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ORIGINAL ARTICLE

Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study

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Objective: To provide an overview of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) design, with particular attention to its quality control procedures. Other important methodological aspects are described in detail throughout this supplement.
Design: Description of the HELENA-CSS sampling and recruitment approaches, standardization and harmonization processes, data collection and analysis strategies and quality control activities.
Results: The HELENA-CSS is a multi-centre collaborative study conducted in European adolescents located in urban settings. The data management systems, quality assurance monitoring activities, standardized manuals of operating procedures and training and study management are addressed in this paper. Various quality controls to ensure collection of valid and reliable data will be discussed in this supplement, as well as quantitative estimates of measurement error.
Conclusion: The great advantage of the HELENA-CSS is the strict standardization of the fieldwork and the blood analyses, which precludes to a great extent the kind of immeasurable confounding bias that often interferes when comparing results from isolated studies.
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Introduction

The early development and maintenance of healthy lifestyles are advocated as important precursors for reduction of nutrition-related diseases and disorders, such as cardiovascular diseases. These lifestyles also influence a large number of physiologic risk factors. Adolescence is a critical period for the development of obesity and other associated disorders.[1] Lifestyle habits also start to be consolidated during this period of life, and nutrition-related disorders such as obesity, anorexia–bulimia nervosa or iron deficiency anaemia are also highly prevalent.[2–4]

The Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) is a randomized multi-centre investigation of the nutritional and lifestyle status of adolescents in 10 European cities. The study also aims to measure variation in lifestyle habits and nutritional status by region, cultural background, socioeconomic status,

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[15]See Appendix at the end of the supplement on page S82.

age and gender. The background of the HELENA Study has been reviewed extensively.[5]

Up to now, there has been no study at the European level that has investigated the nutritional status and lifestyle of adolescents using a central standard methodology across a consortium of several participating countries.[6] This can be considered the 'primum movens' for the establishment of the HELENA concept. An earlier cross-sectional multi-centre Spanish study—called AVENA—was designed in 1999 to evaluate the nutritional status of a representative sample of Spanish adolescents.[7] This study can be considered as the precursor and reference of the HELENA Study, as the experience acquired in the project has been of great assistance in establishing the HELENA Study design and protocol.

The basic objective of the HELENA cross-sectional multi-centre study was to obtain reliable and comparable data from a selected cohort of European adolescents (boys and girls aged 13–16 years) on a broad battery of relevant nutrition and health-related parameters: dietary intake, food choices and preferences, anthropometry, serum indicators of lipid metabolism and glucose metabolism, vitamin and mineral status, immunological markers, physical activity, fitness and genetic markers.[8]

The purpose of this paper is to provide an overview of the HELENA-CSS design, with particular attention to its quality control procedures. Other important methodological aspects are described in detail throughout this supplement.

Management

The management of the HELENA-CSS was designed to ensure effective collaboration and communication among the 10 study centres and the other partners involved in the cross-sectional study. Investigators from each participating centre were involved in the planning and development of the protocol, which included the study design. All the study sites adhered to a common study protocol for training, implementation of fieldwork, data collection and data management and quality control procedures.

The HELENA-CSS coordination is ensured by the general HELENA coordinator (LAM). The continuous monitoring of the study was performed by the HELENA Core Group, composed of five members, including the coordinator. There is also a Steering Committee in which all the research areas are represented. The Steering Committee outlined the scientific strategy and monitored the progress in view of the overall project timeline and key milestones and assisted the coordinator on an *ad hoc* basis for all decisions that need to be taken within a time frame that does not allow a full meeting of all partners, or in cases when immediate contact with one or more partners is not possible.

The work package leaders managed the day-by-day coordination of their work packages. They ensured an appropriate collaboration between participating members, provided sufficient and appropriate information on progress to the other partners, cooperated with the project coordinator to ensure that key milestones were achieved and material for reports were supplied in a timely manner. The structure and function of each study centre was designed to facilitate the development of the assigned tasks.

Sampling and recruitment

A random cluster sampling (all pupils from a selection of classes from all schools in 10 European cities) of 3000 adolescents aged 13.0–16.99 years, stratified for geographical location, age and socioeconomic status, was carried out. Ethical issues and respect for good clinical procedures will be addressed in another paper in this supplement.[9] The sampling and recruitment processes are summarized in Figure 1.

Selection of the European cities

The basis for the selection of the European cities was first of all a practical one. It was not realistic to include a random sample of the European adolescents. We decided to study a city-based sample, striving for representativeness on the level of these cities. The rationale behind this is that nowadays people living in European cities often come from different country areas and partly also from non-EU countries. For those objectives of the project where we plan only to describe the adolescent's characteristics, this procedure is anticipated to give a fair approximation of the average picture of the situation. However, for objectives trying to identify relationships between different variables, the best option is to have a well-defined population, such as that of the adolescents living in the selected cities. From the statistical point of view, the group is considered a homogeneous one without the added variability caused by the habitat.

We considered choosing 10 European cities of more than 100 000 inhabitants located in separated geographical points in Europe: Austria, Belgium, France, Germany, Greece (two cities), Hungary, Italy, Spain and Sweden. The geographical distribution was not fortuitous (random) and not represented by strata, but it was decided according to the following criteria: representation of territorial units (countries) of Europe according to geographical location (N/S/E/W), cultural reference and socioeconomic situation; and selection of a territorial unit (town) in the country, which is representative of the average level of demography, cultural, social and economic markers. The towns are equivalent and comparable between countries. Their size is sufficiently large to ensure that diversity of population is guaranteed.

Males and females aged 13.0–16.99 years from 10 cities (cluster) in Europe (Vienna, Ghent, Lille, Dortmund, Athens, Heraklion, Pécs, Rome, Zaragoza and Stockholm) were the basis for the sampling selection. For this study, it has been

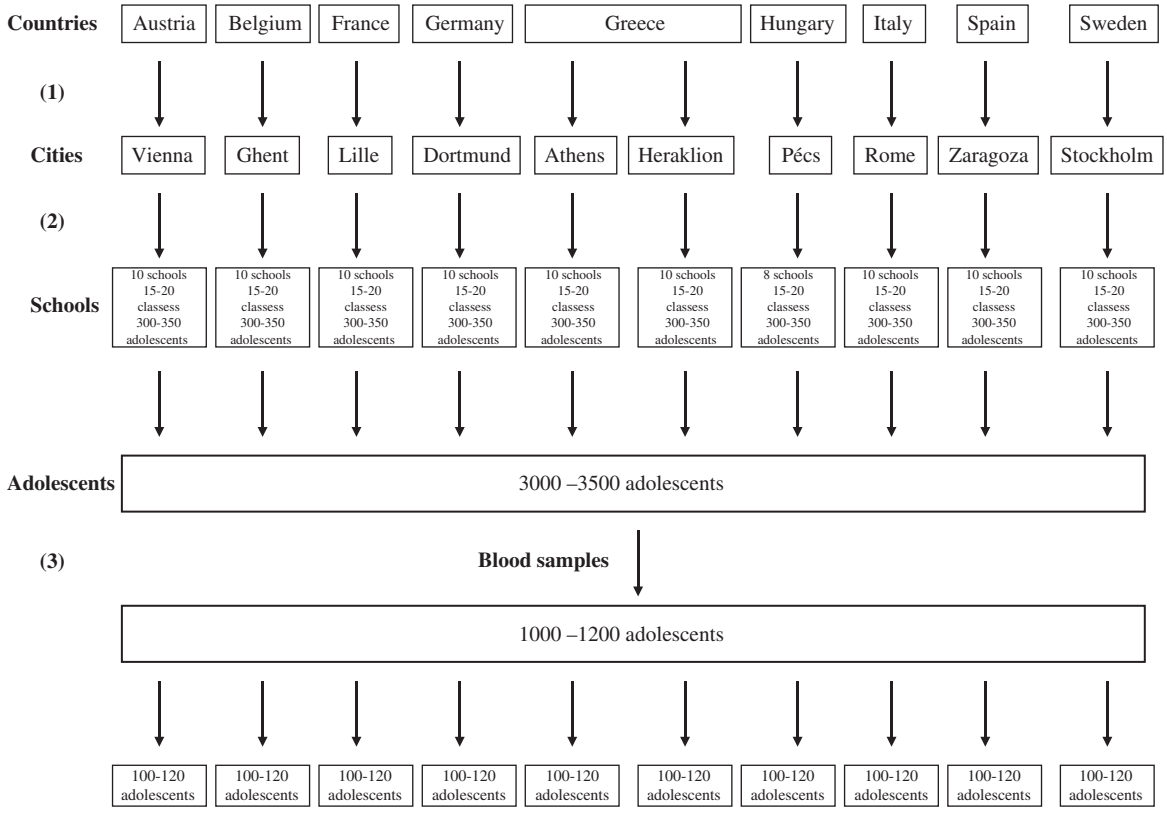

Figure 1 Sampling procedure schemes. (1) Considering geographic balance and the presence of an experienced research group. (2) Strata by age, sex and school are in every city. (3) Strata by age, sex and school are in every city.

considered that the valid option that offers reliability (for comparison) and viability (from the practical point of view) is to recruit the participants at schools. In all the countries where the study was performed, at the ages of 13–16 years it is mandatory for the adolescents to attend schools, and the school population is therefore supposed to be the total population in this age group.

Sampling size

The sampling size was calculated with a confidence level of 95% and ± 0.3 error in the worst of situations for the parameter body mass index. From earlier studies conducted in Europe, the variability of the different variables under study was observed. The variance of body mass index has been chosen as the factor that has the greatest dispersion in the studied population with regard to the problem that is going to be studied. The sampling has been determined by means of this dispersion. The information about demographic data was obtained from the corresponding National Institute for Statistics in each country. We used the body mass index means and standard deviations published earlier in the literature.[10,11] As we decided to evaluate complete school classes (cluster) to randomize as much as possible, we believed that the classes had a minimum of 20 students per class, the distribution by sex is similar to that of the population in general, and that in both schools and classes there are boys and girls. We assumed that age is similar because of attending the same class, but not identical, that there would be a low percentage of students who would refuse to participate in the study, and that there would also be a small percentage of invalid questionnaires. The average number of adolescents to be studied was estimated as 300, with not more than 350 in each centre. The process for selecting which schools and classes would participate in the study was determined by taking into consideration the different school zones in each city. Once the random selection was completed, the selected schools were invited to participate in the study. In case the selected school was unwilling to participate, a reserve school was already foreseen. The classes with blood samples were also randomly selected by district.

The sample size was adopted with proportional linkage to the size of the strata (sex and age). Diversity of the sample in all cultural and socioeconomic aspects was achieved by making a random proportional distribution of all the schools taking into account their characteristics and their localization within the diverse zones of the towns. A total number of at least $300 \times 10 = 3000$ adolescents was included. A subgroup of approximately 1000 adolescents from the 10 cities was chosen to participate also in blood sampling. As

blood parameters have much less variability, a smaller sample size is sufficient to be representative. The size of the subgroup (around 100 adolescents in each city) was chosen by means of the immunological parameters,[12,13] which were those with the highest variability within the blood measurements that were included in the study.

Selection of schools, classes and pupils

The age range from 13 to 16 years has been chosen because the familial bounds that have been present during childhood are less restricting and lifestyle habits are fully established.

For practical reasons and considering both educational and psychological reasons, complete school classes from the four grades that theoretically best correspond to the selected age group were identified. Hereby, we supposed that the random selection of classes resulted in a sex distribution similar to that in the general population. If necessary, the corresponding statistically corrective measures can be introduced during statistical analyses.

To guarantee that the heterogeneity of social background of the population would be represented, a representation of the different school types in the respective cities was considered. However, because of the differences in school systems in the European countries, it was decided to rather use residence—place of school attendance—to guarantee diversity of the sample in cultural and socioeconomic aspects. Hence, schools were randomly selected after stratification on school zone or district.

In practice, random selection of schools and classes was executed centrally by the partner at Ghent University for all cities. The number of adolescents per strata was weighed for the dimension of the strata with the exception that at least one class should be randomly selected from the list of private schools. A list of 10 schools was provided to each centre. In case the selected schools refused to participate, a second list of substitute schools was already foreseen. Overall, 10 schools were involved in the survey in each city with the exception of Hungary where only eight schools participated. The selected school classes were randomly selected after stratification for grade. Up to three classes or class groups from two grades were selected per school, with not more than 60 adolescents per school. In each city, approximately 15–20 classes (350–400 adolescents) were randomly selected taking into account that there would be invalid questionnaires and refusals. In addition, the classes with blood samples were randomly selected.

A class was considered eligible if the participation rate was at least 70%. If less than 70% of a class accepted to participate, the class was excluded and another class in the same age group was chosen in the same school. If this second class of the same school again failed to fulfil the same criteria of eligibility, the corresponding school in the second school list was approached and the same procedure was repeated. If at the end of this procedure still no class fulfilled the criteria of eligibility, for practical reasons, all approached adolescents from the last school who were willing to participate were included.

The final database included those participants who met the following criteria: age of 13.00–16.99 years, schooling in one of the 10 European cities, informed consent signed, has at least weight and height measured and completed at least 75% of the other tests. The exact criteria are shown in Table 1. Participant exclusion criteria include, participants participating simultaneously in another clinical trial, age <13 or $\geqslant 17$ years and an acute infection lasting less than 1 week before the inclusion. In every school class, all adolescents wishing to participate were, for psychological reasons, accepted to participate even if it was known that they were not eligible. Exclusions from the study were carried out *a posteriori*, without the knowledge of the affected participants, to avoid non-desired situations.

Non-participant management

Non-response is a problem in any cross-sectional study in terms of representativeness of the population being investigated. It is important to know as much as possible not only about the participants, but also about the non-participants. Therefore, the non-participant study was based on different levels in the sampling procedure just described. The current ethical norms in most European countries limit, in a very important way, data capture of non-participants.

Schools. As for every selected school there was also chosen by lottery a reserve school in the same municipal district,

Table 1 Criteria to consider an adolescent as an eligible participant

Essential for each participant	
1. Inclusion criteria	
2. Weight and height	
3. Signed informed consent	
Optional (each participant must complete at least 12 measurements)	
1. Clinical examination	(Validity assessed by the medical doctor)
2. Anthropometric measurements	(All the measurements)
3. Bioelectrical impedance analysis (BIA)	
4. General questionnaire for adolescents	(Not fully blank)
5. Nutrition knowledge (NKT-C)	75% completed
6. Eating behaviour (EWI-C)	75% completed
7. Food choice and preference	75% completed
8. Determinants of physical activity (PA)	(Not fully blank)
9. Physical activity+sedentarism	(Not fully blank)
10. Self-European socioeconomic status	(Not fully blank)
11. Questionnaire for parents (QP)	75% completed
12. Determinants of healthy eating (HE)	(Not fully blank)
13. Young Adolescents Nutrition Assessments on Computers (YANA-C)	(2 days)
14. Accelerometry assessment	(Minimum criteria defined into the protocol)
15. Physical fitness tests	(All the tests)
16. Blood sampling (including questionnaire)	(All the tubes)

there should not be a bias concerning socioeconomic and sociodemographic factors. Nevertheless, differences between collaborating schools and non-collaborating schools will be studied, and the reasons given for not participating in the study will be analysed.

Individuals. Gender, age, self-reported height and weight (if this information was obtained) and reason for non-participation were reported on a non-participant form. Differences between participants and non-participants will be analysed statistically.

Standardization and harmonization

Both during the feasibility study and for the cross-sectional study, there was a strict standardization of the fieldwork. All blood samples were analysed centrally (in several laboratories for different analyses)[14] and the processing of questionnaires was also carried out centrally by the database centre in Ghent. This is the only way to exclude, from the beginning, the huge amount of confusing variables that appear when data from isolated studies are compared.

To validate and harmonize the methodology, a general training workshop was held in Zaragoza (Spain), from 16th to 20th January 2006. The scientists in charge of every research tool attended the workshop. Theoretical courses were held in the morning sessions, and the different tools were applied in a group of volunteer Spanish adolescents, who were fluent in English. The national teams participating in the training were composed of at least two persons, one with public health or statistical expertise and the other with medical or nutrition skills. The main topics dealt with were the relevant aspects for the following issues: (a) the sampling procedure, (b) the non-respondent's management, (c) the data collection techniques and procedures and (d) the data management and transfer procedures. At the end of this training, the national teams were in charge of translating the field protocol into the local language. Before the start of the final data collection, a feasibility phase was conducted. Although a general framework was established for sampling and data collection, some adaptation was necessary depending on the local special conditions of the different countries. The logistic functioning of the procedure was also tested with the pilot study.

Data collection and analysis

To match with a variety of institutional facilities, operational procedures and staff capabilities at the participating centres while providing the necessary standardization and quality assurance in data collection and processing, the data management system was required to be well considered. Necessary functions included entry and validation, transfer, security and confidentiality, retrieval and archiving of all data, as well as database updating and closure.

A manual of operations to be followed by all participating centres was prepared. This manual includes the whole set of data collection methodology and a detailed description of all instruments for the core study data.

The database architecture, questionnaires of multiple response format and easy-to-enter user-friendly data-input software were centrally developed in Teleform and MS-Access. The questions from the multiple response format were scanned centrally, whereas the open entry data were entered and archived by each local field centre. The standard formats featured a unique label for each and every item to be collected across all participating centres. These labels were described in a codebook including all the codes and their unambiguous interpretation. A protocol for data entry included specifications aimed at minimizing coding errors. A standardized protocol for quality control and first round of data cleaning to be applied by all participating centres was prepared. A central protocol for anonymous data cross-linkage was created. A central analytical plan was also developed.

The central database architecture and management, completed with a partly distributed data management system, provided the capabilities required for data entry and management of an elaborate international study such as the HELENA Study. Staff participating in the training sessions were evaluated and certified in the use of the data entry system.

Quality control activities

Quality is the first element that should be considered when planning a study. Quality means to guarantee the results and conclusions that are obtained in the study. In a multi-centre study such as HELENA, quality control must analyse the whole process to control the possible critical points and risks. All the dimensions of HELENA were submitted to quality control.

The data management system allowed field centres to locally generate a variety of summary reports on data completeness, outstanding questionable values and so on, enabling each centre to monitor the quality of its performance. This facilitated the timely identification and resolution of problems in data collection and processing.

To ensure accurate, standard and consistent measurements throughout the multi-centre study, a variety of standard procedures were used. These included the documentation of measurement protocols, preparation of detailed manuals of operations, establishment of training and certification procedures and performance monitoring.

Manuals of operations

The persons in charge of the different study components prepared the manual of operations. All measurements require a manual of operations outlining the procedures

needed to implement the protocol. The documentation ensures standardized procedures across the sites throughout the duration of the study. Manuals contained requirements for cohort identification, random selection procedures when necessary, equipment and material. Each measurement component had its own manual. They also specified detailed measurement procedures, training and certification requirements, forms describing procedures for data recording and all quality control procedures associated with measurements.

Training and certification

Quality control for body composition assessments. The first training sessions were held during the general training workshop in Zaragoza, Spain (16th–20th January 2006). After the feasibility study, another training workshop was held in Pécs, Hungary (October 2006). The detailed quality control procedure and the results on the reliability assessment are reported in this supplement by Nagy *et al*.[15] Each trainee was certified for all body composition measurements after training and completing reliable measures on approximately 10 participants not in the cohort. For skinfolds and circumferences, intraobserver reliability must be higher than 95%; interobserver reliability must be higher than 90%.[16]

Dietary interviewer training and certification procedures for 24-h recall data collection. Central training on dietary intake assessment was included in the general training workshop that was held in Zaragoza, Spain (16th–20th January 2006). The workshop included exercises designed to help interviewers to become familiar with the recall questionnaire, the way of motivating participants to fill in the recalls with accuracy, and with domestic kitchen size measures and recipes. Exercises also promoted skills in checking the recall questionnaires for completeness and accuracy, as well as promoted skill in data entry and data management. Quality assurance was guaranteed as all dietary recall forms were checked for completeness and accuracy immediately after they were filled in by the participants. Notes and missing foods were resolved. Food and nutrient data outliers were tabulated and the 24-h recall data file was examined further for possible entry errors.

Quality assurance for administration of questionnaires. The knowledge, attitudes, behaviours and physical activity questionnaires were administered in the classrooms. At least one researcher from each field centre participated in the central training. Certification was granted to those attending the training who were judged capable of fulfilling the required positions. Quality control requirements included the collection of questionnaires from at least 80% of the eligible cohort and 70% with no more than five missing responses.

Quality assurance for blood sampling and laboratory measurements. A standardized protocol was followed for blood sampling and management. The partner responsible for this part of the study (University of Bonn) instructed HELENA staff members in charge of this part of the study at the field sites on correct manipulation of blood sampling and procedures. Laboratory measurements were submitted to the internal quality standards of the certificated laboratories where analyses were performed. Blood sample management and quality control are described in detail in this supplement by González-Gross *et al*.[14]

Performance monitoring

The group responsible for good clinical practice (University of Lille 2) made a site visit to each study centre during the data collection periods. During these site visits, observations were made on (1) adherence to measurement protocols; (2) participant identification and labelling forms; (3) recording, collation, processing and filing of data and (4) confidentiality of procedures to preserve data. In addition, they verified participant consent; trained and certified data collectors on the measurement teams and ensured that all quality control procedures were appropriately followed. They met with each measurement leader during their visit and discussed any significant deviations from protocol or other concerns to ensure immediate action and resolution.

Each field site had a measurement coordinator who oversaw the body composition measurements, administration of questionnaires, data collection on physical activity and fitness, blood sampling and procedures and a nutrition coordinator who oversaw the 24-h dietary recall data collection. These supervisors were responsible for assuring that their site followed all appropriate procedures for specific data collections and quality control procedures, and for conducting subsequent additional training when necessary.

Feasibility study

Pilot studies were organized on a small scale in each country to check every step of the procedure, from sampling to data processing. The objectives were to assess: (a) the collaboration of the school staff in the context of the sampling and data collection procedure; (b) the acceptability of the different blocks of data collection and (c) the importance of the different causes of missing data (refusal rate, loss of questionnaires, outliers or missing values). The data collected were also used to check the good functioning of the data control and analysis programmes. The feasibility study is described in this supplement by Iliescu *et al*.,[17] and some results from this pilot study are also described elsewhere. The schools used for the pilot studies were chosen in the suburbs of the 10 cities so that there would be no consequence on the randomization for the study itself. Following the experience of the feasibility study, a survey strategy was developed that was further adapted to the local conditions in each city (Table 2).

Table 2 Assessment of the different components included in the HELENA Cross-Sectional Study

Day	*Activity*	*Time per adolescent (minutes)*	*Place*	*Number of adolescents per day*
1	Non-participants questionnaire	5	Classroom	45 maximum
	Case report form	10	Classroom	
	General questionnaire	10	Classroom	
	Socioeconomic status questionnaire	10	Classroom	
	Parents questionnaire	0	Home	
	Nutrition knowledge questionnaire (EWI-C)	15	Classroom	
	Eating attitudes questionnaire (NKT-C)	15	Classroom	
	Food choices and preferences questionnaire	15	Classroom	
	24-h dietary recall using Young Adolescents Nutrition Assessments on Computers (YANA-C) (day 1)	45	Computer room	
	Accelerometer placement	5	Classroom	
2	Clinical examination	15	Clinical examination room	20 maximum
	Blood pressure	20	Clinical examination room	
	Anthropometry	20	Clinical examination room	
	Bioelectrical impedance	20	Clinical examination room	
3	Fitness tests	90	Gymnasium	45 maximum
	Physical activity questionnaire	15	Classroom	
	Parents questionnaire collection	5	Classroom	
	Accelerometer collection	5	Classroom	
4	Diet determinants questionnaire	15	Classroom	45 maximum
	Physical activity determinants questionnaire	15	Classroom	
	24-h dietary recall using YANA-C (day 2)	45	Computer room	
5	Blood sampling questionnaire	5	Clinical examination room	20 maximum
	Blood sampling	15	Clinical examination room	

Comments

The HELENA-CSS is a multi-centre collaborative study conducted in European adolescents located in urban settings. The HELENA-CSS cohort is not a fully European representative sample, because it is restricted to 10 geographic areas. The choice of the study population must be seen as a compromise between what is scientifically desirable and what is practically feasible and methodologically justifiable in a large international study. The data management systems, quality assurance monitoring activities, standardized manuals of operating procedures and training and study management have been addressed in this paper.

We are interested not only in the description of the nutrition and lifestyle variables, but also in the measurement process as well. Various quality control procedures to ensure collection of valid and reliable data are discussed in this supplement, as well as quantitative estimates of measurement error. The following articles present results of methodological approaches developed for the different aspects included in the HELENA-CSS, as preparatory work for the final study.

Undeniably, the great advantage of the HELENA-CSS is the strict standardization of the fieldwork and the blood analyses, which precludes to a great extent the kind of immeasurable confounding bias that often interferes when comparing results from isolated studies. The outstanding collaboration between centres and the efficiency with which material and data were interchanged and processed led to an overall result that can only encourage future studies of the same kind.

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Conflict of interest

Frédéric Gottrand has received consulting fees from Numico Clinical Nutrition, lecture fees from SMS and grant support from Danone Research. The remaining authors state no conflict of interest.

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