**Fels Longitudinal Study**

A detailed description of the Fels Longitudinal Study has been previously published. {Roche, 1992 #2882}. Briefly, the study began in 1929 for the purpose of examining growth, maturation, and body composition of individuals residing in southwest Ohio. In many cases, participants were enrolled as newborns, infants or small children, and then followed throughout their lifespans. Others were added during adolescence or adulthood, with a total sample of approximately 1200 active, serial participants. Although the study is closed to additional recruitment, data collection in a substantial number of participants is ongoing. Participants follow specific visit schedules depending on age and sex; for participants ≥18 years of age, visits occur every 2–5 years. Fels participants are overwhelmingly of European descent and typically live in southwest Ohio, roughly mirroring that region’s distribution of socioeconomic status, although Fels participants are well-educated with approximately 80% having at least some college education. Participants are not targeted for study enrollment because of any particular health conditions, diseases or body composition, and as such, the study can be considered to approximate normal population variation within the represented demographic. Each participant is followed from enrollment (usually birth) until death or infirmity rendering their continued participation impossible. Participants are not examined when menstruating, pregnant, or having other transient conditions (e.g., ill with infection) that could affect data quality. Of the 1,259 currently active participants, ~70% live within a half-day’s drive of the Lifespan Health Research Center. The remainder are typically examined when visiting friends or relatives in the Dayton, OH area, or have been flown in during past grant cycles.

**Data collection**

 Since the beginning of the Fels Longitudinal Study, and especially since the current grant began in 1976, new data collection methodologies have been regularly incorporated for increasingly sophisticated quantification of body composition, adipose tissue distribution, and other risk factors. For convenience, in this renewal application the primary type of examination is referred to as a “body composition examination.” Body composition examinations take place at the Lifespan Health Research Center and are scheduled for individuals 8 years and older. We also collect a subset of data on children from birth to age 18 years once annually to capture the rapid changes occurring during growth and maturation.

***Components of a body composition examination:*** A body composition examination currently includes the measurements described in **Table** **C-2** below. Reliability for all measurements is rigidly controlled and monitored.

***Anthropometry:*** Anthropometric data include weight, stature, sitting height, trunk depth, circumferences of the midarm, abdomen, hip, mid‑thigh and maximum calf, and triceps, biceps, subscapular, suprailiac, mid‑thigh and lateral calf skinfold thicknesses. All measurements are taken using techniques similar to corresponding measurements in the Anthropometric Standardization Reference Manual {Lohman, 1986 #880;Lohman, 1988 #3207} or to corresponding measurements in NHANES III and the current NHANES. Stature and weight have been collected since 1929.

***Grip strength:*** Grip strength is measured using a Jamar hydraulic hand dynamometer (Model 5030JL, JLW Instruments, Chicago, IL) with participants seated, elbow flexed to 90 degrees, and the dynamometer resting comfortably on the participants’ thigh. Three maximal effort measures are obtained for each hand, with no more than 10% variation between attempts. Grip strength data have been collected since 1985.

***Dual energy x‑ray absorptiometry (DXA):*** Body composition is currently assessed by DXA using a Hologic Discovery A densitometer following the manufacturer’s protocol. The total body scan is analyzed to yield measures of bone, fat, and lean tissue for particular body regions (e.g., arms, legs, trunk, etc.). DXA measures bone mineral content and density, fat mass, and fat-free mass (FFM) using exponential attenuation due to differential absorption by body tissues of photons emitted at two energy levels. The precision and reliability of DXA is well documented{Economos, 1996 #55;Kroger, 1996 #454}. DXA technology has been in place at the Lifespan Health Research Center since 1989.

***Short Physical Performance Battery (SPPB)****.* The SPPB is a validated and widely used physical performance test that is sensitive to change. {Guralnik, 1994 #669} {Pahor, 2014 #247} It has a composite score of 0-12 derived from tests of timed chair stands (described above), usual gait speed, and static balance in older adults. Static balance consists of tandem, semi-tandem, side-by-side stances. The chair-stand tests consists of five timed full stands with a single chair stand done initially to demonstrate ability to attempt the test. SPPB data have been collected on participants over 40 years old since 2013.

***Long Distance Corridor Walk:*** We will follow the Health, Aging and Body Composition study methods for the 400-meter long distance corridor walk as described by Simonsick {Simonsick, 2001 #1731}. The 400-meter long distance corridor walk is considered a proxy of cardiorespiratory endurance and has been validated against treadmill testing {Simonsick, 2001 #1731}.

***Inflammatory, coagulation, and endothelial function, and other biomarkers:*** All biochemical assays are performed on fasting (>8 hours) venous blood samples.Fasting levels of GCSF, interleukin-1 receptor antagonist (IL-1RA), Interleukin-1 (IL-1), (IL-2), Interleukin-8 (IL-8), monocyte chemotactic protein 1 (MCP-1), regulated upon activation, normal T-cell expressed, and secreted (RANTES), TNFα, TNFR1, TNFR2, vascular endothelial growth factor (VEGF), and IL-6 are assayed using multiplex immunoassay technology using commercially available kits from Millipore (Billerica, MA). Multiplex kits will be used on the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA) utilizing Multi-Analyte Profiling (MAP) technology (Luminex Corp, Austin, TX). P-selectin, MMP-3 (matrix metalloproteinase-3, BioSource, Camarillo, California); TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), leptin, VCAM, paraoxonase, PXN and D-dimer are measured by radioimmunoassay using commercially available kits (R&D Systems, Inc. Minneapolis, MN). Plasminogen Activator Inhibitor-1 (PAI-1) concentrations will be assayed using commercially available enzyme-linked immunosorbent assay (ELISA) (Invitrogen Corporation, Camarillo, CA). All assays are conducted according to manufacturer’s protocols using appropriate calibration standards. Leptin and some of the inflammatory markers have been routinely assayed in blood samples collected and stored from 1991 to the present. The use of stored samples for these types of markers has been conclusively demonstrated {Pai, 2002 #882;Kayaba, 2000 #883;Pearson, 2003 #884}.

***Lipids, lipoproteins, insulin, glucose, glycosylated hemoglobin, and free fatty acids:*** Fasting levels of triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A1, apolipoprotein B, glucose and insulin are measured according to standard laboratory protocols{Nagele, 1984 #118;Stein, 1986 #173;Hogle, 1988 #886;Friedewald, 1972 #887}. CRP, insulin (fasting), glycated hemoglobin (HbA1c), glucose, and complete blood cell count levels are collected as part of the regular blood draw at the Lifespan Health Research Center and are assayed at Labcorp (Dublin, Ohio). Serum levels of free fatty acids (i.e., non-esterified fatty acids) will be measured using enzymatic colorimetry (Wako Chemicals USA, Inc, Richmond, VA) at Labcorp. Fasting lipid and lipoprotein concentrations have been measured in samples from Fels Longitudinal Study participants since 1976.

***Sex steroid hormones:*** Serum levels of total and free (directly measured) testosterone, estradiol and dehydroepiandrosterone sulfate (DHEA-S) were measured at Medical Research Laboratories (March 1989 – February 2006) using radioimmunoassay methodology. Since February 2006, serum DHEA-S assay and follicle-stimulating hormone (FSH) assay (only on females >40 years old) are conducted at LabCorp using electrochemiluminescence immunoassay.

***Health history, reproductive history, smoking, alcohol use, health-related function, and physical activity:*** Immediately prior to their examination, the participant is mailed a series of questionnaires to complete. These are carefully reviewed for completeness and accuracy by the staff, and clarifications are made at the time of the examination. These data are used to describe and categorize participants into demographic, life-stage, and behavioral groups, to document outliers, and to elucidate possible confounding interactions and associations within the data. Questionnaires are used to collect the following data: demographic and personal information, medical history (a detailed inquiry of current health status including chronic, acute and infectious conditions, and medication use), tobacco and alcohol use (a 3‑page inquiry relating to current tobacco and alcohol usage and exposure), and reproductive history (two 3‑page inquires, depending upon the age of the women, relating to gynecological health and status and supplemental hormone use). Physical activity is measured using the modified Baecke questionnaires {Baecke, 1982 #541}. Habitual physical activity is estimated for the previous year by calculating a total activity score from work, sports, and non‑sport leisure time activity indices. The tobacco forms include questions about smokeless tobacco and exposure to environmental smoke. Short Form-36 (SF-36) health survey data are collected serially from adults to assess physical, social, and cognitive function and well-being in eight domains {Ware, 1992 #19}. The SF‑36 is an accepted and validated assessment tool{McHorney, 1993 #403} for measuring a comprehensive set of defined health concepts applicable to the aging process. Out of the domains, the physical functioning sub-scale will be used as the self-reported physical functioning outcome, and the mental health sub-scale will be used as one of the cognitive markers. The Patient Health Questionnaire-9 (PHQ-9) is administered to ascertain depressive symptoms, and will be used as a measure of mental well-being {Kroenke, 2001 #5451}. Health and behavior data have been collected in the Fels Longitudinal Study since 1929. Physical activity data have been collected since 1988. SF-36 health survey data have been collected since 1997.

***Dietary assessment:*** Each participant completes the semi-quantitative self-administered Food Frequency Questionnaire developed by Harvard University. Typical nutrient and food intake during the preceding year are assessed. Additional items include vitamin and mineral supplementation. The reproducibility and validity of nutrient and food intake measurements have been thoroughly described{Willett, 1985 #890;Rimm, 1992 #891;Feskanich, 1993 #892;Willett, 1998 #893}. Nutrient intakes are adjusted for total caloric intake, but in addition general dietary patterns (e.g., “prudent” vs. “Western”) can be validly identified using this instrument{Hu, 1999 #894}. The Food Frequency Questionnaire has been collected since 2004.

***BP and endothelial function:*** Seated and resting SBP, and K4 and K5 DBPs are measured in a standardized manner{Perloff, 1993 #897}. Peripheral pulse wave velocity between the brachial and posterior tibial arteries is measured using an oscillometric method, the Vascular Profiling System-1000 (Omron Inc, San Antonio, TX) since 2006. BP data have been collected since the early 1930s.

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***Data management:*** Data quality control in the Fels Longitudinal Study is a high priority. All data are checked carefully for errors and corrected as necessary before being added to the master database at 6-month intervals. All recorded data are double entered into computer files to minimize error. Continuous checks of data quality are performed and regularly scheduled standardization and re-training of data collection staff is conducted. Reliability for anthropometric, BP, body composition and other variables is examined using the technical errors and coefficients of reliability (CR) calculated from a 5% replicability sample. The technical error and CR of our data consistently remain at or above the level of reliability reported in the literature.