) and Keller et al. (2014) reported a higher percentage of daily energy intake from sugars in tasters compared to non-taster children aged 4–5 years: At a palatable test-meal, taster children consumed more energy from the food group "sweets" than non-taster children, but this was not seen in another study of Keller et al. (2010). Two studies of Keller et al. (2010; 2014) found no effects of TAS2R38 genotype on food selection, while in a study of Joseph et al. (2016) children with two bitter sensitive alleles in the TAS2R38 gene reported a higher sugar intake than children with less sensitive alleles.

We further explored the difference of the effect of the TAS2R38 genotype on sugar intake - no effect - and energy intake from sweet products by comparing the effect on energy from sweet tasting products with high and low energy density. We observed that children with genotype PP or PA have a significantly higher energy intake from sweet products with a high energy density (≥200 kcal/100 g), but not from sweet products with low energy density. The higher energy intake from sweet tasting foods of PP/PA children can be explained by the added energy intake provided by fat, other carbohydrates and protein contained in higher amounts in energy dense sweet products. In that food group many products generally identified as unhealthy like sweets or pastry products are contained,

Table 4
Fixed and random effects on energy intake from sweet tasting foods from a mixed model with random intercept and slope including fixed cubic age terms (3127 observations).

Variable	Estimates (kJ) (95% CI)	p value
Fixed effects		
Genotype PP/PA[a]	83 (21–146)	0.009
Female[b]	−128 (−188 to −68)	<0.001
Country[c]		
Germany	−182 (−274 to −90)	<0.001
Belgium	−178 (−282 to −73)	0.001
Italy	−511 (−597 to −424)	<0.001
Poland	387 (302–472)	<0.001
Age	53 (46–60)	<0.001
Age^2	−1 (−2 to −1)	<0.001
Age^3	0 (0–0)	<0.001
Constant term	1333 (1212–1455)	<0.001
Random effects		
Variance (intercept)	103 (78–135)	
Variance (constant term)	87496 (65668–116579)	
Covariance (intercept, constant term)	−804 (−1501 to −107)	

[a] Baseline: genotype AA.
[b] Baseline: male.
[c] Baseline: Spain.

hence children with TAS2R38 genotype PP or PA tend to have a less balanced and healthy diet. The overall effect of the observed difference is low if purely the energy aspect is considered. However, our observation points also to differences in the dietary quality, which is known to influence lifelong health aside from high energy intake and obesity. As we did not find differences in total energy intake by genotype, the differences consist of a shift in-between food groups, which could influence dietary quality via intake of micronutrients, fiber or phytochemicals. In a report of the United States Department of Agriculture it is summarized that dietary patterns characterized by lower consumption of sugar-sweetened foods and beverages and sweets are associated with lower risk for cardiovascular diseases, type 2 diabetes and some forms of cancer. (United States Department of Agriculture [USDA], 2015).

4.2. Association of gender and country with sweet food intake

In accordance with our findings, Bjelland et al. (2013) also reported that boys have a significantly higher sweet intake namely of sweetened beverages at 18 months of age than girls. Similar in a US study in high school students: boys had a greater odds ratio for high intake of sugar sweetened beverages than girls (Park, Sherry, & Blanck, 2012).

Differences in consumption of sweet tasting foods across European countries were also reported in the HELENA study in adolescents that found the highest energy intake from sweet tasting beverages in Germany (1792 kJ/d) and the lowest in Italy (834 kJ/d). Spanish and Belgian adolescents had an intermediate intake of sweet beverages (Duffey et al., 2012). Previous analyses in the CHOP cohort showed that the consumption of energy providing liquids in infancy was highest in Poland (Schiess et al. 2010). Traditionally, Poland has a high consumption both of meat dishes as well as cakes and pastries, which appear to contribute to the high energy intake from sweet tasting foods in Polish children.

4.3. Association of TAS2R38 genotype with BMI

Negri et al. (2012) found an association of taster status (determined by threshold tests) with BMI in boys but not girls: among the obese boys or girls there was no supertaster, but 32% of the normal weights were supertasters. Inoue et al. (2013) reported that homozygote carriers of the non-taster variant are taller and heavier, but that BMI does not differ between TAS2R38 genotypes. We could not find an effect of TAS2R38 genotype on BMI z-score just as in a German adult population where no associations between TAS2R38 genotype and BMI were found either (Sausenthaler, Rzehak, Wichmann, & Heinrich, 2009). Baranowski et al. (2010) examined the BMI of children in the age of 9–10 and 17–18 years and found that PROP supertasters had the largest BMI percentile and z-score in children with the highest socioeconomic status, while in children with lower socioeconomic status the influence of PROP taster status is covered by other factors. Similar results were found by Burd,

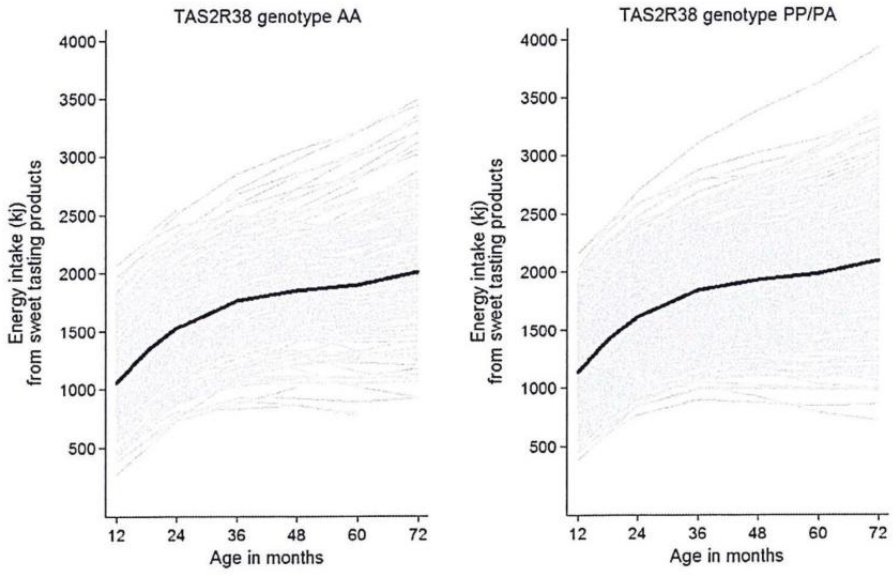

Fig. 1. Subject-specific and population averaged development of energy intake from sweet tasting products adjusted for country and gender by TAS2R38 genotype from 12 up to the age of 72 months (results of a mixed model with random intercept and slope with quadratic and cubic age terms).

Senerat, Chambers, & Keller (2013), who examined the interaction of children's BMI, the food environment and PROP taster status: Non-taster children from unhealthy food environments had higher BMI z-scores than all other groups. Obviously, taster status is only one factor influencing children's BMI and interacts with socioeconomic factors.

4.4. Association of TAS2R38 genotype with other variables of food intake

While a Japanese study on female college students aged 19–21 years found a higher intake of total energy and carbohydrates in carriers of homozygote non-taster variant (Inoue et al., 2013), O'Brien, Feeney, Scannell, Markey, and Gibney (2013) found no association either between TAS2R38 genotype nor PROP sensitivity and energy intake, nutrient intake or food group selection in Irish children aged 7–13 years. Similarly, we did not find a significant effect of TAS2R38 genotype on total energy intake, nor on the total intake of sugar and other nutrients or single food groups. In elderly women in Brazil, no associations of the rs713598 polymorphism of the TAS2R38 gene were found with any food group, except for bitter tasting vegetables (Colares-Bento et al., 2012). The lack of associations between TAS2R38 genotype and the intake of single food groups may also be due to our tool for nutritional assessment (three-day dietary records) which is appropriate for evaluation of macronutrients and large food groups but less appropriate for evaluation of rarely consumed food groups (Magarey et al., 2011).

4.5. Strengths and limitations

An obvious strength of our investigation is the study of children from five countries across Europe; thus our results are indicative of effects across populations. Furthermore, we assessed dietary intake via detailed three-day dietary records which is considered to be the most precise method of dietary intake assessment in young children. We used a standardized approach with quality assurance to data collection and evaluation that is expected to reduce bias and errors.

Although there are three SNPs in the TAS2R38 gene, which are associated with bitter sensitivity, it is considered sufficient to genotype the first one because they are in strong linkage disequilibrium (Kim et al., 2003).

Though studies relating TAS2R38 genotypes with PROP/PTC phenotypes find an intermediate sensitivity to the bitter compounds in heterozygous individuals (Behrens, Gunn, Ramos, Meyerhof, & Wooding, 2013) with a wide range of sensitivity to PROP and similar bitter compounds (Lipchock, Mennella, Spielman, & Reed, 2013), studies that try to find relations between TAS2R38 genotypes and food intakes or food preferences mostly find the same relations for homozygous and heterozygous individuals (Colares-Bento et al., 2012; Mennella et al., 2005). Furthermore, heterozygous children seem to be more bitter sensitive than heterozygous adults (Mennella et al., 2005; Negri et al. 2012). For this reason, we decided to pool homozygotes (genotype PP) and heterozygotes (genotype PA) together in one group. The distribution of TAS2R38 genotype differed slightly in all five study countries, but is comparable to results from other studies in Caucasian populations (Kim et al., 2003).

We did not test the bitter sensitivity via PROP solution threshold tests, as this was done in several similar studies. The TAS2R38 genotypes explains up to 85% of the variation in PROP sensitivity depending from the examined population (Kim et al., 2003). So it might be a limitation that there could be children in the PP/PA genotype group, which would be classified as non-tasters by phenotype and vice versa. In the study of Keller et al. (2010) in 4–6 years old children in 75.8% of the children genotype predicted PROP phenotype, but in 24.4% not. Other factors influencing PROP sensitivity are not well defined: e.g. Timpson et al. (2007) found no association with social factors or depression.

The German food database BLS was selected as basis for the calculation of nutrient intakes from the three-day dietary records in all participating countries as it is the largest food database available in the participating countries. For typical local products or any other products not contained in the BLS the dieticians transferring the dietary records had the possibility to add new food items to the database. Thus, the database we used for this study contained pure food items (as vegetables, fruits or meat) and also valid nutrient data for local products and recipes.

The number of participants decreased from 574 at the age of 12 months–382 at the age of 72 months, partly due to the time consuming and demanding task of completing the three-day dietary records, in addition to the request to complete multiple other questionnaires as part of the large multicentre CHOP trial. Children who stayed in the study came generally from families with a higher socio-economic status. Thus, some bias might be possible despite the fact that we did not find any effect modification by age.

We did not investigate food groups with the affected bitter compounds but sweet tasting foods. We hypothesized that children that are more bitter sensitive consume more sugar and sweet tasting food to compensate for the bitter taste perception. As expected, we can only find a modest effect of TAS2R38 bitter receptor variants on food selection and intake: First of all only part of the variation in the sensitivity to the bitter glucosinolates is explained by the analysed genetic variants (Kim & Drayna, 2005; Kim et al., 2003), secondly, the TAS2R38 bitter receptor is only one of 25 bitter receptors (TAS2Rs) (Hayes, Feeney, & Allen, 2013), and thirdly, individual differences in perception of taste and odours have also been associated with polymorphisms in other bitter receptor genes and in genes involved in umami and sweet tastes or odors perception (Nothlings, Murphy, Wilkens, Henderson, & Kolonel, 2007). Therefore, TAS2R38 is expected to explain only a small part of total genetic taste variance.

The detected effects of genetic variants of the TAS2R38 gene on the intake of sweet foods and particularly on sweet products, generally considered as unhealthy may affect long-term health outcomes such as obesity, adiposity and other nutrition related diseases, which deserve further studies.

Conflict of interest statement

The authors declare no conflict of interest.

Contribution of authors

IP collected, analysed and interpreted data, searched for relevant literature and wrote the paper.

RC, AS, EV, AX collected genetic and dietary data in their respective countries.

VG analysed and interpreted data.

ER performed the genetic analysis.

PR helped analysing data.

BK designed the study.

All authors had final approval of the submitted and published version of the paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.appet.2016.07.034.

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4. SUMMARY:

Objectives

Obesity and its health consequences are currently the greatest public health challenge in western countries. Several factors, thereunder high energy intake via hidden fat and added sugar, promote the development of obesity. Other health components, affected by high sugar consumption are intake of other food groups, and by this, possibly nutrient intake (19), dental health (32) and, independent of overweight, plasma triglyceride concentration (30).

The dissertation aimed to determine total sugar intake, main sugar sources as well as associated factors in children aged 1 to 8 years in five European countries. Furthermore, the association of genetic variants of the bitter receptor gene TAS2R38 with the intake of (energy dense) sweet tasting foods, total energy, macronutrients, sugar and energy from single food groups as well as BMI was investigated.

Methods

The dissertation was conducted in the framework of the CHildhood Obesity Project CHOP, a double-blind, randomized, controlled trial in five European countries (Germany, Belgium, Italy, Poland and Spain).

Anthropometric measures were obtained at visits to study centers and dietary intake via 3-day dietary records regularly until the age of 96 months.

Recorded food was classified by its composition into subgroups, with further classification generated into sweet/not sweet. Mono- and disaccharides were summarized as sugars. Further factors possibly associated with sugar or sweet food intake (gender, birth order, mother's education, age, marital status and BMI pre-pregnancy) were gathered at study entry.

Individuals were grouped by variants of the first SNP (analysed by MALDI TOF (matrix assisted laser desorption/ionisation time of flight mass spectrometry)) of the bitter receptor gene TAS2R38 (rs713598), with PA and PP representing the taster group and AA representing the non taster group.

Results

Total sugar intake increased from 65 g/d at 12 months of age to 83 g/d at 96 months of age, while energy contribution of sugar decreased. Four food groups could be determined as main sugar sources (representing about 80 % of total sugar intake): milk and dairy products, fruits and fruit products, confectionary and sugar sweetened beverages (SSB). Country of residence was associated with total sugar intake, intake of energy dense food and dietary sugar sources. Boys had a significantly higher total sugar consumption and higher intake of sweet tasting foods than girls. SSB consumption was significantly higher in children from young mothers while sugar intake from fruit products was lower in children from mothers with lower educational status and in children with higher birth order(40).

Children with PP and PA genotypes consumed more energy from (energy dense) sweet tasting foods than children with AA genotype. TAS2R38 genotype was not associated with the intake of energy, macronutrients, single food groups and BMI z-score(41).

Conclusions

The results of the two studies on intake of sugar and sweet tasting food showed that sugar and sweet food consumption varies with several factors: mainly gender and study country, intake of sweet food is also associated with genotype of the bitter receptor gene TAS2R38 while sugar sources vary with maternal age and education as well as with birth order.

Complex and diverse reasons lead to inappropriate eating behaviour and its consequences. Country's eating culture has great influence on personal food selection, while the genetic influence is not that great. To improve the nutrition of children from young and low educated mothers it is necessary to offer low-threshold services to reach this population group.

5. ZUSAMMENFASSUNG:

Ziele

Übergewicht und seine Folgekrankheiten stellt eine der größten gesundheitspolitischen Herausforderungen der Industrieländer dar. Viele Faktoren, darunter eine hohe Zufuhr versteckter Fette und zugesetzten Zuckers tragen zur Entwicklung von Übergewicht bei. Neben der Förderung von Übergewicht und dessen Folgeerkrankungen beeinflusst eine hohe Zuckerzufuhr auch die Aufnahme anderer Lebensmittelgruppen und somit möglicherweise die Nährstoffversorgung (19), die Zahngesundheit (32) und, unabhängig vom Zusammenhang mit Übergewicht die Plasmatriglyceridkonzentration (30).

Ziel der vorliegenden Dissertation war es, die Gesamtzucker-Aufnahme, die Hauptzuckerquellen sowie damit assoziierte Faktoren bei Kindern im Alter von 1 bis 8 Jahren in fünf europäischen Ländern zu bestimmen. Desweiteren wurde untersucht, inwieweit genetische Varianten des Bitterrezeptorgens TAS2R38 mit der Aufnahme von süß schmeckenden Lebensmitteln (mit hoher Energiedichte), von Hauptnährstoffen, Gesamtenergie, Energie aus einzelnen Lebensmittelgruppen und Zucker sowie der BMI.assoziiert sind

Methoden

Die Doktorarbeit wurde im Rahmen des CHildhood Obesity Projects CHOP angefertigt, eine doppelblinde, randomisierte, kontrollierte Studie in fünf europäischen Ländern (Deutschland, Belgien, Italien, Polen und Spanien).

Anthropometrische Daten wurden bei Besuchen im Studienzentrum, Verzehrsdaten via 3-Tages-Ernährungsprotokollen regelmäßig bis zum Alter von 96 Monaten erhoben. Die verzehrten Lebensmittel wurden gemäß ihrer Zusammensetzung in Gruppen eingeteilt. Eine weitere Einteilung erfolgte nach süß/nicht süß. Als Zucker wurden Mono- und Disaccharide erfasst. Weitere, möglicherweise mit der Zuckeraufnahme assoziierte Faktoren (Geschlecht und Stand des Kindes in der Geschwisterfolge; Ausbildungsstand, Alter, vorgeburtlicher BMI und Familienstand der Mutter) wurden bei Studienbeginn erhoben.

Die Probanden wurden anhand des ersten SNP rs713598 des Bitterrezeptorgens TAS2R38 gruppiert (genotypisiert mittels Massenspektrometrie (MALDI TOF: matrix

assisted laser desorption/ionisation time of flight mass spectrometry)), dabei stellten die Genotypen PP und PA die Gruppe der taster und die Allelle AA die der non taster dar.

Ergebnisse

Die Gesamtzuckeraufnahme stieg von 65 g/Tag mit 12 Monaten auf 83 g/Tag mit 96 Monaten, während der Energieanteil des Zuckers sank. Hauptzuckerquellen (rund 80% der Gesamtzuckeraufnahme) waren: Milch und Milchprodukte, Obst und Obstprodukte, Süßigkeiten und gesüßte Getränke. Die Gesamtzuckeraufnahme, die Zuckerquellen sowie die Energieaufnahme aus süß schmeckenden Lebensmitteln unterschieden sich zwischen den Studienländern signifikant.: Jungen nahmen signifikant mehr Gesamtzucker als auch mehr Energie aus süß schmeckenden Lebensmitteln auf als Mädchen(40).

Der Verzehr von gesüßten Getränken war bei Kindern jüngerer Mütter signifikant höher, während die Zuckeraufnahme aus Obst und Obstprodukten bei Kindern von weniger ausgebildeten Müttern und bei Kindern mit mehr Geschwistern signifikant niedriger war (40).

Kinder mit dem Genotyp PP oder PA verzehrten mehr Energie aus süß schmeckenden Lebensmitteln (mit hoher Energiedichte) als Kinder mit dem Genotyp AA. Der TAS2R38 Genotyp war nicht assoziiert mit der Aufnahme an Gesamtenergie, Makronährstoffen, einzelnen Lebensmittelgruppen und dem BMI z-score (41).

Schlussfolgerungen

Die Ergebnisse der beiden Studien zur Aufnahme von Zucker und süß schmeckenden Lebensmitteln zeigten, dass verschiedene Faktoren mit dem Verzehr von Zucker und süß schmeckenden Lebensmitteln assoziiert sind: Das Geschlecht und die Studienländer können als Hauptfaktoren ausgemacht werden. Die Aufnahme von süßen Lebensmitteln ist auch mit dem Genotyp des Bitterrezeptorgens TAS2R38 assoziiert, während die Zuckerquellen auch mit dem mütterlichen Alter und Ausbildungsstand sowie der Geschwisterfolge variierten.

Die Ursachen für ungünstiges Essverhalten und seine Folgen sind sehr vielschichtig. Das Essverhalten ist stark kulturell beeinflusst; der Einfluss der Gene ist jedoch eher

als gering einzustufen. Um die Nahrungsmittelauswahl bei Kindern von jungen und wenig ausgebildeten Müttern zu verbessern, sind besondere Anstrengungen und die Schaffung niederschwelliger Angebote notwendig, um diese Bevölkerungsgruppen zu erreichen.

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6. EIDESSTATTLICHE VERSICHERUNG

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema 'Sugar intake, sugar sources and associated factors in European children' 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.
Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

Eichenau, 11.02.1019 Ingrid Pawellek

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8. PUBLICATIONS AND PRESENTATIONS

Publications

Pawellek I*, Grote V*, Theurich M¸ Closa-Monasterolo R, Stolarczyk A, Verduci E, Xhonneux A, Koletzko B; European Childhood Obesity Trial Study Group. Factors associated with sugar intake and sugar sources in European children from 1 to 8 years of age. Eur J Clin Nutr. 2017 Jan; 71(1):25-32.
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Presentations

Pawellek I, Escribano J, Grote V, Reischl E, Rzehak P, Stolarczyk A, Verduci E, Xhonneux A, Koletzko B, European Childhood Obesity Trial Study Group. Influence of genetic variants of the TAS2R38 bitter receptor gene on the intake of sweet tasting foods, nutrient intake and body weight of European children. Early nutrition conference Munich. 20 - 22 March 2014 (poster of distinction)