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The European Youth Heart Study—Cardiovascular Disease Risk Factors in Children: Rationale, Aims, Study Design, and Validation of Methods

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Background: The aim of the European Youth Heart Study (EYHS) is to establish the nature, strength, and interactions between personal, environmental, and lifestyle influences on cardiovascular disease (CVD) risk factors in European children. *Methods:* The EYHS is an international study measuring CVD risk factors, and their associated influences, in children. Relationships between these independent factors and risk of disease will inform the design of CVD interventions in children. A minimum of 1000 boys and girls ages 9 and 15 y were recruited from four European countries—Denmark, Estonia, Norway, and Portugal. Variables measured included physical, biochemical, lifestyle, psychosocial, and sociodemographic data. *Results:* Of the 5664 children invited

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to participate, 4169 (74%) accepted. Response rates for most individual tests were moderate to high. All test protocols were well received by the children. *Conclusions:* EYHS protocols are valid, reliable, acceptable to children, and feasible for use in large, field-based studies.

Key Words: lifestyle, diet, physical activity, fitness, psychosocial factors, methods, validity

The European Youth Heart Study (EYHS) addresses the issue of cardiovascular disease (CVD) risk factors in children. The study has three distinct features. First, it investigates a wide range of factors that might influence the progression of CVD risk factors in children. Second, the study utilizes both new technology and laboratory-based methods adapted for field use. Third, the study is an international multi-center study, necessitating the incorporation of comprehensive quality control procedures.

Strong evidence exists supporting the hypothesis that many adult disease processes—including CVD—begin in childhood. For example, the Bogalusa heart study reported that fatty streaks and even atheromatous plaques are present in young children.[1] Both the Bogalusa and Muscatine studies have shown that CVD risk factor levels in childhood predict the amount of atheroma found at post mortem and radiographically visible coronary calcification.[1–3] Further, there is evidence that major physiological risk factors for CVD persist (track) from childhood to adulthood.[4]

It is becoming increasingly accepted that an accumulation of adverse environmental exposures, combined with the effects of psychological factors throughout life, plays a major role in eventual CVD morbidity and mortality.[5] While individual factors, for example, diet, exercise, sexual behavior, and drug abuse will have important effects, other factors lying outside the individual's control can also influence health. Such factors include poverty, social factors, employment, housing, education, and the physical environment. Whereas not all of these might apply to children, some of them certainly do, emphasizing the importance of the childhood period. Figure 1 describes a hypothetical model of how CVD risk factors and associated influences might be related.

Adult susceptibility to ill health from chronic disease is related to genetic factors and also to exposure to risk throughout the lifecourse. The main risk exposures could be either sociodemographic (e.g., low socioeconomic position) or lifestyle related (e.g., smoking, diet, sedentary living) and are likely to operate throughout the lifecourse. The childhood period might therefore be an important focus for CVD prevention programs. To intervene effectively, however, it is necessary to know which predisposing factors operate and how these factors vary in nature and degree of influence within subgroups of children. Children remain a relatively unstudied group with respect to CVD risk and its pre-disposing factors and therefore EYHS seeks to generate data to explain further the etiology of CVD risk factors in children.

The primary objective of the EYHS is to study the nature, strength, and interactions between personal, environmental, and lifestyle influences on CVD risk factors in a large population of children of differing age, sex, culture, and ethnicity. The secondary objective is to assess the age-, sex-, and time-specific prevalence of personal, environmental, lifestyle, and physiological risk factors.

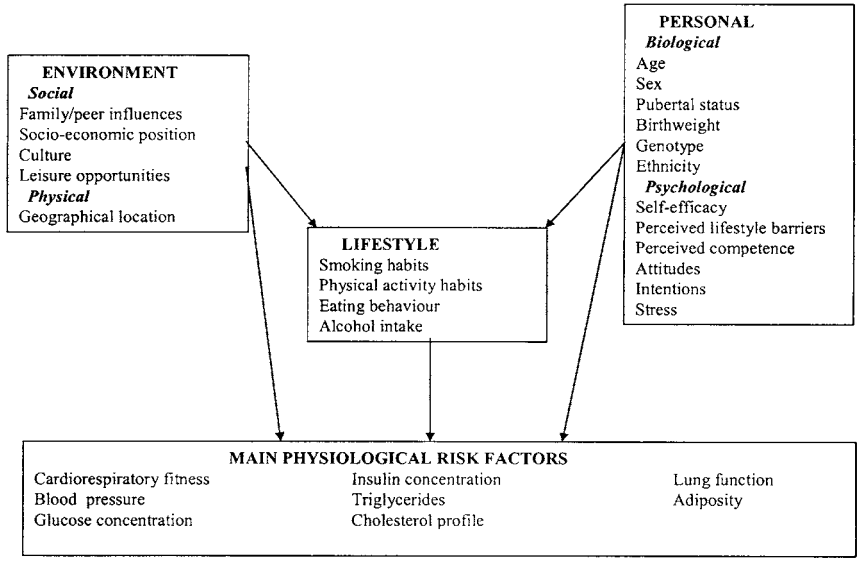

Figure 1 — Schematic representation of candidate CVD risk factors and associated influences in children

It was hypothesized that children who experience more positive personal, environmental, and lifestyle circumstances exhibit lower levels of CVD risk factors than children who experience more negative circumstances.

Study Design and Methods

Design

Overview. An international, multi-center design was chosen for a number of reasons. First, the participation of different countries facilitates investigation of the influence of ethnic and cultural differences. Second, multiple study centers enable a larger sample size to be obtained within a defined time period. This in turn endows greater ability to detect small but possibly critical differences and relationships. Third, gathering data from multiple centers leads to a greater heterogeneity within the final sample, thus increasing the variability and range of key exposures and outcomes. A multi-center design also introduces limitations, however. These include increased cost, the need for an effective and efficient management system, and the need for comprehensive and effective quality control procedures.

The EYHS is designed as a cross-sectional survey of children conducted in four countries—Denmark (Odense), Estonia (Tartu), Norway (Oslo), and Portugal (Madeira). A minimum of 1000 boys and girls ages 9 and 15 y were recruited from each study location. Samples of children were drawn in a similar fashion within each study location. At each study location, a sampling frame of schools using official

lists was compiled. Schools were stratified by the sociodemographic characteristics of their local areas. Each school was given a weighting according to the number of children enrolled and a minimum of 20 schools were randomly selected using probability proportional to school size.[6] Children of appropriate ages were sampled randomly (random number tables) within schools using the school register.

School-based testing was used, with a mobile test team. Where local conditions dictated, however, children were transported to a central testing venue. Because of the large number of variables measured and the sophisticated nature of testing, approximately 10 children per day could be tested. In a second phase of the study, 6-y follow-up data will be collected, which will incorporate repeat measures on existing children and the addition of a new cohort of 9 year-olds, thus extending the study to a mixed longitudinal design. This will facilitate the isolation of age, cohort, and time-of-measurement effects, and provide important data for monitoring trends in risk factors and their associated influences over time.

Sample Sizes. Sample sizes were estimated separately for three types of analysis: a) assessment of the prevalence of risk factors with a sampling error of 2%; $1\text{-}\beta = 0.80$; and 2-tailed $\alpha = 0.05$; b) assessment of pre-specified target differences in risk factors between subgroups of 5 mmHg in blood pressure, 0.5 mmol/L in total cholesterol, 0.2 mmol/L in HDL, and 0.5 $kcal \cdot kg^{-1} \cdot d^{-1}$ of energy expenditure (physical activity), and c) the need to achieve minimum cell-sizes for multivariable analyses.

From the above considerations, it was established that at each study location 200 children per age/sex group (total $n = 800$) would give an acceptable level of power for most projected analyses. Because cluster (school) sampling has been used, a design effect of 1.25 was incorporated, giving a final sample size of 250 children per age and sex group. Therefore, within each country, a sample size of 1000 children was recruited to achieve the aims of this study. Nonresponse has been estimated at a maximum of 20%, and oversampling at this level was incorporated into the sampling design.

Methods

Testing was conducted throughout the majority of the school year to account for seasonal effects on the assessed parameters, and daily order of testing was standardized to minimize between-test interaction. Many of the tests and protocols utilized in the EYHS are standard procedures and have been well validated for children of this age. Where this is not the case, validation studies were performed. The EYHS design minimizes testing error within the constraints of finance, human and physical resources, and testing time available.

Physical Measures. Height and weight were assessed by standard anthropometric procedures.[7] Pubertal status was assessed according to Tanner stages.[8] Pubertal stage was assessed by a researcher of the same gender as the child, using brief observation. In both age and gender groups, pubertal stage was identified using a 5-point scale of pictures—girls according to breast development and pubic hair growth and boys according to genital development and pubic hair growth.

Skinfold thicknesses were measured with Harpenden fat calipers at the biceps, triceps, subscapular, suprailiac, and medial calf sites.[9] Two measurements were taken at each position, and if the difference between the two measurements was

more than 2 mm, a third measurement was taken. The mean value of the two closest measurements was used for analysis. The sum of skinfolds has been shown to correlate highly with DEXA-measured percentage body fat.[10] Waist circumference was measured twice with a metal anthropometric tape midway between the lower rib margin and the iliac crest, at the end of gentle expiration. The mean value of the two measurements was used for analysis.

Blood Pressure. Resting systolic and diastolic blood pressure were measured in the sitting position using the child's left arm, with a Dinamap adult/pediatric and neonatal vital signs monitor (model XL, Critikron, Inc., Tampa, FL). Measurements were taken using a standard pressure cuff of the correct size. The children were introduced to the monitor and allowed to sit quietly by themselves for at least 5 min prior to measurement. Five measurements were then taken at 2-min intervals. The mean of the last three measurements was used for analysis.

Blood Sampling and Analysis. Fasting (overnight) intravenous blood samples were taken at the start of the day from the antecubital vein, 1 h after application of an anesthetic cream (lidocaine/prilocaine; EMLA cream, AstraZeneca). Breakfast was provided immediately after blood sampling. Blood samples were aliquoted and separated within 30 min of venipuncture and samples were stored at –80°C until transported to a WHO-certified laboratory for analysis.

Total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were measured by enzymatic methods. Glucose was analyzed by the hexokinase method. Each of these was measured on an Olympus autoanalyser (model AU600, Olympus Diagnostica GmbH, Hamburg, Germany). Insulin was measured by enzyme immunoassay (microtitre plate format; Dako Diagnostics Ltd., Ely, England). The HDL-C:TC ratio was subsequently calculated, and low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedwald equation.[11] Spun white blood cells were stored at –80°C for DNA extraction.

Genetic Analyses. The European Youth Heart Study offers a unique opportunity to investigate the independent and combined roles of genotype and environment in the etiology of CVD risk. The proposed approach for studying main genetic effects and gene–environment interactions in the EYHS is based on an approach, which relies on a priori biological knowledge, derived from functional evidence or previous epidemiological findings to select suitable candidate genes.

Food Intake. Dietary intake was assessed using a 24-h recall face-to-face interview supported by a parent-assisted food record. This method provides valid estimates of children's food intake for the purpose of group comparison.[12] The advantages of the 24-h recall are that the burden on the child to complete the food-intake interview is relatively minor, and that reading or writing skills are not required. Disadvantages are that the subject could have difficulty remembering what and how much they ate during the last 24-h, and that a single recall represents only a snapshot of a child's usual intake as the result of day-to-day variations. This method has been found, however, to be a suitable instrument for cross-sectional measures of dietary intake among children from the age of 8 y.[13]

To aid in estimating food amounts during the interview, different-sized drinking glasses, plates, spoons, etc. were shown to the children together with pictures of different portion sizes of the most common foods and food groups. The interview

lasted 20 to 30 min, and the quality of each interview was rated on a scale of 1 (very satisfactory) to 5 (very unsatisfactory) by the interviewer. Interviews rated 4 or 5 were noted for potential future exclusion from analyses.

Physical Activity. The MTI accelerometer (model 7164; former Computer Science Applications) was chosen for use in this study. The MTI accelerometer is an electronic motion sensor comprising a single plane (vertical) accelerometer. The monitors are small (4.5 × 3.5 × 1.0 cm) and light (about 43 g) and are worn in an elastic belt around the waist. Verbal and written instructions were given to both the children and their parents regarding its use. All children were instructed to wear the monitor continuously during the day, except when doing water-based activities. The monitors were pre-programmed to start recording at 0500 on the first day of measurement. The activity counts detected by the accelerometer were averaged and stored every 60 s for 4 consecutive days, including 2 weekdays and 2 weekend days. After receiving accelerometers back from children the collected data were downloaded to a computer and derivative variables were calculated using customized software. Over 100 derivative variables were calculated comprising measures of all relevant dimensions of children's activity.

The MTI accelerometer has been validated in both children and adolescents using heart rate telemetry,[14] indirect calorimetry,[15,16] observational techniques,[17] and doubly labeled water.[18] In the latter study, accelerometer counts were significantly related to energy expenditure as measured by total energy expenditure ($r = 0.39$, $P < 0.05$), activity energy expenditure ($r = 0.54$, $P < 0.01$), and physical activity level ($r = 0.58$, $P < 0.01$). Furthermore, in a mechanical setting, the MTI accelerometer demonstrates good intra-instrument reliability (coefficient of variation 4.4%) and reasonable inter-instrument reliability (coefficient of variation 5%) within the "normal" range of human locomotion.[19-21]

Quantification of acceleration (mechanical validity) is, however, affected by increasingly greater filtering at higher movement frequencies but this has only minor implications in vivo for between-subject reliability. During walking and slow running, the MTI accelerometer provides a valid estimate of intensity. Because of the known biomechanical limitations of vertical accelerometry, the instrument does not discriminate between running speeds.[22,23] Although fast running is therefore underestimated by the MTI accelerometer, such activity is likely to constitute only a very small proportion of the average child's activity. Thus, the error arising from this is unlikely to compromise the precision of the total volume of habitual physical activity.[18]

With a behavior as complex and varied as physical activity, there are bound to be sources of residual error. We have identified these and they are summarized as follows:

- Reactivity: Wearing the accelerometer might modify the child's normal activity behavior.
- Loss of data: During swimming, contact sports, showering, and bathing the instruments must be removed. This activity is therefore not measured. Also, during cycling, which involves no vertical movement, the accelerometers do not register accurately.
- Underestimation of vigorous activity: To obtain 4 d of recordings, activity counts must be averaged over 1-min epochs in order not to exceed the

instrument's memory capacity. This level of averaging dilutes the child's vigorous activity[24] because activity is rarely sustained for as long as 1 min.[25]

It is important to be aware of these residual limitations in the interpretation of results. We believe that the measurement error potentially introduced, while being problematical, is not at the level whereby the validity of the primary variable—total volume of physical activity—is seriously compromised.

Cardiorespiratory Fitness. Cardiorespiratory fitness, defined as maximal power output per kilogram of body weight, was measured using a graded maximal exercise test on an electronically braked cycle ergometer (Monark Ergomedic 839, Vansbro, Sweden), according to a previously reported protocol.[26]

Two test protocols were used: a) for 9 year-olds, initial and incremental workloads were 20 W for children weighing less than 30 kg and 25 W for those weighing 30 kg or more; and b) for 15 year-olds, initial and incremental workloads were 40 W for girls and 50 W for boys.

Workload was programmed to increase after every 3 min. Heart rate (HR) was recorded continuously (Polar Vantage, Kempele, Finland) throughout the test, and the test continued until the subject was no longer able to continue. Criteria for a maximal effort were a) HR ≥185 or b) subjective observation from the researcher that the child could not continue (after vocal encouragement if necessary). If the child's pedaling rate dropped below 30 rpm, the child was considered to have stopped the test. The cycle ergometer was electronically calibrated once every test day and mechanically calibrated after being moved between schools.

Self-reported Variables. A computer-based child's questionnaire was used to gather information about children's smoking habits, alcohol intake, diet preferences, and various types of physical activity. Questions addressing psychosocial and environmental factors that influence children's physical activity were also asked. A computer-based questionnaire is feasible in this study because of the small number of children tested daily ($n < 10$).

A separate questionnaire was completed by the parents, based on the questionnaire used in the Northern Ireland Childhood Coronary Prevention Study.[27] Data collected separately from both parents included demographic data, self-reported health status, family income, level of education, ethnicity, level of CVD risk, family history of CVD, the child's birth weight, and breast-feeding practices. Socioeconomic position was assessed using parental education, occupation, and family income data.

Both questionnaires were translated into the relevant languages and back-translated to ensure accuracy. Discussions with researchers from each country ensured that the true meanings of questions were not lost during the process.

Statistical Analysis

Descriptive data will be reported using standard statistical measures of central tendency and variability. Differences between subgroups will be assessed using independent *t*-tests and analysis of variance. Relationships between independent predictor variables and CVD risk factors will be assessed by multivariable and

logistic regression. Structural equation modeling and multilevel modeling will be used to assess the influence of country of origin on identified relationships.

Ethical Issues

The protocols and procedures of the EYHS conform to the ethical guidelines on biomedical research of the World Medical Association's Declaration of Helsinki. The ethical procedures operating in each of the four countries were adhered to prior to the commencement of the study. The written consent of the child's parent or legal guardian was obtained. Written explanations of the study aims and the possible hazards, discomfort, and inconvenience of the test procedures were provided for both the parents and children.

Results

Response Rates

Of the 5664 children invited to participate in the study, 4169 (74%) actually took part. In a small number of children, gender was not reported, reducing the effective total to 4072 (72%). Response patterns were similar across age/gender groups. Tables 1A and 1B give detailed response rates for individual components of testing by country, age, and gender.

Validity of Cardiorespiratory Fitness Test

Within the EYHS, validity studies were undertaken using study-specific procedures and equipment. Separate validity studies were carried out on different children in Denmark and Norway. The studies used different cycle ergometers, but of the same make and model. In all other respects the protocols were the same.

In the first study, 21 nine-year-old children and 9 fifteen-year-old children participated. The protocol comprised two maximal tests on a cycle ergometer separated by a period of 2 d. In one test, maximal power output was measured following a standard protocol.[26] In the other test, using the same protocol, VO_{2max} was measured (in $mL^{-1} \times kg^{-1} \times min^{-1}$) by indirect calorimetry. Order of measurement was randomized between children. In both tests, heart rate was monitored by heart rate telemetry (Polar Vantage, Polar Electro, Kempele, Finland). In the power output test, the final workload achieved (in watts), time at the final workload (in seconds) and final (maximal) heart rate were recorded. Maximal power output was calculated for each child according to the formula:[26]

$$[W1] + W2 * T/180$$

where W1 = workload (in watts) at last fully complete stage, W2 = workload increment (in watts) at final incomplete stage, and T = time (in seconds) at final incomplete stage.

Correlations between maximal power output and VO_{2max} were $r = 0.92$ ($P < 0.001$) for 9-year-olds and $r = 0.96$ ($P < 0.001$) for 15-year-olds. Linear regression was performed, with measured VO_{2max} as the dependent variable and maximal power output, gender, and age as the independent variables. Maximal power output

Table 1A Response Rates for 9-Year-Olds, by Gender, Within Elements of Testing in the European Youth Heart Study

		Physical tests		C-R fitness		Blood sample		Physical activity		Question-naire	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Denmark	Boys	280	100	259	93	247	88	194	69	275	98
	Girls	310	100	281	91	278	90	223	72	303	98
Portugal	Boys	274	100	273	100	250	98	238	87	160	58
	Girls	255	100	245	96	250	97	219	86	173	68
Estonia	Boys	278	100	276	99	270	97	177	64	272	98
	Girls	303	100	300	99	295	97	177	58	294	97
Norway	Boys	210	100	195	93	33	16	193	92	200	95
	Girls	196	100	184	94	63	32	187	95	190	97
	All boys	1042	100	1003	96	800	77	802	77	907	87
	All girls	1064	100	1010	95	886	83	806	76	960	90
	All boys and girls	2106	100	2013	96	1686	80	1608	76	1867	89

Table 1B Response Rates for 15-Year-Olds, by Gender, Within Elements of Testing in the European Youth Heart Study

		Physical tests		C-R fitness		Blood sample		Physical activity		Question-naire	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Denmark	Boys	206	100	197	93	200	97	136	66	202	98
	Girls	224	100	202	91	214	96	167	75	219	98
Portugal	Boys	294	100	294	100	250	85	119	40	289	98
	Girls	300	100	299	96	250	83	123	41	295	98
Estonia	Boys	260	100	258	99	260	100	129	50	251	97
	Girls	333	100	333	99	333	100	200	60	326	98
Norway	Boys	167	100	157	93	134	80	111	66	164	98
	Girls	182	100	170	94	153	84	146	80	180	99
	All boys	927	100	906	96	844	91	495	53	906	9
	All girls	1039	100	1004	95	950	91	636	61	1020	98
	All boys and girls	1966	100	1910	96	1794	91	1131	58	1926	98

($watt_{max}$) was the only significant predictor that entered the model ($R^2 > 0.9$): 9-year-olds $VO_{2max} = (12.44 \times watt_{max} + 250)$; 15-year-olds $VO_{2max} = (11.87 \times watt_{max} + 365)$. The standard error of estimation for predicted VO_{2max} was 2.5%.

In the second study, 21 nine-year-old children participated. The protocol was identical to Study 1, but only one test was performed. During this single test, maximal power output and VO_{2max} were measured concurrently. The correlation between maximal power output and VO_{2max} was $r = 0.93$ ($P < 0.001$). The standard error of estimation for predicted VO_{2max} was 4.8%. Taken together, these data suggest a high validity of the maximal power output test of cardiorespiratory fitness.

Reliability of Fitness and Anthropometry

During EYHS testing, both inter- and intra-tester reliability data were recorded for selected tests where the skill of the tester might influence the results. Tests selected were cardiorespiratory fitness, skinfold measurements, and waist/hip circumference measurements. The majority of reliability tests were conducted at all four study locations, and we report a summary of the combined data in Table 2.

These data suggest that both inter- and intra-tester variability are within acceptable limits and that it is unlikely that significant between-country testing errors have been introduced.

Table 2 Reliability Data for Selected EYHS Tests

Test	Measurement	Reliability (r)
Cardiorespiratory fitness – intra-tester	Time at last workload	0.809
	HR_{max}	0.785
Cardiorespiratory fitness – inter-tester	Test duration	0.609
	HR_{max}	0.945
Skinfolds – intra-tester	Biceps	0.956 – 0.980
	Triceps	0.921 – 0.989
	Subscapular	0.915 – 0.996
	Suprailiac	0.957 – 0.991
	Medial calf	0.950 – 0.991
Skinfolds – inter-tester	Biceps	0.902 – 0.998
	Triceps	0.835 – 0.998
	Subscapular	0.953 – 0.998
	Suprailiac	0.992 – 0.998
	Medial calf	0.995 – 0.999
Waist:hip ratio – intra-tester		0.910 – 0.999
Waist:hip ratio – inter-tester		0.994 – 0.998

Note. All figures are Pearson correlations (r).

Table 3 Laboratory Quality Control Data

Measurement	Between batch coefficient of variation
Total cholesterol (TC)	0.9% (at 7.52 mmol/L)
High-density lipoprotein cholesterol (HDL-C)	1.1% (at 1.88 mmol/L)
Triacylglycerol (TG)	1.0% (at 4.04 mmol/L)
Glucose	1.2% (at 3.26 and 14.67 mmol/L)
Insulin	6.9% (at 15.9 mIU/L)
	5.9% (at 51.3 mIU.L)

Note. Figures are precision data [coefficient of variation (%) between batches].

Biochemistry

Precision data for laboratory procedures are detailed in Table 3.

Quality Control

Comparability of data between study locations is fundamental to the success of a large, multi-center study. In studies of this type, intra- and inter-center systematic or random errors in data collection can occur. It is therefore essential to establish and maintain quality control procedures to ensure standard methods of data collection, treatment of samples, and handling of data. Also, a single laboratory should be used for all biochemical analyses.

Quality control procedures within the EYHS include monitoring of test administration and data collection at study centers, standardization of all research protocols and equipment, standardization of training of new researchers and consistent organization and handling of data within countries.

Specific EYHS quality control procedures include:

- pre-study training programs (5 d) for all researchers;
- researcher training by attachment to a team in an existing study center;
- performance monitoring and data-logging in each center;
- quality control visits by a researcher external to the study team; a minimum of 3 visits are made during the year of data collection;
- a detailed test protocols manual;
- standardization of all equipment and calibration procedures;
- a single, accredited laboratory, used for all biochemical analyses;
- wherever possible, measurements are taken by the same researcher (where this is not possible, researchers are trained in 2 or 3 methods and inter-tester reliability scores assessed"; and

- within the restrictions of working in a field-based setting, the order of testing and environment is standardized with respect to the physical and psychological effects of venipuncture, blood pressure assessment, the need for breakfast after an overnight fast, and cardiorespiratory fitness testing.

Discussion

This article provides the background and justification for the design and protocols of the EYHS. The EYHS design and methods have yielded precise measures, using sophisticated measurement techniques, in a large group of children. The methods have been shown to be valid, reliable, and appropriate for children and field-based testing situations. The balance achieved between precision, cost, acceptability to children, and ethical constraints was shown to be appropriate. Accelerometer output has been shown to be a valid estimate of both volume and intensity of physical activity. Critically, this method captures and records the great majority of movements made by children.

The cycle ergometer test accurately reflects cardiorespiratory fitness assessed by direct measurement of respiratory gas exchange.

We have established a high level of inter- and intra-tester reliability in key tests and have ascertained that laboratory biochemical analyses have acceptable between-batch variability.

Quality control procedures were effective and detected a high level of consistency in test administration between sites.

The purpose of this article has been to demonstrate that a high level of measurement precision can be achieved within the constraints of cost, ethical demands, and logistics in a large, multi-center epidemiological study. The main design issues faced by the EYHS design team have been highlighted together with the steps taken to manage potential weaknesses. As is the case for any epidemiological study, the final design is a compromise that has both strengths and weaknesses. It is important to clarify these and ensure that forthcoming data are interpreted within an appropriate scientific framework. The principal design strengths and weaknesses can be summarized as follows:

Strengths:

1. high precision of measurement, utilizing sophisticated techniques, in the key exposures and outcomes;
2. a multi-national design, enabling cross-cultural comparisons to be made and the relative importance of influencing factors to be assessed both within and between countries;
3. a multi-disciplinary approach, combining knowledge and expertise from complementary scientific fields;
4. a geographical distribution of countries covering both Northern and Southern Europe as well as an "emerging" country from Eastern Europe;
5. measurement of a comprehensive range of factors that potentially influence CVD risk, thus enabling an informed judgment of the more important factors and how they vary between populations and subgroups. The

wide range of measurements facilitates investigation of a broad range of potential confounders within individual analyses; and

6. a high level of statistical power.

Weaknesses:

1. generalizability is limited to the specific populations defined within each country. The samples are large, however, and the populations selected can be easily compared with other areas of the different countries; the costs and logistical problems of a larger population base would have been prohibitive;
2. despite rigorous intra- and inter-tester reliability checks made in controlled situations, differential application of study protocols in the field cannot be discounted; and
3. translation of questionnaires can never be perfect despite thorough quality control procedures. Change of meaning of questionnaire items is always a possibility.

In conclusion, the EYHS has demonstrated that it is possible to measure large numbers of children with technologically advanced methods. A wide range of potential influencing factors on childhood CVD risk factors have been measured and comprehensive quality control measures were applied across the four study sites. With this level of precision, the EYHS is well placed to provide further insight into key critical questions regarding the determinants of children's cardiovascular health and to what extent risk factors are prevalent.

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