CRELES Pre-1945 METHODOLOGY

The Costa Rican Longevity and Healthy Aging Study (CRELES, or Costa Rica Estudio de Longevidad y Envejecimiento Saludable) is a nationally representative longitudinal survey of health and lifecourse experiences of 2,827 Costa Ricans ages 60 and over in 2005. Baseline household interviews were conducted between November 2004 and September 2006, with 2-year follow-up interviews. The study was conducted by the University of Costa Rica's *Centro Centroamericano de Población* (CCP) in collaboration with the *Instituto de Investigaciones en Salud*, with the support of the Wellcome Trust (grant 072406). The Principal Investigator is Luis Rosero-Bixby, with Co-Principal Investigators Xinia Fernández (University of Costa Rica) and William H. Dow (University of California, Berkeley).

The sample was drawn from Costa Rican residents in the 2000 population census who were born in 1945 or before, with an over-sample of the oldest-old (ages 95 and over). The main study objective was to determine the length and quality of life, and its contributing factors in the elderly of Costa Rica. Vital statistics indicate that Costa Rica has an unusually high life expectancy for a middle-income country, even higher than that of the United States, but CRELES is the first nationally representative survey to investigate adult health levels in Costa Rica. CRELES public use data files contain information on a broad range of topics including self-reported physical health, psychological health, living conditions, health behaviors, health care utilization, social support, and socioeconomic status. Objective health indicators include anthropometrics, observed mobility, and biomarkers from fasting blood and overnight urine collection (such as cholesterol, glycosylated hemoglobin, C-reactive protein, cortisol, and other components of integrative allostatic load measures). Mortality events are tracked and conditions surrounding death are measured in a surviving family interview (longitudinal follow-up data are not yet publicly available).

Design of the sample

In the first stage of the design model, a random selection was made from the database of the Census of Population of the year 2000, totaling 9,600 individuals 55 years of age or older, after a stratification by five-year age groups that assures a sufficiently large number of observations for advanced ages. The sampling fraction in this selection varies between 1% for the ones born in 1941-1945 and 100% for the ones born before 1905. For the detailed longitudinal follow-up, including the baseline CRELES survey to which the present report refers, a sub-sample was selected consisting of 60 "Areas of Health" (from a total of 102 in the whole country) aggregated into sub-regions. The sample covers 59% of the national territory. The map in figure 1 shows the zones in the country included in CRELES and the location of the participants.

The sub-sampling for the longitudinal study originally included nearly 5,000 individuals from the census of 2000; of those, it was possible to locate and to interview 2,827. The non-interview rates are comprised of: 19% deceased by date of contact, 18% were not located in the field (due mainly to the lack of accurate addresses), 2% had changed residence, 2% declined to be interviewed and 2% of interviews were pending after several visits (that in fact are veiled rejections). Among the interviewees, 95% provided blood samples, 92% urine samples, 91%

were able to complete the anthropometry module and 25% required a "proxy" who could respond to the questionnaire.

The 20% non-interviewed due to change of residence or not-located is concentrated among the younger ages and it varies by urban and social condition. To correct this distortion and to take into account the different sample fractions by age, non-response weights (variable named "ponderador") were determined by age, sex, urban residence and two education groups (schooling less than 6 years and schooling 6 years or more). These weights allow the replication of the structure for sex, age, residence and education of the whole 2005 population of Costa Rica born in 1945 or before. The weights were normalized so they reproduce the sample size of 2,827. These weights varied from a minimum of 0.07 for men 95 years of age and older with low education, to a maximum of 3.85 for rural men age 60-64 with high education.

Figure 1. Map of the health areas of Costa Rica and the interviewees in CRELES

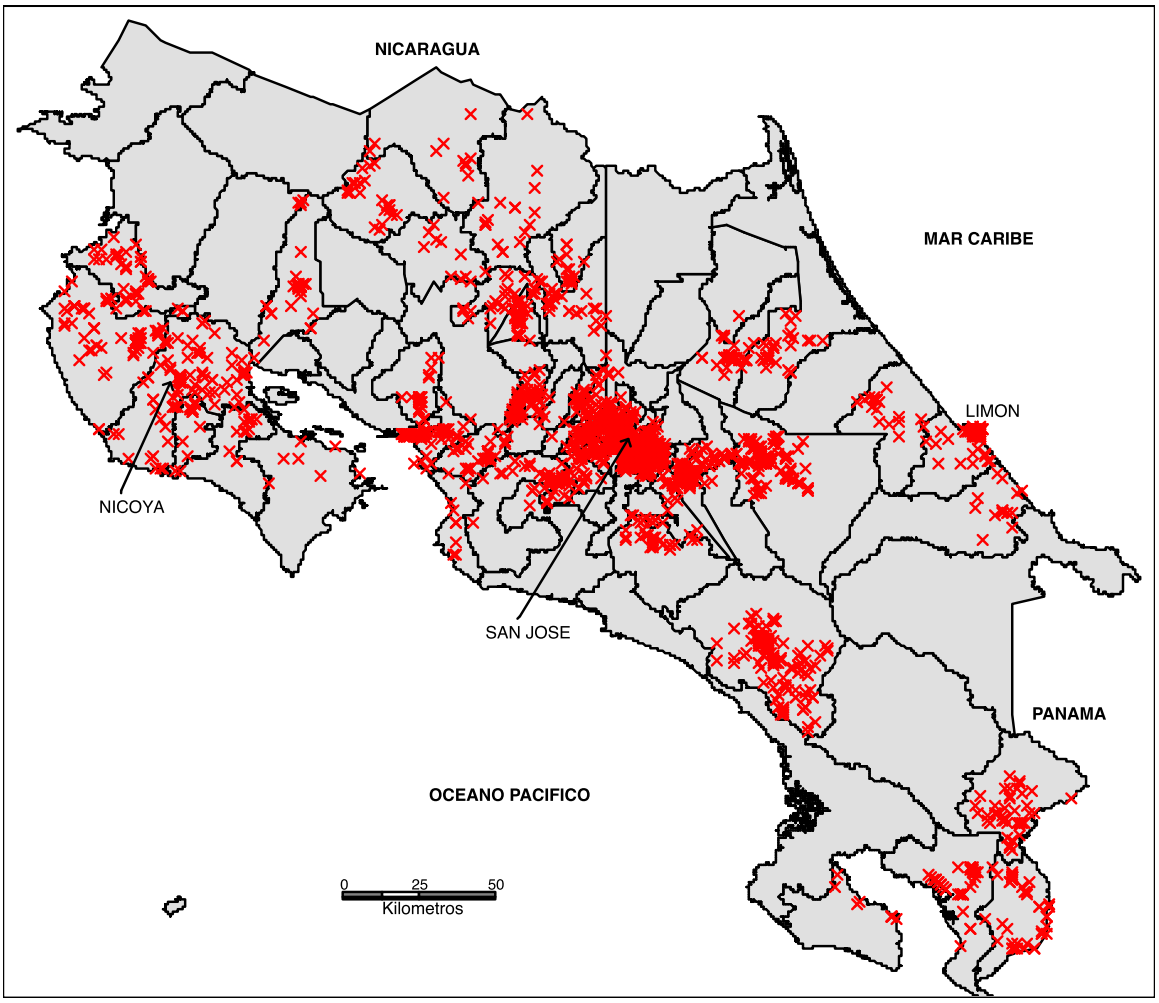

Table 1 compares selected CRELES data (unweighted and weighted) with the July 2005 Costa Rican Household Survey for Multiple Purposes (EHPM), an annual research effort conducted by the Costa Rican Institute of Statistics and Census (INEC) in a national sample representing more than 12,000 homes. A great deal of concordance was found between the two samples after weighting, suggesting the absence of biases that could have damaged the representativeness of the sample. Differences of up to 4 percentage points may well be due to the random selection since they are comparing two different samples of the same population. The only important difference is in the percentage of heads of households: 66% in CRELES compared to 60% in the survey of homes. This difference may be because in CRELES the informant is the an elderlyperson who may perceive themselves as household head, while in EHPM it can be another person with a different perception of who is the head of the household.

Table 1: Comparison between CRELES and 2005 EHPM
(Population of 60 and more years of age)

Indicator	CRELES		EHPM
	Observed	Weighted	2005
(N)	(2,827)	(2,827)	(3,834)
Sex ratio (%)	84.3	90.3	88.3
Average age	76.4	70.4	70.9
% secondary education or +	14.1	21.8	23.1
% in urban area	60.1	62.6	62.2
% in homes 1 or 2 members	41.0	38.5	39.6
% head of household	61.5	65.7	60.4
% married	49.7	60.3	57.2
% widowers	32.2	21.4	22.6
% economically active	20.3	29.4	26.2

EHPM = Costa Rican Household Survey for Multiple Purposes, source database in Internet

Field Work

The study, being longitudinal, consists of baseline data collection and two-year household follow-up surveys. At the time of this report, the 2005 baseline and 2007 follow-up waves have been completed, and the 2009 follow-up is in the field. This report presents the results of the baseline round, in which a structured interview was conducted, anthropometric measurements were taken, physical functioning tests were given and blood and urine samples were taken. All the data and specimens were gathered in the homes of the participants, generally in two visits. In the first visit the participants granted their informed consent by means of their signature, they answered a main questionnaire of around 90 minutes, they answered a short (about 10 minute) diet questionnaire, and blood pressure was measured twice during the survey. In the evening participants began fasting and began a 12-hour overnight urine catch. In the second visit to the participant's home early the following day, fasting blood samples were drawn, the urine was

collected, the anthropometric measurements were taken, and the physical functionality tests were performed (including hand-strength and maximum peak of breathing flow).

At the beginning of the main interview a cognitive evaluation was included that, together with the interviewer's criteria, established whether or not a "Proxy" informant for the participant was needed to help respond to the survey. Of the interviews, 25% were conducted with the help of a Proxy.

The interviews were conducted using handheld Palm computers or "Personal Digital Assistants" (PDAs), with a software application developed at the *Centro Centroamericano de Población* for this study. This included the main questionnaire which featured complex skip patterns and linkages. During CRELES pilot work the questionnaire answers were recorded in the Palm and on paper simultaneously by two interviewers, yielding an extremely high level of concordance (Hidalgo-grass, Rosero-Bixby et al. 2007). The Palm shows on the screen the text of each question that the interviewer should read and, when needed, it also provides instructions. The answers are usually registered in the Palm by pressing on the screen ("tapping") on the selected option from a list, but also it can be registered by entering numbers or text directly in "graffiti" or, if so choosing, into a virtual keyboard. The Palm controls the flow of the interview; that is to say, it skips questions and employs filters based on previous questions. It also executes verifications of consistency programmed ahead of time, and it automatically generates certain variables such as the date and time. The Palm does not allow entering of inconsistent data or date outside of the range, nor does it allow skips in the sequence of questions. The Palms also contained preloaded data on the location and identification of each sampled participant, thus reducing transcription and identification errors. Data were backup daily in the field and uploaded regularly to allow real-time data quality monitoring during fieldwork.

Also registered in the field were the data of the geographical coordinates of the place of each participant's residence, using GPS devices.

The fieldwork to gather the information of the first round was conducted from November 2004 to September 2006; that is to say, during a period of 22 months. A team of 5 interviewers with a supervisor located the subjects in the sample, completing the informed consent and conducted the main interview and diet interview. The team conducted an average of 7 interviews per day. Another team comprised of two phlebotomists and an interviewer from the first team obtained the fasting blood samples, gathered the overnight urine sample, took the anthropometrics measurements (body weight, height, knee height, abdominal circumference, hip circumference, calf circumference, arm circumference, tricipital and subescapular skin-fold measurements), and conducted physical functioning tests. The entire data collection was carried out in the home of the participants.

Physical, anthropometric and mobility and flexibility tests:

The following describes materials, equipment and methods used in the physical measurements: blood pressure, anthropometric measurements, flexibility and mobility tests, hand strength and peak breathing flow.

Blood pressure
It was measured on two occasions during the main interview, with an average interval time of 20 minutes between each; the measurement was taken using OMRON brand digital monitors with automatic inflating, model HEM-711AC, DuPont (precision: ± 3mmHg) that were calibrated periodically. The bracelet was adjusted to the thickness of the adult's arm.

Anthropometric measurements
These were taken by the interviewers who were trained and certified for this purpose, with updated training after a year of fieldwork. The measurements taken and the equipment used are the following:

Body weight: The scale used was the Life Source brand, M&D medical, model UC-321p; it was placed on even floor and without carpets, the measurement was carried out without shoes, nor objects of weight in the pockets of those participants with clothes.

Height: A Seca brand stadiometer was used to measure the height of the senior adults. The measurement was not taken if the person had major deformations of the spine.

Knee height: The measurement was carried out in the right leg whenever the interviewee did not have present a lesion on it. For this measurement an inclinometer was used to indicate the angle of 90 degrees, and then height was measured with a stadiometer manufactured by Shorr Productions (USES Knee-Height Caliper).

Abdominal measurement and Hip circumference: These measurements were made with the participants standing, in a semi-anatomical position (with the feet separated and the palm of the hands resting on the lateral thigh). The metric tapes used were the Dry and the Quick Medical brand tapes.

Calf circumference: The person was seated, with the right leg exposed.

Arm circumference: With the person seated or standing, the circumference was measured in the half point between the acromion (or posterior bone of the shoulder) and the olecranon or protruding bone of the elbow.

Tricipital and sub-scapular skin folds: The interviewer carried out the measurements using his or her thumbs and index fingers in order to make sure to only take the fatty tissue and not muscles or nerves. For this, a Lange Skinfold caliper, from Beta Technology Incorporated, was used.

Hand strength
Two measurements of hand strength were taken (the highest value is used in the analysis) with the interviewee standing with the dominant arm extended beside their body. A Creative Health Products Inc. dynamometer of was used, model T -18.

Peak breathing flow or lung function.

The maximum respiratory volume was measured in liters per minute with Mini-Wright type meters. These are homologated meters; that is to say, they have an acceptable correlation with the following: vitalograph, Sibelmed, PF - control, Clement Clark, Asses and TruZone. Three consecutive measurements of peak flow were taken.

Flexibility and mobility
The flexibility and mobility tests were carried out with the purpose of measuring (1) equilibrium and balance, (2) agility and (3) walking speed. The exercises that were carried out were the following:

Equilibrium and balance: To measure equilibrium and balance two tests conducted, (1) to remain standing with feet together for 10 seconds and (2) to stand up five times from a sitting position, with arms crossed on the chest.

Agility: The agility was measured beginning with the senior's ability to bend over, to pick up a pencil and to straighten out. If the interviewee could not do it in less than 30 seconds the test was not continued. The test was also not conducted if the senior had a cataract operation or another retinal procedure in the six weeks previous to the test.

Walking speed: To measure the senior's ability to rise off of a chair and walk, the interviewee was asked to rise from a chair and walk a distance of 3 meters in the manner that he normally does it; neither slower nor faster. The test was registered with a chronometer, noting the time in seconds that it took to carry out the test.

Laboratory procedures

The blood sample was obtained by venipuncture, normally during the second visit, the day after the main interview, with the participant fasting (for 14 hours). Three tubes of blood samples were collected: One with anticoagulant (VACUTAINER / EDTA) of 3-4 ml that was centrifuged later to separate the plasma of the cells and two tubes without anticoagulant with coagulum activator (VACUTAINER SST, 5 ml) for obtaining serum. In the laboratory a fraction of serum was separated in a conical tube type Eppendorf for total cholesterol tests, HDL, LDL, triglycerides, glucose, and serum creatine and 1 ml of complete blood in the tube EDTA for the analysis of glycosylated hemoglobin. These tubes were sent immediately to the participant laboratories for analysis. The remaining fractions of serum and plasma were aliquoted in red-top cryovials and they were stored in ultra-refrigeration (-140°C).

Urine was gathered in an ice box with a 12-hour sample of urine taken during the night, which was maintained cold with ice gel. After measuring the volume of the urine sample a fraction of it was stored in aliquot at -40°C. In the field, in areas far from San José the project had the cooperation of laboratory units of the CCSS that provided the space for the initial preparation of the blood and urine samples.

The biomarkers measured from blood and urine samples of the CRELES project were analyzed in various different laboratories. The clinical chemistry tests were conducted in the Clinical

Chemistry laboratory of the Department of Clinical Analysis of the College of Microbiology of the University of Costa Rica and in the clinical laboratory of the Hospital San Juan de Dios (HSJD). The measurement of the glycosylated hemoglobin was carried out in the above-mentioned laboratories using automated methods and also in the clinical laboratory of the Office of Health and Student Well-being of the University of Costa Rica (UCR).

Table 2 shows the techniques used in the different laboratory tests and quality control. The variability among laboratories was also evaluated with comparisons between batches of 20 samples taken at random and without the laboratories knowing that this comparison was being conducted (Méndez-Chacón, Rosero-Bixby et al 2007). In all the cases, high correlations were found among the series from different laboratories, but for some markers there were some systematic differences in means that were detected. To eliminate these differences one of the laboratories was established as the standard (Laboratory of the Hospital San Juan de Dios with automated technology) and the results of the others were adjusted with equations estimated by regression in the validation batches. Chart 3 presents the correction equations that were used.

The Laboratory of the Neuro-sciences Research Program (PIN) of UCR analyzed the urine samples to determine epinephrine and norepinephrine. The high yield liquid Chromatography (HPLC) technique was used. Modifications to the protocol of the kit of the commercial firm Bioanalytical Systems were carried out since it did not produce the expected results in the solid phase extraction. The modifications consisted of decreasing of the elusion speed (0.1ml/min) and the quantity of the collected eluent (0.5ml), also modified was the injection flow (1.5ml min), the temperature of the analytic column (28°C) and the intensity of the applied current (10nA in the first 15 min and 20nA in the subsequent time). Both were adjusted by urinary creatinine.

Cortisol and the DHEA-S were determined by an automated process of chemiluminescence using an IMMULITE in the laboratory of the Central American Center of Hormonal Analysis (Cenahce. Ltda). The cortisol was adjusted by urine volume and urinary creatinine.

C-reactive protein was determined in the laboratory of the Central American Center of Hormonal Analysis (Cenahce. Ltda) and in the laboratory of the Hospital San Juan de Dios (HSJD). In the first laboratory a high-sensitivity CRP method was used with the automated equipment KONELAB TM and in HSJD they used the aggregation of particles covered with monoclonal anti PCR antibodies and the Dade Bohering BN System automated equipment. The HSJD was established as the standard laboratory and the Cenahce Ltda. results were adjusted, starting from an equation estimated by regression in a validation lot.

Table 2: Analytic methodologies used by each laboratory.

Analysis	[1]Laboratory A	[2,3]Laboratory B	[4]Laboratory C	[5]Laboratory D
Glucose	Enzymatic	Enzymatic	Enzymatic	N/A
Cholesterol total	Enzymatic	Enzymatic	Enzymatic	N/A
HDL-Cholesterol	Precipitation y determination enzymatic	Method direct	Method homogeneous	N/A
Triglycerides	Enzymatic	Enzymatic	Enzymatic	N/A
Creatinine (serum & urine)	Jaffé Reaction	Jaffé Reaction	Jaffé Reaction	N/A
Glycosylated Hemoglobin	N/A	High yield Liquid Chromatography of (HPLC)	Inhibition with latex agglutination	Turbidimetric Immunoinhibition
Internal quality control	Daily Control of two levels, equipment calibration	Daily Control of two levels, equipment calibration	Daily Control of two levels, equipment calibration	Daily Control of two levels, equipment calibration
External control of quality	A. Quality Assurance Program (INCIENSA) B. External Quality Evaluation Program (PEEC) of the School of Microbiologists and Clinical Chemicals, CR	A. Beckman NYSSTATH Programs B. External Quality Evaluation of Lipids and Glucose Program (INCIENSA) C. BIORAD in England (for glycosylated Hemoglobin)	A. External Quality Evaluation of Lipids and Glucose Program (INCIENSA)) B. External Quality Evaluation Program (PEEC) of the School of Microbiologists and Clinical Chemicals, CR	RIQAS-England (Randox International Quality Assessment Scheme) for glycosylated hemoglobin

[1] Manual method, FOB WIENNER
[2] Automated Method, SYNCHRON CX7
[3] Automated Method, BIORAD Variant II hemoglobin A1C
[4] Automated Method, ACE Alpha Wassermann
[5] Automated Method, TUB QUANT

Table 1: Corrections to standardize laboratory results.

Biomarker	Lab. A.	Lab. C.	Lab. E.
Glucose mg/dl	*-20.204 +1.2218*x*	*6.687 +0.930*x*	
Cholesterol HDL mg/dl	*0.926*x*		
Triglycerides mg/dl	*-2.056+x*	*0.924*x*	
Creatinine urinaria[1] mg/dl	*0.896*x*	*-3.126+1.112*x*	
Glycosylated Hemoglobin %		*0.888*x*	
C-reactive protein (CRP) mg/dl			*0.073+0.731*x*

C-reactive protein (CRP) mg/dl
[1] *the adjusted values < 0 are replaced by 0.5*

Nutrients in the diet

Data on the diet of the participants were gathered with an abbreviated food frequency questionnaire (FFQ) developed specifically to evaluate the ingestion of macronutrients in the adult population in Costa Rica. It was developed and validated from a Costa Rican coronary health study that contained a full FFQ (Ek-Sohemy, Baylin et al 2001; Kabagambe, Baylin et al 2001; Kabagambe, Baylin et al 2005).

The original full FFQ in the coronary health study contained 147 foods in the Costa Rican diet, and it required approximately 45 minutes of interview time (Kabagambe, Baylin et al 2005). That study collected information on about 2000 residents in the Central Valley of Costa Rica, ages 60 and older who were the population control group in a case-control study of myocardial heart attack patients (the heart attack patients were not used in the FFQ validation study). The FFQ asked for the average consumption during the year prior to the survey, providing 9 possible answers to categorize the consumption frequency, which range from "never or less than once a month" to "6 or more times a day." The frequencies were converted by computer to a daily number of servings to estimate the quantity consumed. The FFQ also asked for the consumption of vitamins and nutritional supplements, the brands of cooking shortening, oil margarine used, and certain kinds of food preparation.

The coronary health study estimated for each individual the energy ingestion of several dozens of nutrients by multiplying the frequency of consumption of each food by the nutritional content of the respective portion using values of composition of the foods from the database of the Department of Agriculture of the US, in addition to data from producers and published reports as well as specific data for Costa Rica regarding the nutritional content of foods and local food preparation practices.

Using stepwise regression to optimize the goodness-of-fit and the parsimony of the model that explains the nutrient with the foods, CRELES researchers reduced the original FFQ to a 10 minute interview by identifying the minimum number of foods that maximizes the variance explained in a selection of macronutrients of interest to CRELES. In this way 27 tracer foods were identified for the abbreviated CRELES FFQ, which together with the brand of oil or shortening and the food preparation practice, explain 85% or more of the variance in seven macronutrients and 75% or more in a total of 17 macronutrients (Table 4). The abbreviated FFQ of CRELES was defined with these purposes: (1) to have valid estimates at the individual level of the consumption of key nutrients of interest and (2) to minimize the interview time.

The ingestion of these 17 macronutrients by the CRELES participants is estimated based on the abbreviated questionnaire of tracer foods in combination with the regression equations estimated from the detailed coronary health study data. The tracer foods defined in this way are sometimes counterintuitive or go against previous knowledge. For example, although rice is an important determinant of the consumption of calories in Costa Rica, this food is not a good tracer because its consumption is practically universal in the country. After consolidating all the tracer foods of the different nutrients, the result was the list of 27 foods of the abbreviated FFQ. The correlation coefficients among the 18 nutrients estimated in this way and the originals in the coronary study

are on the average of 0.90, with range from a minimum of 0.84 for iron to 0.94 for cholesterol and 0.99 for alcohol.

Table 4: Goodness-of-fit (R2) of ingestion of selected macronutrients with an abbreviated questionnaire of 27 tracer foods.

Nutrient	R^2
Total energy, kcal/d	0.81
Proteins, g/d	0.80
Carbohydrates, g/d	0.76
Glycemic Load, g/d	0.78
Total fats, g/d	0.85
Saturated fats, g/d	0.84
Monounsaturated fats, g/d	0.88
Polyunsaturated fats, g/d	0.82
Omega-6 fatty acid, g/d	0.81
Omega-3 fatty acid, g/d	0.85
Trans fats, g/d	0.85
Cholesterol, mg/d	0.94
Fiber, g/d	0.76
Alpha-Tocopherol, mg/d	0.78
Gamma-Tocopherol, mg/d	0.86
Calcium, mg/d	0.84
Alcohol, g/d	0.99

N = 2,200 (decreased slightly in some nutrients due to missing values)
Source: database of the Costa Rican Study of Coronary Health

Research Ethics

The study was approved by the Ethical Science Committee of the University of Costa Rica in the March 17, 2004 session (reference: VI-763-CEC-23 -04), research project number 828-A2 -825. All the databases of the study have been made anonymous (the name or identifier has been removed) to avoid risks to the privacy of the participants.

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