

# Healthy Lifestyle by Nutrition in Adolescence (HELENA). A New EU Funded Project

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## Keywords:

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**Abstract** – The key to health promotion and disease prevention in the 21st century is to establish an environment that supports positive health behaviour and healthy lifestyle from childhood. The HELENA project includes cross-sectional, crossover and pilot community intervention multi-centre studies, as an integrated approach to the above-mentioned problem. Dietary intake, nutrition knowledge and eating attitudes, food choices and preferences, body composition, biochemical, physical activity and fitness and genotype (to analyse gene-nutrient and gene-environment interactions) assessment will provide the full information about the nutritional and lifestyle status of the European adolescents. The requirements for health promoting foods will be also identified, and three sensory acceptable products for adolescents will be developed. Harmonization and standardisation of the assessments for both scientific and technological objectives should result in reliable and comparable data of a representative sample of European adolescents. This will contribute to understand why health-related messages are not being as effective as expected in the adolescent population. A realistic intervention strategy will be proposed in order to achieve the goals of understanding and effectively enhancing nutritional and lifestyle habits of adolescents in Europe.

## Mots clés :

obésité ;  
composition corporelle ;  
génétique ;  
activité physique ;  
intervention  
communautaire

**Résumé** – **Habitude de vie, nutrition et santé chez l'adolescent (étude HELENA). Un nouveau projet soutenu par la Communauté européenne.** L'un des enjeux de la promotion de la santé et de la prévention des maladies du XXI<sup>e</sup> siècle est de mettre en place dès l'enfance des comportements et un style de vie favorables à la santé. Le projet HELENA inclut une étude multicentrique transversale, une étude croisée et des études pilotes interventionnelles chez une population d'adolescents, dans une approche intégrative de la prévention pour la santé. Les apports alimentaires, les connaissances et les habitudes nutritionnelles, les choix et préférences alimentaires, la composition corporelle, l'évaluation de paramètres biochimiques, de l'activité et des performances physiques et des analyses génétiques centrées sur les interactions gènes-nutrition et gènes-environnement permettront d'apporter une information complète sur l'état nutritionnel et le style de vie des adolescents européens. Les besoins en aliments bénéfiques à la santé seront également identifiés et trois produits adaptés aux goûts des adolescents seront développés. Une démarche d'harmonisation et de standardisation de tous les objectifs scientifiques et technologiques de ce projet permettra d'obtenir des données fiables et comparables d'un échantillon représentatif des adolescents européens. Une stratégie interventionnelle réaliste sera proposée pour atteindre les objectifs de compréhension et d'amélioration effective des habitudes nutritionnelles et de style de vie des adolescents européens.

## 1. Introduction

The key to health promotion and disease prevention in the 21st century is to establish an environment that supports positive health behaviour and healthy lifestyle. Most diseases have

their origin during childhood and adolescence, but the relationship between the development of non-communicable diseases and the adolescence process is poorly understood. Adolescence is a crucial period in life and implies multiple physiological and

psychological changes that affect nutritional needs and habits. The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) project includes cross-sectional, crossover and pilot community intervention multi-centre studies, as an integrated approach to the above-mentioned problem. The following aspects will provide the full information about the nutritional and lifestyle status of the European adolescents: 1) dietary intake, nutrition knowledge and eating attitudes; 2) food choices and preferences; 3) body composition; 4) plasma lipids and metabolic profile; 5) vitamin status; 6) immune function related to nutritional status; 7) physical activity and fitness and 8) genotype (to analyse gene-nutrient and gene-environment interactions). The requirements for health promoting foods will also be identified, and three sensory acceptable products for adolescents will be developed. Both scientific and technological objectives should result in reliable and comparable data of a representative sample of European adolescents, concerning: foods and nutrients intake, food choices and preferences, obesity prevalence, dislipidemia, insulin resistance, vitamin and minerals status, immunological markers for subclinical malnutrition, physical activity and fitness patterns, and variations of the nucleotide sequence in selected genes. This will contribute to understand why health-related messages are not being as effective as expected in the adolescent population. A realistic intervention strategy will be proposed in order to achieve the goals of understanding and effectively enhancing nutritional and lifestyle habits of adolescents in Europe. The partners participating in the HELENA project are summarized in table I.

The HELENA general goal will be achieved through the following specific objectives:

- to develop and harmonise innovative methods for the assessment of lifestyle habits of adolescents across Europe with special focus on diet, nutrition and physical activity;
- to assess dietary and physical activity patterns as well as nutritional status among European adolescents;
- to investigate knowledge and attitudes towards nutrition and physical activity among adolescents and to establish the main determinants of their food choice and preference;
- to describe regional, cultural, social, genetic and gender differences and similarities across Europe;
- to identify adolescents at risk of eating disorders, dislipemia, obesity and/or type 2 diabetes;
- to develop a number of healthy foods and identify marketing strategies for consumers, in order to improve the diet of adolescents;
- to develop a lifestyle education programme and test its efficacy for improving adolescents' health.

Therefore, the main objective of this paper is to present to the scientific community, the most relevant issues of this project.

## 2. Previous knowledge and project relevance

The biomedical, behavioural, social, and political sciences are increasingly recognising the interactions and interdependence among genetic, biological, behavioural, environmental, and socio-cultural processes involved in health, disease, and public health practice.

Non-communicable diseases, such as coronary heart disease, stroke, obesity, hypertension, type 2-diabetes, allergies, and various cancers are still the most common causes of morbidity and mortality in European countries. These highly prevalent diseases occur in the face of increasing knowledge, awareness and education about chronic diseases and their risk factors. It has been suggested that a paradigm shift is necessary if future progress is to be made.

Most of these diseases that affect a huge number of people in western countries have their origin during childhood and adolescence,<sup>[1-3]</sup> but the complex relationship between all these processes and the development of non-communicable diseases is poorly understood in the adolescent group. Moreover, the interactions between the environment, the genetic predisposition and growth in children and adolescents have not yet been studied.<sup>[4,5]</sup> Adolescence is a crucial period in life and implies multiple physiological and psychological changes that affect nutritional needs and habits. Adolescence is characterised by an intense growth and development; sexual maturation is followed by important changes in body composition of human subjects, and it introduces definitive sex and gender differences.<sup>[6]</sup>

Existing research indicates that risk factors for non-communicable diseases have to be placed in an ecological context, which calls for more than simple education about risk factors and needs a collaborative strategy with the multiple sectors that affect the diseases. Diet habits and physical inactivity are the major preventable risk factors for the occurrence of non-communicable diseases. Cardiovascular disease is the main cause of death in the European Union as a whole, accounting for 1.5 million deaths each year or expressed in another manner, for 42 percent of all deaths. The reduction of non-communicable diseases risk factors in childhood and adolescence may reduce morbidity and mortality in adulthood.

To identify adolescents at risk and find solutions, known risk markers have to be measured and new biological markers or indices should be developed combining biological and lifestyle/environmental data. This is only possible doing research with well-defined study protocols, as will be done in HELENA. Up to now, there has been no study at European level investigating the nutritional status and lifestyle of adolescents aged 13 to 16 with the same methodology throughout several countries.<sup>[7]</sup>

**Table I.** Groups participating in Healthy Lifestyle in Europe by Nutrition in Adolescent (HELENA) project.

Partic. no.	Participant name	Group	Participant short name	Country
1	Universidad de Zaragoza	E.U. Ciencias de la Salud	UNIZAR	Spain
2	Consejo Superior de Investigaciones Científicas	Instituto del Frio. Departamento de Metabolismo y Nutrición	CSIC	Spain
3	Université de Lille 2	Clinique de Pédiatrie, Hôpital Jeanne de Flandre et Centre d'Investigation Clinique CIC9301 CH&U-INSERM	UL2	France
4	Research Institute of Child Nutrition Dortmund		FKE	Germany
5	Pécsi Tudományegyetem	Department of Paediatrics. Medical Faculty	PTE	Hungary
6	University of Crete	Department of Social Medicine, School of Medicine, Preventive Medicine and Nutrition Clinic	UOC	Greece
7	Rheinische Friedrich Wilhelms Universität	Institut für Ernährungs- und Lebensmittelwissenschaften- Humanernährung	Uni Bonn	Germany
8	University of Granada	Department of Medical Physiology, Faculty of Medicine, Research Group EFFECTS 262	UGR	Spain
9	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione	Human Nutrition Unit, INRAN	INRAN	Italy
10	University of Napoli "Federico II" Department of Food Science	Department of Food Science	DSA-UNINA	Italy
11	Ghent University	Department of Public Health. Faculty of Medicine and Health Sciences. Unit of Nutrition and Food Safety	BE-TUG	Belgium
12	University of Vienna	Department of Pediatrics, Division of Clinical Nutrition	AKH-WIEN	Austria
13	Harokopio University	Department of Nutrition and Dietetics	HUA	Greece
14	Institut Pasteur de Lille	INSERM-U508 et Service d'Épidémiologie et de Santé Publique	IPL	France
15	Karolinska Institutet	Department of Biosciences, Unit for Preventive Nutrition	KI	Sweden
16	Asociación de Investigación de la Industria Agroalimentaria	Laboratorio de Análisis Físico y Sensorial	AINIA	Spain
17	Campeen & Chorleywood Food Research Association	Department of Consumer and Sensory Sciences	CCFRA	United Kingdom
18	SIK – Institutet foer livsmedel och bioteknik	Flavour and sensory evaluation	SIK	Sweden
19	Meurice Recherche & Developpement asbl	Institut Meurice – Departement de Technologie Alimentaire	Meurice R&D	Belgium
20	Campden & Chorleywood Food Development Institute, Hungary		CCFRA – Hungary	Hungary
21	Productos Aditivos SA	Research and Development	PASA	Spain
22	Carnicas Serrano SL	Research and Development	SERRANO	Spain
23	Cederroth International AB	Research and Development	Cederroth	Sweden
24	Cerealia R&D AB		Cerealia	Sweden
25	EUFIC		EUFIC	Belgium

### 2.1. Nutrition knowledge, food and nutrient intake and micronutrient status

Up to now, seventy-nine surveys of dietary intake from 23 countries have been collected by an ILSI (International Life Science Institute) Europe Task Force<sup>[8]</sup> as they satisfied a defined set of quality criteria. Data on energy, protein, total fat and carbohydrate were given in a large number of surveys, but information was very limited for some micronutrients. No surveys gave information on fluid intake and insufficient gave data on food patterns. A variety of collection methods were used, there was not consistency in the ages of children surveyed or the age cut-off points, but most surveys gave data for males and females separately at all ages. Just under half of the surveys were nationally representative and most of the remainder were regional. Males had higher energy intakes than females; energy intake increased with age but levelled off in adolescent girls. Intakes of other nutrients generally related to energy intakes. Some north-south geographical trends were noted in fat and carbohydrate intakes, but these were not apparent for other nutrients. Some other trends between countries were noted, but there were also wide variations within countries.

A number of validation studies have shown that misreporting is a major problem in dietary surveys of children and adolescents and so all the dietary data collected should be interpreted and evaluated with caution. That is why it has been claimed for the need of harmonisation and standardisation of diet and nutrition methods of nutrition surveys in Europe. Therefore, it is prior to design short, easily administered, inexpensive, accurate instruments that can be used in a broad range of adolescent sub-populations.<sup>[9]</sup> In this regard, it seems that the computer-based dietary assessment tools are the future.<sup>[10]</sup>

Concerning micronutrients, *i.e.* folate, iron and calcium, their nutrient intake seems to be at risk in most of the European countries.<sup>[11-14]</sup> Deficit of these micronutrients is associated with increased risk of cardiovascular disease and insufficient bone mass accrual.<sup>[15,16]</sup> Food and nutrient intake data are not enough to establish nutritional status and must be complemented with biochemical data. For micronutrient status, it is necessary to establish reference values obtained in large population samples analysed under the same methodological conditions. This will permit a correct interpretation of the results in further nutritional studies. As there are no nutritional studies on adolescents at European level as proposed in HELENA, these reference values and cut-off points for most parameters, and specifically for vitamins, are missing.

Presumably, as it has been observed in adults, nutrition knowledge is associated with healthy eating.<sup>[17]</sup> However, studies in children and adolescents are scarce.

### 2.2. Physical activity and physical fitness

Physical activity is a multidimensional human behaviour. Because of its complex nature, physical activity is difficult to assess precisely under free-living conditions. As a result, no single method is available to quantify all dimensions of physical activity. Two methods of objectively assessing physical activity are the use of motion sensors, which are based on accelerometry, and the doubly labelled water technique. Motion sensors measure the acceleration of the body, *i.e.*, body movement, in one or more directions and can quantify physical activity data in terms of time and intensity.<sup>[18]</sup> At present, several international research groups are working on the elaboration of valid, international and comparable questionnaires for monitoring physical activity such as International Physical Activity Questionnaire (IPAQ), the European context of Health Interview Surveys (EUROHIS), and the European Physical Activity Surveillance System (EUPASS)<sup>[19]</sup> focusing on the member states of the European Union. The IPAQ (International Physical Activity Questionnaire) was developed as an instrument for cross-national monitoring of physical activity and inactivity, showing acceptable measurement properties; at least as good as other established self-reports.<sup>[20]</sup>

Within lifestyle habits, it seems that sedentary behaviours are strongly related to nutrition-related diseases and disorders, already in the adolescent period.<sup>[21,22]</sup> Obese adolescents are less physically active than are normal-weight adolescents,<sup>[23]</sup> but physical activity-related energy expenditure was not significantly different between groups. The data suggest that physical activity is not necessarily equivalent to the energy costs of activity.<sup>[22]</sup>

Which is clear is that higher level of physical activity participation is associated with better physical fitness.<sup>[24,25]</sup> In Spanish boys involved in 3 hours of extracurricular physical activity over 3-yr period increased their cardio-respiratory fitness over adult values.<sup>[26]</sup> In addition, physical fitness is associated with general health<sup>[27]</sup> mainly depending on cardio-respiratory and neuromuscular adaptations. The EUROFIT (European research program started in 1993 to harmonize fitness assessment) Test Battery has been developed for evaluating health-related physical fitness<sup>[28]</sup> and is widely used across Europe. In Swedish adolescents, it has been observed that cardio-respiratory fitness showed only small changes between 1987 and 2001 among boys, with no changes in girls.<sup>[29]</sup> Neuro-muscular fitness, as measured by three functional tests, was lower in 2001 compared to 1987. The most pronounced changes in these functional tests were found in the arm-hang test, while changes in lower body and trunk strength tests were less.<sup>[29]</sup> It is therefore time to look for European consensus.

### 2.3. Obesity in adolescents

Rates of cardiovascular diseases and diabetes have been found to increase in both men and women who were obese during adolescence.<sup>[30]</sup> Approximately 50% of obese adolescents with a body mass index at or above the 95th percentile become obese adults.<sup>[30]</sup> The prevalence of obesity in children and adolescents in US has increased dramatically in the last decades.<sup>[31]</sup> In Europe, there are little representative data about obesity prevalence in adolescents and the existing ones are not comparable, because different definitions for obesity were used. However, available results point out that there is also a dramatic increase in the prevalence of obesity in European adolescents, with gender and socio-economic status differences.<sup>[32]</sup>

Recently, Lobstein and Frelut<sup>[33]</sup> have reported estimates of the prevalence of overweight in children and adolescents in various European countries based on 20 surveys with the data recalculated where necessary to conform to the international definitions. They have detected two apparent trends. The first is the general lower levels of overweight in the countries of central and Eastern Europe whose economies suffered varying degrees of recession during the period of economic and political transition in the 1990s. The second trend apparent in the data is for the prevalence of overweight to be higher among the southern countries of Europe, especially those outside of the former eastern bloc. These data have been also included in a very recent report from the IASO (International Association for the Study of Obesity) International Obesity Task Force.<sup>[34]</sup>

In cross-sectional, nationally representative school-based surveys in 1997-1998, by means of identical data collection methods, other authors<sup>[35]</sup> have assessed overweight prevalence in 13 and 15 years old adolescents, in 13 European countries, Israel and the United States. The highest prevalence was found in the United States and the lowest in Lithuania. The highest prevalence in Europe was found in Ireland, Greece and Portugal.

The Working Group Report of the 2nd World Congress of Paediatric Gastroenterology, Hepatology, and Nutrition has recommended that large-scale prospective population studies should incorporate careful anthropometric measurements and regular monitoring of selected obesity-associated complications using some or all of the following tests: fasting blood tests (glucose, insulin, lipids, liver function tests), blood pressure measurement, glucose tolerance test, liver ultrasound, measurements of psychosocial functioning, lung function tests, and biomechanical or podiatric assessment.<sup>[36]</sup>

Atherosclerosis starts in childhood and adolescence, but clinical manifestations can appear 30 to 50 years later. This is the reason why it is so important to identify risk factors as early as possible. Risk factors for several of the major chronic diseases,

such as cardiovascular diseases, hypertension, diabetes, obesity, and cancer, are often observed during childhood. Metabolic syndrome X seems to be present already during adolescence, especially if there is a predisposing genetic background. Prevalence of multi-metabolic syndrome among obese children and adolescents in Hungary was 7.7% for boys and 9.1% for girls. Only 14.4% of obese children and adolescents were free from any cardiovascular risk factors.<sup>[37]</sup> Adolescents at risk for this condition could easily be identified.<sup>[38]</sup>

### 2.4. Nucleotide polymorphisms and phenotype heterogeneity

The study of genetic markers in adolescents and their relationship to several phenotypic characteristics of the population will permit a better understanding of the pathogenic mechanisms that are involved in non-communicable diseases, specifically, cardiovascular diseases and also to approach the mechanisms of variability in nutritional responses. Indeed, some individuals appear to be relatively sensitive to dietary or lifestyle intervention, whereas others are quite resistant. There is evidence suggesting that this variability to changes in environmental factors and especially nutrition intervention is partly determined by genetic factors.

This can be relevant for obesity at least at three different levels: they could be involved in determining the susceptibility to gain fat in response to environmental risk factors (high fat diet, low physical activity, etc.), they may influence the response of the phenotype to interventions, and they can be involved in the susceptibility of obese individuals to develop co-morbidities associated with obesity. The molecular pathogenesis of paediatric obesity remains unknown for the vast majority of overweight children and adolescents. More than 100 candidate genes or regions are potentially implicated.<sup>[39]</sup> Single formal genetic studies suggest a higher heritability of body weight in adolescence and genes that influence body weight in adulthood might not be the same as those that are relevant in childhood and adolescence. Multiple measurements of BMI from childhood to adulthood showed considerably greater heritability than a cross-sectional measurement. Significant and suggestive linkage with long-term burden and trend of BMI was observed on chromosomes 1, 5, 7, 12, 13 and 18. Candidate genes involved in the pathophysiology of obesity are identified in all regions on six chromosomes except for 7q11.1.<sup>[40]</sup> There are more than 430 chromosomal regions with gene variants involved in body weight regulation and obesity development. Polymorphisms in genes related to energy expenditure – uncoupling proteins –, related to adipogenesis and insulin resistance – hormone sensitive lipase, peroxisome proliferator-activated receptor gamma, beta adrenergic receptors 2 and 3 and alpha tumor

necrosis factor  $\alpha$ , and related to food intake – ghrelin – appear to be associated with obesity phenotypes.

Obesity risk depends on two factors: genetic variants in candidate genes and biographical exposure to environmental risk factors. The genotype-environment interactions could also be involved in the susceptibility of obese individuals to develop comorbidities associated with obesity (diabetes, hyperlipidemia, hypertension and coronary heart disease). Definition of these interaction effects for phenotypes related to obesity is therefore important because it will eventually allow the identification of individuals at risk of the development of complications and the identification of those likely to be resistant to dietary interventions. There are also several genetic factors, which determine blood lipid profile, specifically several apoproteins like apoE and apo(a). These apoproteins, which are synthesized by polymorphic genes, present several isoforms that are relatively frequent among the population and can influence blood lipid profile interacting with specific exogenous factors like dietary habits.

Finally, a number of pathways have been identified that may be involved in muscle mass development, the ability to perform resistance physical activity or to affect pancreas function. Among these pathways those influenced by nutritional habits will be explored preferentially in adolescent from HELENA.

## 2.5. New foods

A lack of understanding about how to communicate dietary messages effectively is hindering the innovation of products that can contribute to consumer health, well-being and enhanced industrial competitiveness.<sup>[41]</sup> Food small medium enterprises (SMEs) play an important role in producing the great diversity of foods in Europe and the retail sector increasingly contributes to strengthening the links between production, processing and the consumer. Therefore, there is a need for a better understanding of consumer requirements and to provide a healthy, safe and high-quality food supply, in this case, specifically for adolescents. A multidisciplinary research effort bringing together a wide range of expertise is essential to address the whole problem surrounding nutrition and lifestyle of adolescents in Europe.

The glycemic index has proven to be a useful nutritional concept, providing new insights into the relationship between foods and chronic disease. Observational studies suggest that diets with a high glycemic index load are independently associated with increased risk of type 2 diabetes and cardiovascular diseases.<sup>[42]</sup> Some evidence suggests that a low glycemic index diet may also protect against obesity, colon cancer and breast cancer. In adolescents, the effects of an *ad libitum*, reduced-glycemic load diet have been compared with those of an energy-restricted, reduced-fat diet. At 12 month, the body mass index and the fat mass de-

creased more in the low-glycemic index diet compared with the reduced-fat diet. In *post hoc* analyses, the glycemic load was a significant predictor of treatment response among both groups, whereas dietary fat was not.<sup>[43]</sup>

Low-glycemic index diets influence body weight and resting energy expenditure independently of caloric intake. In a short crossover study,<sup>[44]</sup> it was compared a high-glycemic-index-energy-restricted diet with an isocaloric low-glycemic-index diet in moderately overweight young men and showed that resting energy expenditure declined by 10.5% on the high-glycemic-index diet compared with 4.6% on the low-glycemic-index diet.

## 2.6. Lifestyle education interventions

New and modern tools for lifestyle education in adolescents need to be developed focusing on this specific population and considering gender differences. Adolescents need a food culture based on foods to eat rather than foods to avoid, and an understanding of suitable weight-control measure.<sup>[45]</sup> Most studies dealing with nutritional status and physical activity of European adolescents conclude that nutritional interventions and interventions to enhance physical activity are strongly needed.<sup>[46]</sup> Computer-tailored nutrition and physical activity education is an innovative, promising and cost-effective tool to motivate people to make healthy dietary and physical activity changes. The available evidence indicates that computer tailored education is more effective in motivating people to make changes than general nutritional and physical activity education.<sup>[47]</sup> Until now most computer tailored nutrition programmes focus on one or a few aspects of the nutrition behaviour as fat intake and fruit and vegetable intake. Most programmes are aimed at adults.

The present project will develop and evaluate a computer-tailored intervention focusing on the usual eating habits and the physical activity of adolescents. Data of the cross sectional study will make it possible to validate the dietary assessment instrument necessary for the development of the computer tailored intervention. The “fuzzy logic” approach, not used until now in computer-tailored interventions, will make it possible to write an individualised advice to optimise the usual eating habits of adolescents. Despite of the encouraging results of a health and nutrition education programme for changing certain chronic disease risk factors, observed after a 6-yr school-based intervention,<sup>[48]</sup> there is no experience in the assessment of the efficacy of such computer tool in Europe. The majority of trials have been conducted in the United States, and there are doubts if these can be extrapolated to the great diversity of the European area.<sup>[49]</sup>

In conclusion, HELENA will provide, for the first time in Europe, harmonised and comparable data among male and female

**Table II.** Cities (alphabetic order) where the epidemiological studies are carried out.

	HELENA-CSS	HELENA-LSEI	HELENA-COMS
Athens (Greece)	×	×	×
Birmingham (UK)	×*	-	-
Dortmund (Germany)	×	×	-
Ghent (Belgium)	×	×	-
Heraklion (Greece)	×	×	-
Lille (France)	×	-	-
Madrid (Spain)	-	-	×
Naples (Italy)	-	-	×
Pecs (Hungary)	×	-	×
Rome (Italy)	×	-	-
Stockholm (Sweden)	×	×	-
Vienna (Austria)	×	×	-
Zaragoza (Spain)	×	-	-

\* Only food choice and preference analysis.

**CSS:** cross-sectional studies; **LSEI:** lifestyle education intervention; **COMS:** crossover multi-centre studies.

13 to 16.99 years old European adolescents about:

- food intake, micronutrients reference values, as well as attitudes towards nutrition, and the main determinants of the food choice and preference,
- physical activity and physical fitness,
- obesity prevalence and body composition,
- relationship between genetic markers and phenotypic characteristics,

Therefore, HELENA will taking advance of computer-based dietary assessment tools in developing, a computer-tailored lifestyle education intervention, adapted to male and female European adolescents.

### 3. Work plan

The HELENA proposal is a STREP (Specific Targeted Research Project) designed taking into account the prescriptions of the 6th Framework Programme of the European Commission and the experience from the HELENA partners have integrated in specific research areas of the proposal.

The work has been broken down according to types of activities: Research, technological development and innovation related activities; demonstration activities; project management activities; and quality control activities.

The main part of the work plan is devoted to research, technological development and innovation related activities, focused on four main studies (see below): cross-sectional studies (CSS), behavioural studies and development of new foods (BEFO), lifestyle education intervention (LSEI), and crossover multi-centre studies

(COMS). Aiming to optimise the resources and the duration of the project, all these studies will run in parallel during 36 months. The project started in May 2005 and will be finished in May 2008. The studies are performing across 13 European cities included in one or more studies as it is represented in table II.

CSS/LSEI studies started in May 2005 in developing collection data support form as: Case Report Form, Questionnaires, providing a manual of operation describing management of each test/exam and blood samples, a protocol to submit to Ethical Review Committee's and other regulatory authority/organisation in order to achieve a high level of quality assurance relating to ethical issues. We followed the good clinical practices (GCP) described at the International Conference on Harmonisation (ICH), which we adapted to the national and local situations of each of the 11 participating cities in 10 European countries (see table II). Inclusion of adolescents started in February to May 2006 by a pilot study (about 160 adolescents) and the field work began in October 2006.

COMS studies started in May 2007 and are on going.

BEFO part of the project began in May 2005 and several new food are now available to be tested.

#### 3.1. Cross-sectional study (CSS)

Nutrition, body composition, physical activity and fitness, biochemical parameters and genetics are going to be investigated by a multi-centre, cross-sectional study design in 10 cities in Europe (table II). Using a school-based multiple steps stratified random sampling selection, and doing a strict standardization of the fieldwork, centralised of blood samples and questionnaires, as well as the evaluation of the data. Then, at least 3000 (300 per

city) adolescents (male and female) aged 13.0 to 16.99 will be involved in the investigation. Before to start de complete CSS, a pilot study will be carried out with the same plan, on a small scale in each country (table II) in order to check every step of the procedure, from sampling to data processing, to anticipate any feasibility problems resulting from the implementation on the field.

Apart from the specific aims of the study, a major goal is the standardization and harmonization of all the measures as the only way to exclude from the beginning the huge amount of confusing variables that appear when data from isolated studies are compared.

### 3.1.1. Measurements

The study includes several measurements.

- *Subject description* comprising personal data and anamnesis by questionnaire.
- *Maturation status* will be evaluated following Tanner staging protocol.<sup>[50]</sup>
- *Nutrition knowledge* will be measured Nutrition knowledge will be measured by the Nutrition Knowledge Test for Children and Adolescents (NKT-C).<sup>[51]</sup>
- *Eating attitudes* will be assessed by the Eating Behaviour and Weight Problems Inventory for Children (EWI-C).<sup>[51]</sup>
- *Food choice and preference.* Attitudes and behaviours of adolescents regarding food choice and preference will be quantified using a questionnaire based on information obtained in the focus group discussions conducted in five countries (Belgium, Hungary, Spain, Sweden and the UK). The questionnaire will include the key issues identified in the focus groups and will be administered in the classroom.
- *Food and nutrient intake.* Dietary and nutrient intake data will be obtained from a validated 24-h recall questionnaire, following the European Food Consumption Survey Method (EFCOSUM).<sup>[52,53]</sup>
- *Body composition.* There are several methods that can be used for evaluating body composition in adolescents. The most adequate for epidemiological studies with high number of subjects are anthropometry and bioelectrical impedance analysis (BIA).<sup>[54]</sup> However, also dual energy X-ray absorptiometry (DXA) and (Bod-Pod) technology will be used in this study in a subgroup of adolescents, in order to developed new equations for body fat (%) assessment in adolescents.
- *Physical activity and physical fitness.* A draft protocol for assessing physical activity will be developed based on existing methods such as the Health Behaviour in School-aged Children (HBSC) questionnaire and the International Physical Activity Questionnaire (IPAQ).<sup>[20]</sup> While the assessment of the physical fitness includes six tests included in the EUROFIT

test battery, validated and standardized by the European Council and several others that have been proven to be of a specific value.<sup>[55]</sup>

- *Biochemical parameters.* We will analyse those blood parameters that are at risk in the adolescent period or for further nutrition related disorders, as lipid profile, iron and vitamin status using specific techniques.
- *Genetics.* In addition to the traditional collection of clinical and biological information, a blood sampling for DNA extraction purposes from white blood cells will be taken. DNA extraction will be performed at the Lille's Genopole using various molecular biological techniques. Each DNA sample will be blinded to the investigators, aliquoted and made available for the participants for polymorphism analyses.

### 3.1.2. Data collection and analysis

With the aim of satisfy the harmonization and standardization a manual of operations to be followed by all the participating centres will be prepared. This manual will include the whole set of data collection methodology and a detailed description of all instruments for the core study data. A standardised software format for data-input, tailored to the different types of data that will be collected will be developed. These standard formats will feature a unique label for each item to be collected across all participating centres. These labels will be described in a codebook including all the codes and their unambiguous interpretation. A protocol for data-entry will include specifications aimed at minimising coding errors. Training sessions with all the technical staff involved in the fieldwork in the different centres, aiming at harmonize data collection, will be organised. Standardised protocols for the quality control and first round of data cleaning to be applied by all participating centres will be prepared. It will be designed a central database architecture for centralisation of data from the different participating centres and for the different aspects of the study. A central protocol for anonymous data cross-linkage will be created. A central analytical plan will be developed. It will also provide the analyses and reports of the HELENA study data in line with existing public health databases on EU level.

### 3.2. Behavioural studies and development of new foods (BEFO)

In order to aid the understanding of behaviours and attitudes regarding food choice and preference among European adolescents, a series of focus groups will be conducted in five countries (Belgium, Hungary, Spain, Sweden and the UK). Thirty-four groups will be undertaken, which will include the views of approximately 300 adolescents. Information from these groups will

be reported both on a country-by-country basis and as a summary report investigating similarities and differences between countries. Key issues will be identified and incorporated into a food choice and preference questionnaire to be undertaken within the cross sectional study. The group work will also provide valuable information guiding the recommendations for the lifestyle education programme. In addition, the results from this work will help determine the products and ingredients to be used for the development of new improved foods targeted specifically at the adolescent population.

Types and sources of health promoting ingredients will be identified, mainly by contacting ingredient suppliers. On the basis of the above mentioned information, and on information regarding attitudes and habits of adolescent consumers and also considering the special competences and interests of the participating companies the types of food products to develop and the modification/improvement of their nutritional properties will be decided. Plans will be prepared for the product development experiments and it will be decided what methods, *e.g.* chemical, physical, microbiological and sensory analysis, to use to measure the quality characteristics of the new products. Health promoting products will be developed.

Product development experiments will initially be carried out on a laboratory scale in order to define preliminary conditions for the pilot plant trials. Methods for experimental design will be used to plan pilot-plant scale experiments, which will aim at finding optimal process settings. Participating companies according to those plans will manufacture different versions of the different types of products. The relationships between processing conditions and quality characteristics will be determined by use of different statistical methods. A few, typically 3, versions of each type of product will be chosen and manufactured for the acceptability study. The acceptability of different versions, typically 3, of the selected food products to adolescent consumers will be studied in the following countries: Belgium, Hungary, Spain, Sweden and UK. For these studies, representative samples of 120 consumers in the age 13–16 years will be recruited. Acceptability data will be analysed and a report including EU recommendations will be prepared.

### 3.3. Lifestyle education intervention (LSEI)

A nutrition and physical activity assessment instrument will be developed and culturally adapted. It will be evaluated if exactly the same dietary assessment and physical activity assessment instrument can be used for the cross sectional study and for the tailored educational intervention. The instrument will be developed for use on the Internet; however, for the pilot studies a CD-ROM version will be used. The aim of the instrument is to assess the usual eating habits and the physical activity level of adolescents.

Food frequency questionnaires and questionnaires concerning the physical activity will form the bases of the instrument; these questionnaires will be completed with information on age, sex, length, weight, maturational development, motivation to change the diet and physical activity and determinants of behavioural change.

Software will be developed for the immediate individualised counselling of the adolescents. The software will make it possible to give individual tailored advice on enhancing their eating habits and physical activity.

In the six centres taking part in the intervention study (table II) the first 80 adolescents taking part in the cross sectional study will be used for the validation of the assessment instrument. These adolescents will fill in the questionnaires for the cross sectional study, will provide a blood sample for the biomarkers and will fill in the diet and nutrition assessment instrument used as the bases for the tailored intervention (the physical activity instrument is already validated in previous studies). The two sets of data will be compared to assess the content validity of the diet assessment instrument. Main parameters for the validation study are energy intake, fat intake (saturated and non-saturated fat), fibre intake and calcium intake. If needed, the instrument will be adapted and further tested.

In those centres taking part in the intervention study, at least 200 adolescents taking part in the cross sectional study will be randomly assigned to an intervention group and a control group. At the time of the cross sectional study (T1) the adolescents in the intervention group will also receive the computer-tailored nutrition and physical activity educational intervention. The control group (will receive the cross sectional study questionnaires + the diet assessment instrument and the questionnaire on physical activity. One week (T2) after the intervention and three months (T3) later the intervention group and the control group will fill in again the diet assessment instrument and the questionnaire on physical activity + the knowledge test and determinants questions.

Main outcome parameters for the impact study are: energy intake; fat intake (saturated and non saturated fat); fibre intake and calcium intake.

### 3.4. Crossover multi-centre studies (COMS)

Slightly overweight adolescents of both sexes will be selected for the study. Exclusion criteria will be diabetes mellitus, severe hyperlipidemia or other metabolic disorders. In the first phase, twenty adolescents will participate in the postprandial study. Glycemic index will be determined according to standard procedures for snacks having different glycemic index, *i.e.* containing available carbohydrates with different absorption rate. Sensory properties will also be evaluated. Subsequently, satiety

and voluntary energy intake will be measured after the consumption of snacks with the same energy content but different with respect to their glycaemic index.

Twenty overweight adolescents will participate in the crossover experiment.<sup>[56]</sup> The study will consist of two 21-day dietary periods separated by a 10-day washout period. The two diets will contain the same amount of carbohydrate, but will have different glycaemic index thus defining a low glycaemic index diet and a high glycaemic index diet. Low glycaemic index snacks will be incorporated in the low glycaemic diet. At the end of each of the dietary period glycaemia, insulinemia and satiety will be evaluated throughout the day. Blood lipids and blood pressure will be determined in the postabsorptive state.

#### 4. Dissemination plan

Dissemination activities aim to promote transnational dissemination and exploitation of the HELENA results, especially among SMEs. The promotion would be either in a diffusion basis (final consumer, press, farmers, etc.), as well as in a technical basis (specialised producers, SMEs, food sector professionals, etc.), and in a scientific environment. Effort is allocated through the work plan to disseminate good practices, knowledge, industrial new healthy products and their benefits for the adolescent consumers.

The official **HELENA web site**<sup>[57]</sup> is on line from May 1st 2005, and will have a great charge in the dissemination. This public web site will provide an information resource for the work within the project (including details of partners, objectives, work areas, results, internal results and working papers, public deliverables, etc.) and for related fields of work (links to other projects, services, collaborative efforts, etc., relevant to each part of the project). This will also include a section targeting adolescent consumers, trying to introduce attractive claims.

A major theme of this project is to describe current best practice or usual practice in each area investigated. Documents created in the project will be made public and given permanent URLs linked from the project website, and promoted to the wider community using mailing lists and discussion forum. Reporting on best practice means that the partners will undertake to write up (or where appropriate encourage others to write up) areas of the technology which seem neglected or that will be useful to this project or any other project working in this area.

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