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ORIGINAL CONTRIBUTION

The DONALD Study

History, current status and future perspectives

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Summary Nutrition during childhood and adolescence is an important determinant of development and health, both for the child and the later adult. In industrialised countries as well as in many countries of economic transition, emphasis has moved from combating nutrient deficiencies to research on the effects of overnutrition and food selection. Prevention of chronic diseases including obesity have become a major focus in research. However, the complex relation between infant growth and its related endocrine and metabolic changes on the one hand and the influence of nutrition and nutritional status on the other hand still need to be understood in detail. Studies aiming to elucidate this have to follow children and adolescents during their growth period. The following pages display the features of the German DONALD Study (*DO*rtmund *N*utritional and *A*nthropometric *L*ongitudinally *D*esigned Study) which was specifically designed to address these complex research questions. Finally, comparisons to other studies are made and the specific strength and weaknesses of this study are discussed. As the DONALD study offers unique research opportunities and due to its long follow-up an abundance of data, collaborative research is encouraged.

Key words children – nutrition – cohort study – anthropometry – endocrinology

Introduction

The importance of an adequate nutrition for a healthy development of children, both physically and mentally, has long been recognised. In recent years it has additionally become evident that nutrition during childhood and adolescence is related to adult disease occurrence [1–3]. These findings have underlined the importance of nutritional quality early in life and have changed the type of research in that field. Nowadays, in wealthy societies more emphasis is placed on issues related to chronic disease development rather than combating nutrient deficiencies. This is highlighted by the statement that today the most prominent and frequent nutrition-related disease among children is obesity [4]. However, with respect to children, little is known so far about the physiological changes of the related endocrine and metabolic parameters on the one hand, and on the pathophysiological alterations associated with the development of obesity on the other hand. Correspondingly, data on the influence of dietary behaviour and nutrition on the metabolism and endocrinum of children are scarce.

One difficulty in studying these factors in children are the ongoing changes of the body and its functions associated with growth and development. To take this into account and to obtain insight into the characteristics of these changes, studies are required with repeated assessment of the variables of interest during childhood and adolescence. In the following, a study of that kind, the *DO*rtmund *N*utritional and *A*nthropometric *L*ongi-

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tudinally *D*esigned Study (DONALD) is described in detail. The aim of the present paper is to describe the design features and assessment procedures of the DONALD Study and outline its potential for addressing current research topics in nutritional epidemiology.

Aims of the study

When the study was initially planned, five major research needs were identified which formed the rationale to start a longitudinal study in children focussing on diet, nutrition and development:

- Description of intra-individual and inter-individual trends in dietary intake and nutritional behaviour,
- Analyses of interaction between nutrition and growth,
- Determination of nutritional needs in children and adolescents,
- Metabolic reference data from healthy children,
- Dietary intake data to support the evaluation of the environmental burden.

During the progress of the study its focus has been widened to the complex interrelations between nutritional behaviour, food consumption, growth, development, nutritional and endocrine status, individuality, metabolism and health in children from infancy to adolescence and early adulthood.

Design and methods

The DONALD Study is a longitudinal (open cohort) study collecting detailed data on diet, growth, development and metabolism between infancy and adulthood. Per annum, about 40 infants are recruited and first examined at the age of three months and are then followed until the age of 20 years (girls) and 23 years (boys). During the first year of life, three further visits at the Research Institute of Child Nutrition in Dortmund, Germany, are planned, two in the second year and one thereafter. During puberty, two annual assessments are attempted. The regular assessments include records of dietary intake and behaviour, anthropometry, urine sampling, interviews on life-style and health-related issues and a medical examination. According to the age of the child different additional measurements are performed. At certain points in time data from the parents on socio-demographic issues, life-style, health status and anthropometry are obtained as well (Fig. 1).

Recruitment for the study started in 1985 and is planned to continue. The starting study sample included children and adolescents between age 2 and 18 years with annual anthropometric measurements recruited from earlier cross-sectional studies in schools and kindergartens.

The study which is exclusively observational and non-invasive, has been approved by the Scientific Committee of the Research Institute of Child Nutrition. All examinations and assessments are performed with parental consent and later on with the children's consent.

Study population

Study participants are recruited in the city of Dortmund and surrounding communities via personal contacts, maternity wards or paediatric practices. Eligible are healthy (no prevalent diseases affecting growth and/or diet) German babies (age 3–6 month) whose mothers and/or fathers are willing to participate in a long-term study and of whom at least one parent has sufficient knowledge of the German language. These criteria were and are followed throughout the study, with the only ex-

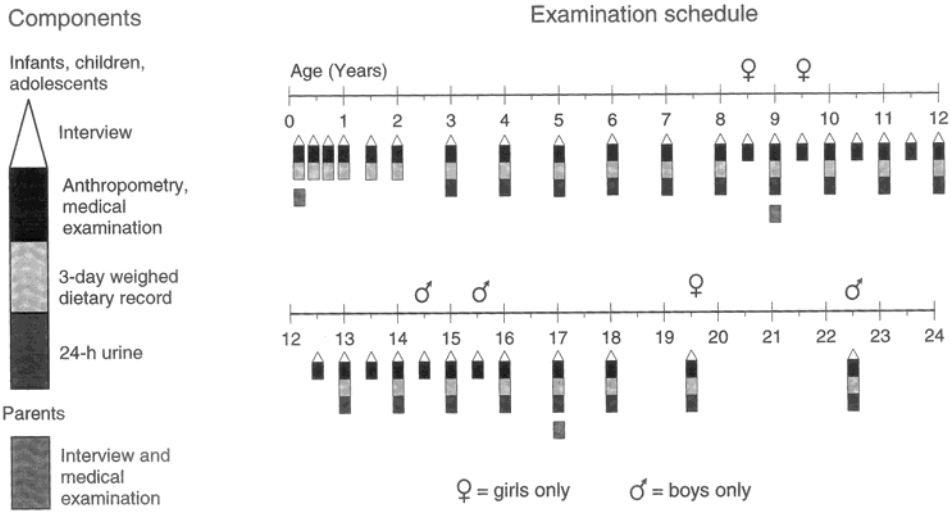

Fig. 1 Examination Schedule of the DONALD Study

ception at the beginning of the study, when older children from other projects were "transferred" to the DONALD Study if all the other criteria applied. Therefore, for some children who entered the study before 1989, data from infancy are missing.

Similar to other cohort studies the DONALD Study uses a convenient sampling scheme. This approach results in a selected, non-representative study sample (Table 1). Compared to the general population the parents of the DONALD Study children are characterised by a higher educational attainment and higher socio-economic status. Comparing body mass index (BMI) to the German reference data, only slight deviations from the percentile curves can be observed (Figs. 2 and 3). From this point of view the selection procedure did not result in a major deviation from the reference population.

The drop-out rates are small and vary by the age of the child, with the highest drop-out rate during puberty.

Table 1 Comparison of educational attainment between the parents of the DONALD Study and the general population in Germany

Highest school education achieved	Men[a]	Fathers[b]	Women[a]	Mothers[b]
Primary School (Volks-/Haupt-Schule)	40.6	23.1	37.2	17.8
Secondary School (Realschule oder Gleichwertiges)	19.9	21.3	26.5	32.9
Technical School/High School[c] (Fachhochschule/Abitur)	29.4	55.6	36.4	49.3

[a] Data from a national survey, 2001 [5];
[b] of the children participating in the DONALD Study
[c] including Technical School (former GDR)

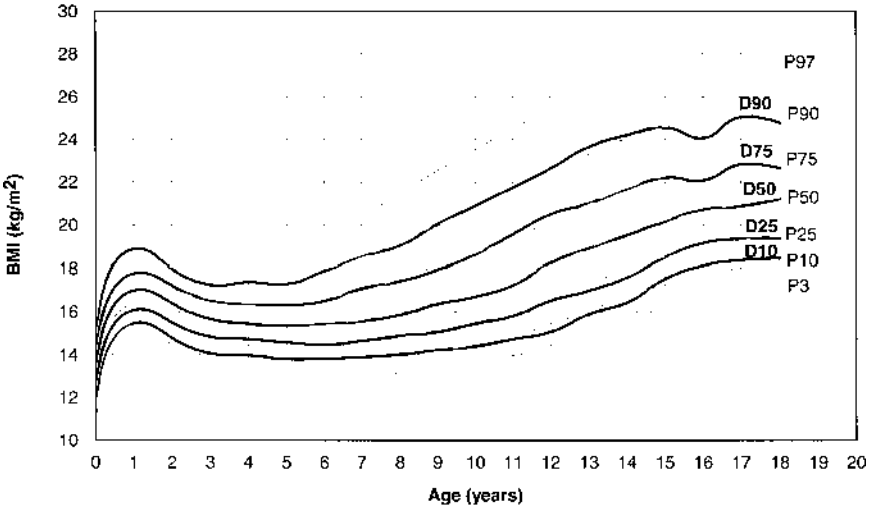

Fig. 2 Percentiles 10, 25, 50, 75 and 90 in girls aged 0–18 years of the DONALD study population (D10-D90) in comparison to the German standard percentiles (6)

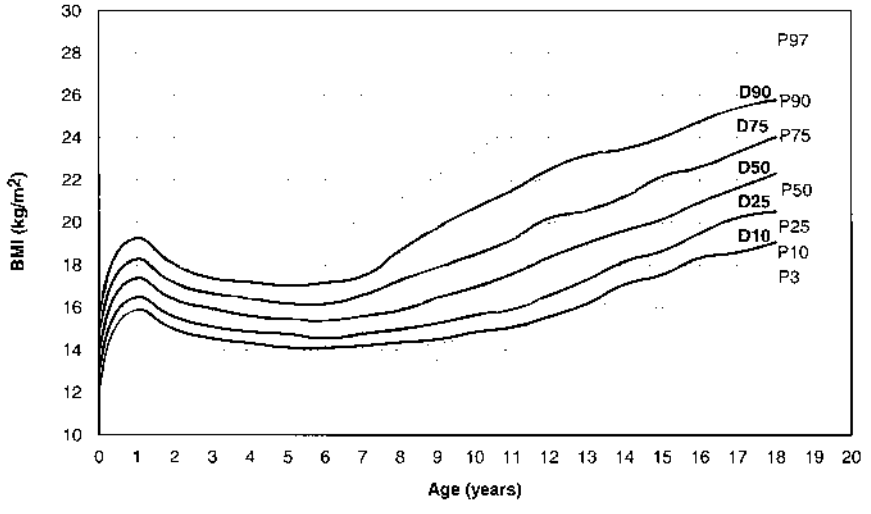

Fig. 3 Percentiles 10, 25, 50, 75 and 90 in boys aged 0–18 years of the DONALD study population (D10-D90) in comparison to the German standard percentiles (6)

Overall, 1137 children have been recruited so far and the currently active cohort consists of 633 children. At least five complete assessments were obtained from 852 children (76 %), at least 10 complete assessments from 510 children (45 %). Table 2 presents information on numbers of assessments per age and sex group.

Data assessment

The study assessment procedures are based on several modules. These modules are the following: recording of dietary intake and behaviour, anthropometry, urine sampling, parental interview and medical examination. Depending on the age of the child different assessments and measurements are performed for each of these modules. Fig. 1 gives an overview of the distribution of the assessments during the time-course of follow-up.

Dietary intake and nutritional behaviour

Three-day weighed dietary records are used to collect information on food and nutrient intake from all children beginning at study entry. The weighing includes beverages as well as away from home eating and snacks. Parents, or the child respectively, are instructed by a dietician on how to use the digital scale (Soehnle Digita 8000 (in former times, now WEDO Digi 2000)) and on recording the relevant information to the nearest 1 g during the three consecutive days of measurement. Semiquantitative recording is allowed when weighing is not possible, e. g. away from home eating. Requested information includes type and brand name of a food item, time and location of eating, and recipes. For commercial food items the packages, or the food labels respectively, are to be collected and the product information thereof is added to the dietary record data. In case of breast feeding, the mothers are supplied with a baby scale (Soehnle multina 8300) and are instructed to weigh the child before and after feeding to the nearest 10 g.

After the three consecutive days of measurements, a dietician visiting the family at home picks up all materials and assesses additional information on the measurement period related to unusual events, changes in dietary behaviour due to the measurement, etc. Also, any missing information is inquired, estimated and completed.

Dietary records are coded and linked to the LEBTAB nutrient database to calculate the intake of 30 nutrients and energy. This nutrient database was developed during the course of the study. LEBTAB contains detailed data on nutrient content of commercial foods, e. g. different formula, weaning foods and fortified food products, and is continuously being updated. Data entries for staple food items are based on the German standard food tables [7]. Missing food items or nutrients are substituted by data from other national food tables, predominantly from the UK [8], the US [9] and The Netherlands [10]. New commercial food products and convenience food items are added by the dietician via a recipe which is simulated from the ingredients listed on the label and controlled by those nutrient contents declared on the label or requested information from the manufacturer. Due to the longitudinal character of the DONALD Study, changes in the supply of commercial food products (e. g. due to fortification) are monitored continuously. To that end, a new or modified product (e. g. due to a modified recipe or new ingredients) receives an entry with a new food code in the nutrient database.

The database now contains 5220 general food items, 1490 infant food products and 400 dietary supplements. The nutrient data refer to the raw food item. To take the loss of vitamins during food preparation into account, corresponding subtractions are made based on average nutrient losses during preparation as published by the German Nutrition Society [11].

Table 2 Number of assessments of diet and anthropometry, and number of assessments with urine collections[a] performed between 1985 and 2003, by age and sex groups. DONALD Study, N = 1137

Age (years)	Girls			Boys			Total		
	Anthropometry	Diet	Urine collection	Anthropometry	Diet	Urine collection	Anthropometry	Diet	Urine collection
≤ 1	1075	934	–	953	850	–	2028	1784	–
1.5–4	1327	1176	311	1244	1119	265	2571	2295	576
> 4–8.5[b]	1642	1164	1007	1258	1134	1000	2900	2298	2007
9–14.5[b]	1380	748	697	1572	772	741	2862	1520	1438
≥ 15[c] y	374	316	309	449	307	311	823	623	620
Sum	**5798**	**4338**	**2324**	**5476**	**4182**	**2317**	**11184**	**8520**	**4641**

[a] Urine collections begin at age 3–4 ; during puberty, only at the full year assessments urine samples are requested
[b] A second annual anthropometric measurement in girls between age 8.5 and 13.5 and in boys between age 10.5 and 15.5
[c] Final anthropometric measurements are planned for girls at age 19–20 and for boys at age 22–23

In addition to the dietary records, qualitative and, if possible, quantitative dietary information is obtained during the interviews at the study centre on iodine salt use, vitamin supplementation, and other dietetic food items.

Anthropometry

A set of different anthropometric measurements are performed according to the age of the child. Body weight and length/height are assessed twice in the parents and at each visit in each child. Beginning at age 1.5 years, more detailed measurements are obtained from the children. Table 3 lists the diverse anthropometric measurements as they are performed at different ages.

For the measurements, children are wearing underwear only and no shoes. Body mass is assessed to the nearest 100 g with an electronic scale, height and circumference to the nearest 0.1 cm, and skinfold thickness to the nearest 0.1 mm. All measurement instruments are calibrated regularly. The measurements are performed by trained nurses who have been working with the study for many years to ensure continuity of procedures and assessments.

Urine sampling and urine analysis

Starting at age 3–4 years when the child has learned to use the toilet, 24-hour urine collections are made. The urine sampling day usually covers the third day of the 3-day weighed dietary record. Parents and children receive personal and written instructions on how to collect complete 24-h urine samples. Children are asked to void their bladder upon getting up in the morning on the third day of recording. This micturition is completely discarded. The time of this micturition is registered and defines the start of the 24-h collection which ends with the first micturition on the following morning. All micturitions are stored immediately in preservative-free, Extran-cleaned (Extran, MA03; Merck Darmstadt, Germany) 1 litre plastic containers at ≤ –12 °C before transfer to the research institute. There, the containers are stored at ≤ –20 °C until analysed. The dietician picking up the dietary record and urine samples inquires parents and children about the completeness of the urine samples and adds this information to the protocol sheet.

After thawing and stirring, all urine samples undergo routine check using a commercial test strip. Total urine volume, pH, osmolality and creatinine are determined in all samples. Further parameters regularly quantified in most samples – partly depending on specific research projects – are shown in Table 4. From the total 24-h urine collections five samples of 20 ml each are stored at ≤ –22 °C in the urine bank for further analysis.

Table 3 Anthropometric measurements in the DONALD Study by age of the child

Anthropometric measurement	Age of the child (years)																							
	¼	½	¾	1	1½	2	3	4	5	6	7	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	15	15½	16	16½	17	18	19[d]	22[e]
Parents																																	
Body height (cm)	•																													•			
Body mass (kg)	•																													•			
Child																																	
Body height[a] (cm)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Body mass (kg)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Abdominal circumference (cm)	•	•	•	•	•																												
Breast + upper arm circumference, elbow and knee width (cm)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Skeletal measures (cm)[b]					•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Head circumference (cm)	•	•	•	•	•	•	•	•	•			•																			•	•	•
Skinfolds[c] (mm)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

[a] body height at age ≥ 2 years, body length at < 2 years; [b] chest sagittal and transversal, shoulder width, pelvis width, sitting height, height of acromion, radialis, styleon, mid-finger, trochanter, tibia; [c] biceps, triceps, scapular, iliac; [d] girls only; [e] boys only

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Table 4 Parameters of 24-h urine analysis and the respective determination method in the DONALD Study

Parameter	Unit	Determination method
Volume	mL	/
pH		pH electrode (KCl + AgCl)
Osmolality	mosm/kg	Osmometer (freezing point)
Titratable acid	mEq/L	Three-phasic acid/base titration
Ammonium	mmol/L	Three-phasic acid/base titration
Bicarbonate	mmol/L	Three-phasic acid/base titration
Total nitrogen	mmol/Ll	Kjeldahl
Creatinine	mmol/L	Photometric (kinetic Jaffé procedure)
Urea	mmol/L	Photometric (enzymatic conductivity rate method)
Uric acid	mmol/L	Photometric (enzymatically generated quinonimine)
Sodium	mmol/L	Flame atomic absorption spectrometry
Potassium	mmol/L	Flame atomic absorption spectrometry
Calcium	mmol/L	Flame atomic absorption spectrometry
Magnesium	mmol/L	Flame atomic absorption spectrometry
Zinc	µmol/L	Flame atomic absorption spectrometry
Chloride	mmol/L	Ion chromatograph
Phosphate	mmol/L	Ion chromatograph
Sulfate	mmol/L	Ion chromatograph
Organic acids	mmol/L	Titration
Oxalate	µmol/L	Ion chromatograph
Citrate	mmol/L	Photometric (enzymatic: citrate lyase)
Iodine	µg/L	Photometric (modified Sandell-Kolthoff)

Interview

At each visit at the study centre a set of core questions and additional age-dependent questions are asked in a personal interview conducted by a paediatrician. The core questions relate to acute illnesses since the last visit; others relate to sleeping habits, dejection, food preferences, child care, sporting activities, preventive measures (vitamin D and fluoride), and use of medical preventive services.

Medical examination

A medical examination is a core part at each visit at the study centre. The main aim of this examination is to receive information about the current and past health status of the child – information needed for the interpretation of the anthropometric, dietary and urinary data. Corresponding to the interview part, some aspects are assessed each time (e. g. infect signs), whereas others (e. g. pubertal development) are only assessed at certain ages of the child.

Additional, project specific assessments

Occasionally, specific additional examinations or assessments are added to the core protocol. For example, isometric grip force and the measurement of peripheral quantitative computer tomography of the forearm were performed in a subsample of the study [12–14]. Another project was the participation in the Euro Growth Study [15]. The determination of circadian rhythms of urine osmolality and renal excretion rates of solutes was the aim of another spin-off project [16].

Results

Overall, during the years, more than 25500 days of weighed dietary records were obtained. They were, for example, used to describe patterns and trends in food and nutrient intake [17–19]. 11184 sets of anthropometric measurements were performed and were, for example, related to adrenocortical activity [20] or used to compare two methods to determine muscularity in prepubertal children [21]. 4641 24-h urine samples were collected and stored. The bank of urine samples now contains aliquots from most of these measurements. Analysis of urine samples were used, for example, to define reference values for urinary creatinine excretion during growth [22], to determine normal values of urinary calcium excretion related to age and sex in healthy children and adolescents [23], and to describe the relation of nutritional status to hormonal changes associated with adrenarche [24].

These are only a few examples of recent results ob-

tained from the DONALD Study. Due to its long duration and the multiple areas of research that are covered by the study a considerable number of results have been obtained so far. Numerous publications in national and international journals document these research activities. For an extended list of publications, please refer to our website at http://www.fke-do.de/publik/publik.html. The following examples of published results were selected to illustrate the range of research topics to be covered by DONALD Study and some of its unique features.

Example 1:

The most important nutrient: defining the adequate intake of water [25]

Data from the DONALD Study on total water intake during 24 hours and 24-h urine volume and osmolality were used to characterise individual hydration status among 479 children (age 4 to 11 years). Adequate intake values for total water intake were estimated based on a maximum urine osmolality of 830 mom/kg as the upper level of euhydration among children consuming an affluent western-style diet. As water is quantitatively the most important nutrient and recommended dietary allowances or adequate intake levels have not been defined so far, these data may prove to be a helpful tool to investigate the health effects of different states of euhydration and to derive recommendations for daily water intake in population groups.

Example 2:

Changes in time-trends of nutrient intake from fortified and non-fortified food in German children and adolescents – 15 year results of the DONALD Study [18]

Weighed dietary record data from 383 boys and 404 girls collected between 1985 and 2000 were analysed. For several vitamins (vitamin A, B1, B2, B6, C, E folate, niacin) and minerals (calcium, iron) the contribution from fortified and non-fortified food items to the recommended daily intake (German Nutrition Society) could be estimated.

Example 3

Role of nutritional status in the regulation of adrenarche [24]

Data from 22 prepubertal (age 3–12 years) and 20 pubertal (age 8–18 years) children with at least five consecutive annual 24-h urine collections were selected. Dehydroepiandosterone sulphate (DHEAS) was measured and related to anthropometric measures and changes in body mass index (kg/m^2; BMI). Tracking of DHEAS excretion over time and the regulating effect of BMI on adrenarche were investigated.

Discussion

The DONALD Study is characterised by design features which allow to address particular research questions about the interplay between growth, metabolism, endocrinology, nutritional status and diet in healthy children. The major advantage of the study are the frequent repeats and detailed assessments of data on nutrition, anthropometry and biological specimens. Due to its elaborate assessment procedures a convenient, non-representative study sample has been recruited. The internal and external validity of the study results, however, are very unlikely to be affected by this selection since the main purpose of the study is to internally compare and relate certain characteristics of the children (e. g. the relation of a nutrient intake to growth patterns). In contrast, an extrapolation of cross-sectional analyses to describe population distributions of dietary behaviour or growth measures of the general population is not possible, at least not for those parameters that are known to be influenced by such selection factors. Therefore, all external comparisons need to be made with caution.

A further disadvantage is the relatively small sample size compared to other cohort studies. However, only few studies in Europe or abroad with comparable detail and assessment frequency do exist. As outlined in the following paragraphs, a set of studies with differing design features have addressed several aspects of these issues. They each have their advantages and disadvantages for addressing certain research topics. There are, for example, birth cohorts that focused on specific outcomes, e. g. allergies, and had therefore tailored the exposure assessment to that specific topic [26] or have followed their participants for limited number of years only. The ALSPAC (Avon Longitudinal Study of Pregnancy and Childhood) study (N = 1335) had its focus on childhood growth between age 0 and 5. Information on diet and anthropometry were obtained from up to 10 occasions [27]. The Bogalusa Heart Study [28] (N = 11564) and the Boyd Orr cohort study [29] (N = 2399) are other examples of complex cohort studies with assessment of anthropometry, diet and other factors. Both studies which are still ongoing have their focus on CVD risk factors. Although nutrition is an exposure that was considered in most of these studies, only few of them have specifically focused on nutrition as their main exposure. The dietary and anthropometric assessment is less detailed and repeated assessment less frequent. Therefore, their ability to describe tracking of these factors during childhood/adolescence and their interplay with other factors is not as good as in the DONALD Study.

In Germany, no entirely comparable study to the DONALD Study exists. One German birth cohort, the German Multicenter Atopy Study (MAS) has collected detailed data on anthropometric development between birth and age 6. Detailed anthropometric data and blood

samples were collected at 8 occasions, but dietary information was restricted to breast feeding and weaning food practices [30, 31]. Another birth cohort (LISA) aims at environmental and behavioural risk factors for allergic diseases but assesses only very limited information on nutrition [32]. Some prospective information on nutritional factors and anthropometry are collected in the context of an obesity intervention study [33] and a family intervention study [34]. However, the studies started with children at age 6 only and had planned only 2 follow-up assessment during a 10 year period. Further data on diet and related factors (e. g. anthropometry) come from cross-sectional studies (e. g. [35–37]).

Also European-wide, a study comparable to the DONALD Study could not be identified. Several European countries have recruited national birth cohorts, which have collected data on early nutrition and subsequent development of various diseases in adulthood. In Britain, for example, three birth cohorts of children born during a one-week period in 1946, 1958, and 1970 respectively were started [38–40]. This tradition in birth cohorts is now being followed by the launch of the Millennium Cohort Study for which the families of 20,000 babies born in 2000/2001 were recruited [41]. Data assessment was made at age 9 months and is planned to continue at age 3 years and 5 years. Norway has just begun the recruitment for a national mother and child cohort study. The target population consists of all pregnant women during a 4 to 5 year period; 100,000 is the target sample size. Data assessment starts at the 17th week of pregnancy and is then repeated in week 30, at birth and when the child is 6 months, 18 months and 6 years old. Nutrition is assessed with self-administered questionnaires. A similar approach is followed by the Danish birth cohort [42]. Two special features of the latter studies are the possibility to link their data to the national registries of diseases, demography, and social conditions, and the prenatal recruitment and collection of blood from the parents as well as from the umbilical cord. This will allow studying hypotheses related to fetal programming and metabolic imprinting [43]. Currently, these aspects cannot be addressed by the DONALD Study. Due to the limited number of study participants and lack of comparable disease registries in Germany, record linkage for the assessment of disease endpoints, for example, is not an option for the DONALD Study.

There are several other cohort studies that started their recruitment during childhood, e. g. the Nurses' Health Study II Offspring Study, the National Heart, Lung and Blood Institute Growth and Health Study [44, 45]. In contrast to the DONALD Study these studies do not provide prospective data on the entire time of childhood and adolescence, which limits their ability to assess early exposures and tracking. In general, the large sample sizes of the above mentioned studies does not allow a data assessment as detailed as and as frequently as in the DONALD Study. With few data on an individual, however, the gradual changes during growth with respect to physiological and pathophysiological changes are hard to describe. On the other hand, due to their sample size, they have the power to study disease-related outcomes in relation to nutrition, which is only to a very limited extent possible in the DONALD Study. Some of the smaller studies in children have therefore more comparable features with respect to the DONALD Study. For example, the Amsterdam Growth and Health Study [46] assessed nutrition and other life-style variables in a cohort of 500 12–13 year old boys and girls who were then followed for 20 years. In a subsample, exposure assessment was made annually until age 18, and later at age 21, 23, 29 and 33. Despite a detailed assessment of diet, anthropometry, and biomarkers in blood the study started only with children at the age of puberty and consequently does not give information on smaller children.

In summary, population-based studies in healthy children are relatively rare. However, they are an indispensable prerequisite to understand the complex interrelation between genetic factors, nutrition, and other behavioural and environmental exposures on the health status of the child as well as the later adult. Studies addressing aspects of these issues vary greatly in their design features and potentials. The data collected in the DONALD Study offer numerous opportunities to study nutritional and metabolic factors during childhood and adolescence, especially those relating to tracking and longitudinal trends. In particular, hot topics such as hypotheses related to the aetiology and development of overweight and obesity can be addressed. For the future it is intended to extend the study to the prenatal period and into early adulthood. This will allow investigating factors like fetal programming on the one hand and long-term effects and outcome related research on the other hand. Compared to other studies conducted in Europe or other parts of the world the DONALD Study has unique features due to its detailed data assessment and frequent follow-up measurements for each individual study participant. Given the abundance of data and the wide variety of hypotheses that can be tested with these data, future external cooperations are necessary to fully exploit the potential of this study. Therefore, we welcome suggestions for collaborative research both nationally and internationally.

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