The HERITAGE Family Study: A Review of the Effects of Exercise Training on Cardiometabolic Health, With Insights Into Molecular Transducers

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The HERITAGE Family Study: A Review of the Effects of Exercise Training on Cardiometabolic Health, with Insights into Molecular Transducers

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ABSTRACT

SARZYNSKI, M. A., T. K. RICE, J.-P. DESPRÉS, L. PÉRUSSE, A. TREMBLAY, P. R. STANFORTH, A. TCHERNOF, J. L. BARBER, F. FALCIANI, C. CLISH, J. M. ROBBINS, S. GHOSH, R. E. GERSZTEN, A. S. LEON, J. S. SKINNER, D. C. RAO, and C. BOUCHARD. The HERITAGE Family Study: A Review of the Effects of Exercise Training on Cardiometabolic Health, with Insights into Molecular Transducers. Med. Sci. Sports Exerc., Vol. 54, No. 5, pp. S1–S43, 2022. The aim of the HERITAGE Family Study was to investigate individual differences in response to a standardized endurance exercise program, the role of familial aggregation, and the genetics of response levels of cardiorespiratory fitness and cardiovascular disease and diabetes risk factors. Here we summarize the findings and their potential implications for cardiometabolic health and cardiorespiratory fitness. It begins with overviews of background and planning, recruitment, testing and exercise program protocol, quality control measures, and other relevant organizational issues. A summary of findings is then provided on cardiorespiratory fitness, exercise hemodynamics, insulin and glucose metabolism, lipid and lipoprotein profiles, adiposity and abdominal visceral fat, blood levels of steroids and other hormones, markers of oxidative stress, skeletal muscle morphology and metabolic indicators, and resting metabolic rate. These summaries document the extent of the individual differences in response to a standardized and fully monitored endurance exercise program and document the importance of familial aggregation and heritability level for exercise response traits. Findings from genomic markers, muscle gene expression studies, and proteomic and metabolomics explorations are reviewed, along with lessons learned from a bioinformatics-driven analysis pipeline. The new opportunities being...
pursued in integrative -omics and physiology have extended considerably the expected life of HERITAGE and are being discussed in relation to the original conceptual model of the study. **Key Words:** EXERCISE GENOMICS, HERITABILITY, EXERCISE TRAINING, CARDIOMETABOLIC RISK FACTORS, EXERCISE AND GENE EXPRESSION, EXERCISE AND PROTEOMICS, EXERCISE AND METABOLOMICS, EXERCISE AND BIOINFORMATICS

The aim of this article is to summarize the findings of the HERITAGE (HEalth, Risk factors, exercise Training And GEnetics) Family Study and their potential implications for cardiometabolic health and exercise performance. HERITAGE has contributed substantially to our understanding of adaptation to exercise and endurance training over the last 25 yr. It started as a collaborative endeavor among scientists from five research institutions in the United States and Canada. Over the years, investigators from institutions around the world have joined the effort to use HERITAGE data to address novel questions of scientific interest.

Scientists who have been involved in the early phases of the study or are currently leveraging the biobank and data of HERITAGE to address underlying biological mechanisms have undertaken the present review. The review covers foundational topics such as background and planning, recruitment, testing and exercise program protocol, panel of quality control measures, and other relevant issues. This is followed by a review of the findings on cardiometabolic traits, including adiposity and specific fat depots, cardiorespiratory fitness, exercise hemodynamics, insulin and glucose metabolism, lipid and lipoprotein profile, blood levels of steroids and other hormones, markers of oxidative stress, skeletal muscle morphology and metabolic indicators, and resting metabolic rate (RMR). Subsequently, reviews of findings arising from genomic markers, muscle gene expression studies, and proteomic and metabolomic explorations are provided, along with lessons learned from a bioinformatics pipeline. Finally, an overview of the strengths and limitations of the study, and considerations on new opportunities offered by advances in -omics and bioinformatics are offered.

**Background.** In a series of experiments performed in the laboratory of C. Bouchard (CB) at Laval University, Quebec, Canada, during the 1980s, two important conclusions were reached. The first was that there were large interindividual differences in the response level to a standardized exercise program at a given dose (1–6). The second was that there was strong indication of a genetic component to the variation in human trainability (4,6,7). Based on the aforementioned observations, it became evident that a major exercise training study encompassing a substantial number of subjects from nuclear families was necessary to test the genetic hypothesis more thoroughly. To this end, CB established a team of seasoned investigators who agreed to work together in the context of a research consortium. They included Arthur S. Leon (ASL) (University of Minnesota), D. C. Rao (DCR) (Washington University), James S. Skinner (JSS) (initially at Arizona State University and then at Indiana University), and the late Jack H. Wilmore (JHW) (University of Texas at Austin).

**Historical notes.** The team began planning the study in 1988 and a series of meetings were held to prepare an application to the National Institutes of Health. The National Heart, Lung and Blood Institute (NHLBI) expressed an interest in the proposed research, and Dr. Millicent Higgins from the Division of Epidemiology and Clinical Applications came forward to provide guidance for a multicenter grant application involving American and Canadian investigators. Our grant application was reviewed in June 1990, and a revision went to a Special Review Committee in May 1991; the latter received an excellent score and was recommended for funding. Dr. Claude Lenfant, Director of NHLBI, and Dr. Higgins met with CB to...
discuss the project funding and to ask for an expansion of the research to a substantial sample of African American families. The budget was adjusted accordingly, and the project was approved for funding (five coordinated R01 grants to CB, ASL, DCR, JHW, and JSS) with a start date of September 1992 (phase 1). Data collection began in February 1993 and was completed in the spring of 1997. The five grants were renewed from 1997 to 2000 (phase 2) and from 2001 to 2003 (phase 3). The grants for the two sites (CB and DCR) with expertise in genetics were renewed from 2006 to 2010 (phase 4).

**Aim of HERITAGE.** The aim of the HERITAGE research consortium was to investigate the magnitude of the individual differences in response to a standardized endurance exercise program, the importance of familial aggregation, and the genetics of the response levels of cardiorespiratory fitness and cardiovascular disease and diabetes risk factors. Figure 1 depicts a schematic of the initial conceptual model underlying HERITAGE.

**Organization of the research consortium.** The HERITAGE Family Study was led by a Steering Committee composed of the five principal investigators (CB (Chair), DCR, ASL, JHW, and JSS) and the Project Director (Jacques Gagnon from 1992 to 1999 and Tuomo Rankinen from 1999 to 2012). An administrator (Jean-Paul Albert) supported the work of the Steering Committee from 1992 to 1999 (Supplementary Table S1, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C482). An Advisory Board supported and advised the Steering Committee (Supplementary Table S2, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C482). During phase 1, the most demanding period of the project, the research was organized around a Consortium Coordinating Center (Laval University), a Data Coordinating Center (Washington University), four Clinical Centers responsible for recruitment, testing, and training of subjects (Arizona State University), four Clinical Centers responsible for participants who self-identified as Black was challenging. The priority shifted to the recruitment of trios and pairs of first degree relatives, which was approved by the HERITAGE Steering Committee, the HERITAGE Advisory Board, and NHLBI. The leadership of these HERITAGE units and their personnel is identified in Supplementary Table S3 (Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C482). By 2021, more than 200 peer-reviewed articles have been published from the HERITAGE Family Study. Most of these articles were based solely on HERITAGE data. In other publications, HERITAGE contributed data along with multiple other cohorts mainly in the context of multicenter genome-wide association studies (GWAS). A list of all HERITAGE-based publications and other general information regarding the study can be found at www.HeritageFamilyStudy.com.

**PROTOCOL AND QUALITY CONTROL MEASURES**

The HERITAGE Family Study enrolled a total of 855 individuals from 101 two-generation nuclear families of European ancestry (hereafter Whites), each with both biological parents and at least two offspring, and from 117 families of African ancestry (hereafter Blacks) (Table 1). The final sample at baseline included a total of 529 White and 326 Black individuals. Participants were within 17–65 yr of age, “sedentary” for at least 3 months before the study, and otherwise in good health. They were evaluated before and after a 20-wk standardized training program, providing an opportunity to assess familial aggregation of responsiveness to regular exercise (8).

Families were recruited from media (newspapers, TV, radio), posted advertisements, personal contacts, and institutional communication services. Telephone and mailed questionnaires were used for initial eligibility screening. All potential families underwent an orientation session to explain the study and answers questions. Participants were compensated up to $1000 in incremental payments for successful completion of the study (i.e., after completing the 20-wk training program, after completing baseline and postraining battery of tests). The study was approved by each participating institution’s institutional review board. Written informed consent was obtained from each study participant, including permission to use individual cell lines, DNA, and collected specimens for the initial project and future collaborative studies.

The recruitment went smoothly for the families of Whites, as more than 90% of the families included both parents and three or more offspring. Recruiting similar types of families for participants who self-identified as Black was challenging. This led to a revision of the recruitment requirements for the latter families, which was approved by the HERITAGE Steering Committee, the HERITAGE Advisory Board, and NHLBI. The priority shifted to the recruitment of trios and pairs of first degree relatives by descent in Black communities at all HERITAGE sites. This revised approach worked well in the end, as we were able to enroll 326 Black adults from 117 families (Table 1). It has been brought to our attention that the description 27 yr ago of the challenge we were facing in the recruitment of families of African ancestry in the first HERITAGE paper devoted to aims, design, and measurement protocols of the study (8) was offensive to some. We agree, and in retrospect, we should have used a more sensitive description of the situation.

The initial screening included a health history, physical examination, a resting electrocardiogram (ECG), and an exercise test with ECG monitoring. At baseline, participants completed a health questionnaire, including habits relating to smoking and alcohol consumption; the ARICBaecke Physical Activity Questionnaire (9,10); the Willett Food Frequency Questionnaire to assess usual food nutrient patterns (11); the Minnesota Eating Pattern Assessment Tool (EPAT) to evaluate dietary fat sources

### TABLE 1. Number of families, men, women, and total subjects at baseline and posttraining in the HERITAGE Family Study.

| Ancestry | Families | Men | Women | Total | Families | Men | Women | Total |
|----------|----------|-----|-------|-------|----------|-----|-------|-------|
| Whites   | 101      | 262 | 267   | 529   | 99       | 233 | 250   | 483   |
| Blacks   | 117      | 117 | 209   | 326   | 105      | 87  | 172   | 259   |
| Total    | 218      | 379 | 476   | 855   | 204      | 322 | 422   | 742   |

*Posttraining numbers include only the subjects who were classified as completers having completed at least 57 (95%) of the 60 exercise training sessions.*
(12,13); a menstrual history; and a detailed family history questionnaire (Table 2). The health habit and EPAT questionnaires were repeated during midtraining (week 10). Participants were instructed at the beginning and at midtraining to not change their baseline health habits. The health habit and EPAT questionnaires were administered again at the end of training (week 20).

Panel of Tests and Measurements

Anthropometric and body composition measurements were made before and after training, as described elsewhere (14). Measurements included standing height, body mass, skinfold thicknesses at eight different sites, and waist circumference (see Ref. [8]). Underwater weighing (the gold standard measure of body composition at the time) was performed in the postabsorptive state before and after training to determine body density, fat mass (FM), fat-free mass, and relative body fat using sex- and ethnic-specific equations (15). Abdominal visceral and subcutaneous fat were quantified before and after training, as described elsewhere (14). Scans were taken between the fourth and fifth lumbar vertebrae (L4–L5 space) (16). Subjects were examined in the supine position, with arms stretched above their heads. Total and visceral (AVF) abdominal fat areas (in cm²) were calculated by delineating those areas with an electronic graph pen and then computing the adipose tissue surfaces by using an attenuation range of −30 to −190 Hounsfield units. The abdominal subcutaneous fat area was calculated as the difference between the total and visceral fat areas.

Resting blood pressure (BP) measurements were made twice on separate days before the start of exercise training and at 24- and 72 h after training. Subjects were tested before 11:00 A.M. in the postabsorptive state with no caffeine-containing beverages and tobacco products for at least 2 h before measurements. BP was determined using a properly fitted cuff connected to a Colin STBP-780 automated unit (17).

To measure plasma lipid and lipoprotein levels, blood samples were collected in the morning after a 12-h fast, kept on ice until centrifugation, and then stored at 4°C until analyzed within 10 d (18). Total cholesterol (TC) and triglycerides (TG) levels were determined in plasma and lipoprotein fractions (VLDL, HDL, LDL) by enzymatic methods using the Technicon RA-1000 analyzer plasma. Apolipoprotein (Apo) B and ApoA-I concentrations were measured, and those of LDL cholesterol (LDL-C), LDL-TG, and VLDL-ApoB were calculated. The cholesterol content of HDL₂ and HDL₃ subfractions was also determined. ApoE phenotype was measured by an isoelectrofocusing method. Lipoprotein lipase (LPL) and hepatic-TG lipase (HL) activities were measured in plasma obtained from 12-h fasted subjects, 10 min after intravenous injection of heparin (60 IU·kg⁻¹ of body weight).

An intravenous glucose tolerance test (IVGTT) was performed in the morning after an overnight fast. Blood samples were collected at 16 time points over 3 h to determine plasma glucose, insulin, and connecting peptide (C-peptide) concentrations. The updated MINMOD model was used to quantify the acute response to insulin, insulin sensitivity index (Si), disposition index, and other parameters (19).

The plasma steroid hormone profile was assayed, including androstenedione (DELTA4), testosterone (TESTO), dihydrotestosterone (DHT), androsterone glucuronide (ADTG), androstane-3α, 17β-diol glucuronide, pregnenolone (PREGE) fatty acid esters, dehydroepiandrosterone and its fatty acid, progesterone (PROG), 17-hydroxyprogesterone, cortisol (CORT), aldosterone (ALDO), estradiol (E2), and dehydroepiandrosterone sulfate. Table 3 summarizes the variables measured and the posttraining timing of measurements. Three exercise cardiopulmonary fitness tests all on cycle ergometers were performed before and after the exercise program. The first test was a ramp protocol with increasing workload every 2 min leading to exhaustion and the first estimate of VO₂max. The second test was a submaximal test with workloads of 50 W and then 60% of the power output (PO) at VO₂max, each lasting 8 min in to obtain physiological data in steady state. The third test repeated the conditions of the preceding submaximal tests, then proceeded to a PO corresponding to 80% of VO₂max followed by a progressively increasing workload until exhaustion and the second estimate of VO₂max. Resting and exercise BP, fasting insulin, glucose and C-peptide, lipid and lipoprotein profile, and steroid hormone profile measurements were performed twice before the exercise training program, as well as twice posttraining (24 and 72 h after the last exercise session), as specified in Table 3. The anthropometry, body composition, CT assessment of abdominal fat, IVGTT, postheparin LPL and HL activities, and the plasma levels of various substrates during exercise were obtained only once before and once post training. Additional traits measured in subsamples of the HERITAGE cohort were performed once before and once after the exercise program.

### Inclusion and Exclusion Criteria

**Age.** Participants outside 17–65 yr old were excluded to reduce potential complications from maturation at the low end and aging at the high end.

| Phenotype                                      | Baseline | Midtraining | Posttraining |
|------------------------------------------------|----------|-------------|-------------|
| Age, sex, and ethnicity                        | x        | x           | x           |
| Medical history: previous diseases and physical examination | x        | x           | x           |
| Personal history: medications, smoking, alcohol consumption, level of education, occupation, sleeping habits | x        | x           | x           |
| Family history: myocardial infarction, other cardiovascular disease, stroke, hypertension, hypercholesterolemia, obesity, diabetes, cancer | x        | x           | x           |
| Menstrual cycle: age at menarche, menstrual status (premenopausal and postmenopausal), number of pregnancies, babies over 9 lb, estrogen, and PROG replacement | x        | x           | x           |
| Physical activity habits: leisure time index, work index, sports index | x        | x           | x           |
| EPAT: Score section I and section II           | x        | x           | x           |
| Food frequency questionnaire                   | x        | x           | x           |
| Anxiety levels                                 | x        | x           | x           |

**TABLE 2.** List of the concomitant and behavioral variables available in the HERITAGE Family Study.
Three exercise tests were administered both before and after training: maximal, steady-state submaximal, and submaximal–maximal. All tests were performed on the same cycle ergometer (Ergo-Metrics 800S from SensorMedics; different from the cycle ergometer used for the training program) in the sitting position at approximately the same time of day, with at least 48 h difference between tests at baseline. The first test was to establish the participant’s VO2max, as well as to verify the normality of the exercise ECG at baseline. The maximal test started at an initial intensity of 50 W for 3 min and increased by 25 W every 2 min until volitional exhaustion. For older, smaller, or less fit individuals, the test started at 40 W, with increases of 10–20 W each 2 min thereafter. This was done in to increase the number of stages they could complete before reaching maximum because the results were used to select the PO for the subsequent exercise tests (20). During the second test, participants exercised at 50 W and at 60% VO2max, determined from the initial maximal test, for 8 min at each PO to measure steady-state ventilation (VE), VO2, VCO2, respiratory exchange ratio (RER), systolic (SBP) and diastolic (DBP) BP, heart rate (HR), cardiac output (Q̇), and stroke volume (SV). During the third and final test, each participant repeated the submaximal steady-state exercise at 50 W

### Activity level.
All participants were required to be “sedentary” at baseline. Sedentary was defined as no regular physical activity (occurring no more than once a week) involving an energy expenditure of ≥7 METs for subjects 50 yr and older and ≥8 METs for those younger than 50 yr over at least the previous 3 months.

### Body mass index.
Body mass index (BMI) was <40 kg·m⁻² because of metabolic abnormalities and difficulty in exercising that is associated with extreme obesity. However, a few subjects with a BMI of 40 kg·m⁻² or more were included because it was determined that they could perform the exercise and the testing program.

### BP and medications.
Resting BP was required to be <160/100 mm Hg for two out of three readings. Individuals on diuretic or antihypertensive drugs at the initial interview were permitted to enter the study if they were free of hypertensive complications, their personal physician permitted them to discontinue their medication(s), and their BP level met the aforementioned criteria after at least 3 months off medication. Subjects unable to be removed from their antihypertensive treatment were excluded from the study.

### Absence of significant medical conditions and diseases.
A detailed medical history and physical examination were conducted by a physician or a nurse practitioner under the supervision of a physician after screening. For subjects with suspicious symptoms or suggestive medical histories, their personal physicians were asked to provide additional medical information, test results, and hospital records before inclusion or exclusion. A past history and/or physical or laboratory finding of certain medical conditions required exclusion from the study (8).

### Exercise Testing Protocol
Three exercise tests were administered both before and after training: maximal, steady-state submaximal, and submaximal–maximal. All tests were performed on the same cycle ergometer (Ergo-Metrics 800S from SensorMedics; different from the cycle ergometer used for the training program) in the sitting position at approximately the same time of day, with at least 48 h difference between tests at baseline. The first test was to establish the participant’s VO2max, as well as to verify the normality of the exercise ECG at baseline. The maximal test started at an initial intensity of 50 W for 3 min and increased by 25 W every 2 min until volitional exhaustion. For older, smaller, or less fit individuals, the test started at 40 W, with increases of 10–20 W each 2 min thereafter. This was done in to increase the number of stages they could complete before reaching maximum because the results were used to select the PO for the subsequent exercise tests (20). During the second test, participants exercised at 50 W and at 60% VO2max, determined from the initial maximal test, for 8 min at each PO to measure steady-state ventilation (VE), VO2, VCO2, respiratory exchange ratio (RER), systolic (SBP) and diastolic (DBP) BP, heart rate (HR), cardiac output (Q̇), and stroke volume (SV). During the third and final test, each participant repeated the submaximal steady-state exercise at 50 W

| Phenotype | Baseline | Posttraining |
|-----------|----------|--------------|
|           | Test 1   | Test 2       | Test 3   | Test 4   |
| Body fat and body composition | x | x | x | x |
| Weight, height, and BMI | x | x | x | x |
| FM and fat-free mass (underwater weighing) | x | x | x | x |
| Regional fat distribution and visceral fat | x | x | x | x |
| CT total, subcutaneous, and visceral fat | x | x | x | x |
| Sum of 8 skinfolds | x | x | x | x |
| Waist and hip girths | x | x | x | x |
| BP | x | x | x | x |
| Resting | x | x | x | x |
| Exercise at 50 W and at 60% 80% VO2max | x | x | x | x |
| Insulin and glucose metabolism | x | x | x | x |
| IVDTT: Glucose, insulin, C-peptide | x | x | x | x |
| Fasting: Glucose, insulin, C-peptide | x | x | x | x |
| Lipids, lipoproteins, and lipases | x | x | x | x |
| Postheparin lipolytic activities: LPL and HL | x | x | x | x |
| Fasting lipid, lipoprotein, apolipoprotein profile | x | x | x | x |
| Steroids | x | x | x | x |
| Panel of androgens, estrogens plus SHBG | x | x | x | x |
| Cardiorespiratory endurance | x | x | x | x |
| Submaximal and maximal indicators of cardiorespiratory fitness | x | x | x | x |
| SV, cardiac output, and other hemodynamic indicators during exercise | x | x | x | x |
| Plasma protein, glucose, FFA, lactate at 50 W, 80% VO2max, and 80% VO2max, and max | x | x | x | x |
| Additional phenotypes on subsamples | x | x | x | x |
| Leptin (resting and exercise) | x | x | x | x |
| Insulin-like growth factors 1 and 2 | x | x | x | x |
| Homocysteine, cysteine, glutathione, cysteinyl glycine, folates, vitamins B6, and B12 | x | x | x | x |
| LDL oxidation, TBARS, hemolysis, GSH-Px, α-tocopherol, β-carotene, retinol, phospholipid fatty acids, Lp(a) | x | x | x | x |
| Skeletal muscle fiber types, capillary density, enzymes | x | x | x | x |
| RMR | x | x | x | x |

24 h, 72 h, and 96 h refer to tests performed 24, 72, and 96 h after the last training session, respectively.

Three exercise tests were performed before and after training. First, a maximal cardiorespiratory fitness test was performed. It was a cycle ergometer test with increasing workload leading to exhaustion and VO2max. The data from this test were used to compute the specific HR and PO needed for the exercise prescription of each participant. The second test was submaximal and included exercising for 8 min at 50 W followed by 8 min at the PO associated with 60% of VO2max. The last test repeated the conditions of the second test, followed by a workload corresponding to 80% of VO2max, and then increasing workloads until exhaustion was reached to provide a second estimate of VO2max. In the post-training period, these tests were performed at 24, 48, and 72 h following the last exercise training bout.

### Table 3. List of phenotypes available in the HERITAGE Family Study.

| Phenotype                                      | Baseline | Posttraining |
|------------------------------------------------|----------|--------------|
| Body fat and body composition                  | x        | x            |
| Weight, height, and BMI                        | x        | x            |
| FM and fat-free mass (underwater weighing)     | x        | x            |
| Regional fat distribution and visceral fat     | x        | x            |
| CT total, subcutaneous, and visceral fat       | x        | x            |
| Sum of 8 skinfolds                             | x        | x            |
| Waist and hip girths                           | x        | x            |
| BP                                             | x        | x            |
| Resting                                       | x        | x            |
| Exercise at 50 W and at 60% 80% VO2max         | x        | x            |
| Insulin and glucose metabolism                 | x        | x            |
| IVDTT: Glucose, insulin, C-peptide             | x        | x            |
| Fasting: Glucose, insulin, C-peptide            | x        | x            |
| Lipids, lipoproteins, and lipases              | x        | x            |
| Postheparin lipolytic activities: LPL and HL   | x        | x            |
| Fasting lipid, lipoprotein, apolipoprotein profile | x    | x            |
| Steroids                                       | x        | x            |
| Panel of androgens, estrogens plus SHBG        | x        | x            |
| Cardiorespiratory endurance                   | x        | x            |
| Submaximal and maximal indicators of cardiorespiratory fitness | x | x |
| SV, cardiac output, and other hemodynamic indicators during exercise | x | x |
| Plasma protein, glucose, FFA, lactate at 50 W, 80% VO2max, and 80% VO2max, and max | x | x |
| Additional phenotypes on subsamples            | x        | x            |
| Leptin (resting and exercise)                  | x        | x            |
| Insulin-like growth factors 1 and 2            | x        | x            |
| Homocysteine, cysteine, glutathione, cysteinyl glycine, folates, vitamins B6, and B12 | x | x |
| LDL oxidation, TBARS, hemolysis, GSH-Px, α-tocopherol, β-carotene, retinol, phospholipid fatty acids, Lp(a) | x | x |
| Skeletal muscle fiber types, capillary density, enzymes | x | x |
| RMR                                            | x        | x            |
TABLE 4. Overview of the 20-wk training program in HERITAGE.

| Weeks | Frequency (Sessions per Week) | Intensity (% VO2max) | Duration (Minutes per Session)* |
|-------|-------------------------------|----------------------|---------------------------------|
| 2     | 3                             | 55                   | 30                              |
| 2     | 3                             | 60                   | 35                              |
| 2     | 3                             | 65                   | 35                              |
| 2     | 3                             | 70                   | 40                              |
| 2     | 3                             | 75                   | 45                              |
| 6     | 3                             | 75                   | 50                              |

*Does not include 5-min warm-up or 3-min cool-down.

and 60% VO2max of the second test, after which the PO was increased to 80% VO2max and then continued to increase until the subject reached volitional exhaustion. In addition, a venous catheter was inserted in the left arm to obtain blood samples immediately upon completion of the maximal test. Blood samples were analyzed for glucose, free fatty acids, lactate, and total proteins. Metabolic measurements and VO2 were determined using a SensorMedics 2900 metabolic cart, BP with a Colin STBP-780 automated BP monitor, and HR from the ECG. SV was derived from HR and VO2.

Exercise Training Protocol

The training protocol required a total of 60 training sessions, three per week for 20 wk. All training sessions were held on-site at the participating clinical centers. Each participant trained on a cycle ergometer (Universal Aerobicycles, Cedar Rapids, IA) using the same standardized training protocol at the four clinical centers. Training intensity was adjusted for individual differences in VO2max and began at the HR associated with 55% of baseline VO2max (derived from the first, maximal exercise test) for 30 min for the first 2 wk. The intensity or duration of the training sessions was increased every 2 wk, until subjects were working at the HR associated with 75% of baseline VO2max for 50 min during the last 6 wk (Table 4). The PO of the cycle ergometer was adjusted to maintain each subject’s HR at the prescribed level; this was controlled by direct HR monitoring at all times during all training sessions using the Universal Gym Mednet (Cedar Rapids, IA) computerized system. All training sessions were supervised on-site by trained personnel to ensure that the equipment was working properly, and the participants were compliant with the protocol. A total of 742 participants completed at least 95% of the training sessions (57 sessions) and were defined as “completers.”

Subjects were not allowed to exercise more than four sessions per week or less than one per week and could not get ahead or fall behind by more than two sessions. Adherence was monitored several times per week. If subjects were falling behind, a plan was developed to bring them back on schedule as soon as possible. In premenopausal women, tests and measurements were scheduled during the follicular phase of the menstrual cycle. This required that some women exercised a few or several more sessions until they had reached the proper phase of their menstrual cycle.

Core Laboratories, Cell Lines, Biobank

Four core laboratories were established at Laval University in Quebec, Canada, for assaying lipids, sex steroid hormones, and glucose-insulin–related traits, and to establish cell lines. Lymphoblastoid cell lines were established for each participant to ensure a continuous source of DNA. Cell lines were obtained by transformation of human lymphocytes with the Epstein–Barr virus. Such cell lines grow well in culture, have an infinite life span, and present chromosomal stability over years (21). The procedure requires isolation of monocyte cells, transformation with the virus, and cryopreservation of transformed cell lines.

Training of Personnel and Data Quality

Study personnel were centrally trained on recruitment, measurement, exercise testing, exercise training, and data entry using a detailed Manual of Procedures. Retraining of staff on measurement protocols was implemented as needed at each clinical center under the Project Director on a yearly basis. Appropriate training of new personnel was provided when necessary. These actions were designed to maximize quality of all data acquired at each clinical center and to minimize potential unwarranted data heterogeneity.

Reproducibility, Clinical Center Differences, and Drift over Time

As part of the quality control program of HERITAGE, plans were made to quantify assay and measurement variability, within-subject variability, potential clinical site differences, and drift over time in protocol at each clinical center. As a control group was not deemed necessary by the investigators and external reviewers given the primary aim of HERITAGE, the quality control measures became particularly important and useful. The metrics of these quality control measures have been addressed in several publications (18,22–25), and they are commented upon hereinafter in the various sections of this article. Briefly, four approaches were used to assess assay and measurement reproducibility and within-subject variability. First, for all traits assayed from blood samples, the assay was repeated on split samples from the same blood draw. In addition, the assays were performed twice on 5% of all samples selected at random. Second, for all variables that were measured days apart, twice before and twice after the exercise program, reliability coefficients (intraclass correlations), technical errors (within-subject SD), and coefficients of variation (CV) (100 × SD/mean) could be computed across all subjects. Third, an additional 15 subjects meeting the inclusion criteria of the study were recruited at each clinical center for the sole purpose of being tested three times over 3 wk for most of the HERITAGE panel of tests. Fourth, four subjects (a total of eight individuals over the period) traveled to all four clinical centers in a random order across three yearly cycles under the supervision of the Project Director to take the full battery of tests and measurements at each clinical center.

Table 5 summarizes the test–retest data at baseline plus the quality control study data on subjects measured three times over 3 wk. Intraclass correlations, technical errors, and CV are...
presented for a subset of variables to illustrate the various measurement domains of HERITAGE. Under both designs, the three indicators of reproducibility can be considered highly satisfactory and well within values reported in the literature for these types of measurements.

One aspect of the Quality Control Program was to verify periodically whether there were differences in measurements across clinical sites (24–27). No substantive differences among the four clinical centers were found. Likewise, there were no substantive differences between Blacks and Whites (23). Finally, data were interrogated across six time periods of the data collection phase to verify whether there was any evidence of drift over time (22). None was detected.

**General Overview of Statistical Analyses**

In general, standard statistical methods (e.g., correlations, linear regressions, general linear models, t-tests) were used to assess the associations within and between phenotypes at baseline and in response to exercise training. The specific procedures used were specific to the research questions and tests being performed. Models were run in the total sample or stratified by ethnic, sex, or age group, or post hoc analysis was used to determine differences between subgroups. In most HERITAGE individual studies, statistical significance was established at \( P < 0.05 \). Thus, for any text hereafter referring to results from previously published HERITAGE studies, particularly results related to clinical phenotypes (i.e., non-omics variables), the term “significance” refers to \( P < 0.05 \), unless otherwise noted. Moreover, if text states that a value “increased” or “decreased” without also using the word “significant,” it should be assumed the increase/decrease was significant unless otherwise noted.

**EXERCISE, ADIPOSITY, AND FAT DEPOTS**

The reproducibility of anthropometric and body composition measures in HERITAGE was determined in 60 men and women who completed the entire anthropometric and body composition test battery on three different days within a period of 3 wk (26). A high degree of reproducibility was observed across all traits measured and at each of the clinical centers. For instance, intraclass correlations ranged from 0.95 to 0.99 for anthropometric measures and from 0.97 to 1.00 for body composition measures, all with low technical error variance; this suggests that it would be possible to detect small changes in adiposity phenotypes in response to the endurance training program, with a view to investigate the genetic basis of these changes.

**Baseline observations and effects of training.** HERITAGE participants were slightly overweight, with average baseline BMI values ranging from 24.9 ± 13.9 kg·m\(^{-2}\) in White women to 27.9 ± 6.0 kg·m\(^{-2}\) in Black women (29). Significant ethnic differences were found for most adiposity phenotypes in women (but not in men), with higher values in Black women than in White women. At baseline, higher values of adiposity (sum of eight skinfolds, percent body fat, and FM) were observed in men than in women. Both before and after training, men had more AVF than women, Whites had more than Blacks, and parents had about twice as much adiposity as their offspring (14).

In response to training, there was only a marginal but significant decrease (delta values ± SE) in body weight (−0.2 ± 0.1 kg). Decreases were also observed for the sum of skinfolds (−6.2 ± 0.7 mm), waist girth (−0.9 ± 0.1 cm), percent body fat (−0.8 ± 0.1), FM (−0.7 ± 0.1 kg), abdominal subcutaneous fat (8.9 ± 1.1 cm\(^2\)), AVF (−4.6 ± 0.6 cm\(^2\)), and abdominal total fat (−13.5 ± 1.4 cm\(^2\)) in the total sample. The greatest changes were observed for total FM and percent body fat (>3%) and AVF (6%). There were several differences in training response by sex and ethnicity but not by generation (parents vs children). For example, reductions in FM and AVF were significantly higher in men than in women. For the sum of eight skinfolds, reductions were higher in Whites than in Blacks (14). After controlling for reductions in both BMI and waist girth, there were significant reductions in FM, percent body fat, and all CT abdominal fat measurements in all sex–ethnic groups (30).

**Familial aggregation studies.** The topic of familial aggregation in the amount and distribution of fat was addressed.
The heritability estimates for baseline AVF and FM in 86 White families reached 47% and 55%, respectively, with no sex or generation differences in the familial correlations for these measures (32). Figure 2 displays the CT AVF level adjusted for age, sex, and FM by family ranked on the basis of familial mean value. For a given level of total adiposity, there are families with low levels and others with high levels of visceral fat. The heritability of AVF adjusted for age, sex, and FM was 47% (32).

Familial aggregation of the amount and distribution of subcutaneous fat (skinfold measures of trunk fat, extremity fat, subcutaneous fat, and trunk to extremity fat ratio; waist circumference) at baseline and in response to training was examined in 483 White subjects from 99 families. Significant familial aggregation was found for all phenotypes measured at baseline and for training-induced changes in trunk fat and waist circumference (31). The heritability estimates for baseline skinfold measures of fat ranged from 31% (total subcutaneous fat) to 51% (waist circumference), whereas heritability estimates for the response phenotypes were lower (range, 14%–21%) and not significant for the change in waist circumference (31).

**CARDIORESPIRATORY FITNESS**

This section deals with indicators of maximal and submaximal exercise capacity, including \( \dot{V}O_2 \)max, maximal and submaximal PO (at 60% and 80% \( \dot{V}O_2 \)max), and \( \dot{V}O_2 \) at the ventilatory threshold (\( \dot{V}O_2 \)VT). Because exercise testing was done at four clinical centers, it was deemed important to determine the reproducibility of submaximal and maximal cycle ergometer exercise tests (as described previously). The within-subject SD for repeated \( \dot{V}O_2 \)max measures was 114 mL before training, 108 mL after training in >700 subjects, 137 mL for the 60 subjects tested three times in a period of 3 wk, and 115 mL in 8 traveling subjects tested four times, once at each clinical center, within a period of 2 wk. The CV for repeated measures ranged from 4.1% to 5.0% and the intraclass correlations were >0.96 (Refs. [20,27,33,34] and unpublished data). \( \dot{V}O_2 \)VT was also deemed satisfactorily reproducible with a test–retest correlation of 0.83 (35).

In a study on the accuracy of commonly used age-based prediction equations for maximal HR (HRmax), Sarzynski et al. (36) compared intrinsic (baseline or pretraining) HRmax obtained in 762 subjects who underwent two separate maximal exercise tests and where \( \dot{V}O_2 \)max was directly measured, and compared them with the Fox (220–age) (37) and Tanaka (208–0.7×age) (38) formulas. It was observed that the measured HRmax was not highly correlated with estimated HRmax using these formulas; the standard error of the estimate for the formulas was ~11–12 bpm and largely consistent across sex and ethnicity. These findings highlight the limitations of using age to estimate HRmax.

**Baseline observations.** HERITAGE participants were quite representative of sedentary populations of relevant age groups. As depicted in Figure 3, \( \dot{V}O_2 \)max (in milliliters of \( O_2 \) per kilogram of body mass) averaged 33 in the sample of 493 Whites and 27 in 245 Blacks, with SDs ranging from about 7 to 9. A wide range of cardiopulmonary fitness in the sedentary state was observed in each ethnic group, with a range of \( \dot{V}O_2 \)max values extending from 15 to 55.

**Training response.** The steady-state HR and \( \dot{V}O_2 \) were measured during cycle ergometer exercise tests before and
after training (39). The HR associated with 55%, 65%, 70%, and 75% of each subject’s pretraining \( \dot{V}O_2\text{max} \) was used to prescribe exercise intensity across the 20 wk of training. Using the linear relationship between HR, \( \dot{V}O_2 \), and PO, PO was predicted for each of the 60 training sessions at the respective programmed HR. The average ratio of the actual training HR to programmed HR was 0.99; that is, subjects typically trained within 1–2 bpm of their prescribed HR. One could ask whether minute-by-minute differences in the ratio of actual/predicted PO influenced the \( \dot{V}O_2\text{max} \) training response. Secondary analysis showed that the training program significantly increased mean \( \dot{V}O_2\text{max} \) across all actual/predicted PO classes (39). The minute-by-minute difference between actual and predicted PO accounted for 6% of the variance in gain in \( \dot{V}O_2\text{max} \) expressed in milliliters of O\(_2\). (33).

The training program produced significant improvements in \( \dot{V}O_2\text{max} \) (17%) and PO at 60% \( \dot{V}O_2\text{max} \) (26 W or 28%) (40). There was wide interindividual variation in \( \dot{V}O_2\text{max} \) response, with values ranging from −4.7% to +47.8% or −110 to +1100 mL \( \dot{V}O_2\text{min}^{-1} \), as illustrated in Figure 4A.

The effects of exercise training intensity relative to the VT on changes in PO (in watts) and \( \dot{V}O_2\text{VT} \), as well as changes in \( \dot{V}O_2\text{max} \) were evaluated (35). VT could be determined at baseline and after training in 432 sedentary White and Black men (\( n = 224 \)) and women (\( n = 208 \)). While training started at a fixed intensity according to their \( \dot{V}O_2\text{max} \) (individual’s HR corresponding to 55% of baseline \( \dot{V}O_2\text{max} \)), there was substantial variability in the training intensity relative to VT. The range of participant’s \( \dot{V}O_2 \) at VT to \( \dot{V}O_2\text{max} \) (VT%\( \dot{V}O_2\text{max} = \dot{V}O_2\text{VT}/\dot{V}O_2\text{max} \)) was 34% to 83%, reflecting differences in intrinsic fitness among participants despite everyone being sedentary for at least 3 months before the study. In analyses that related baseline VT%\( \dot{V}O_2\text{max} \) to the changes in \( \dot{V}O_2\text{VT} \) and \( \dot{V}O_2\text{max} \) after training, individuals who trained at higher intensities (relative to VT) had greater improvements in \( \dot{V}O_2\text{VT} \) after training. Overall, training intensity relative to VT accounted for ~26% of the training effect on \( \dot{V}O_2\text{VT} \), whereas the absolute training intensity (measured in watts) accounted for ~56% of the training effect. In contrast, there was no effect in the training intensity relative to VT on \( \dot{V}O_2\text{max} \) changes (35).

In a separate investigation, the changes in HR at a given percent of \( \dot{V}O_2\text{max} \) with training were investigated across the full HERITAGE cohort and by age, sex, and ethnicity (42).
Although HR decreased at the same absolute intensity (PO in watts) with training, HR at the same relative intensity (%VO$_2$) remained constant across all subgroups. These findings in a large and diverse cohort were the first to show that frequent testing and recalibration of HR relative to %VO$_2$max during a training program is unnecessary.

**Age, sex, and ethnicity.** The influence of age, sex, ethnicity, and initial fitness on the response of VO$_2$max to training was examined in 435 Whites and 198 Blacks (287 men and 346 women) (43). As expected, the average VO$_2$max in milliliters of O$_2$ per minute increased significantly for all sex and ethnic groups, but responses in men and women and Blacks and Whites of all ages were slightly heterogeneous (Table 6). However, there were no mean differences in the gains in VO$_2$max expressed as a percent of baseline levels across sex, ethnicity, and age classes. The mean gain was 17% ± 9% (43). Blacks began with lower values but had similar responses to those of Whites. Older subjects had a lower absolute change but a similar percent gain as younger subjects. There were low, average, and high responders at all ages, both sexes, both ethnic groups, and all levels of initial VO$_2$max. In the aggregate, age, sex, ethnicity, and initial fitness had limited influence on VO$_2$max response to standardized training in a large heterogeneous sample of sedentary Black and White men and women. It has been estimated that age, sex, ethnicity, and baseline VO$_2$max explain about 11% of the variance in the response of VO$_2$max to training, with age, sex, and ethnicity, each explaining about 3% and baseline level about 2% (33,44).

**Familial aggregation.** We reported that there was familial resemblance of intrinsic VO$_2$max levels (i.e., VO$_2$max in the sedentary state), with 2.7 times more variance between families than within families based on 86 White families, and a heritability level of 51% for VO$_2$max (in milliliters per minute) adjusted for age, sex, body mass, FM, and fat-free mass (45). A significant spousal correlation in intrinsic VO$_2$max (0.14 ≤ r ≤ 0.26) was observed, consistent with results from prior studies (46,47). Importantly, the variability in VO$_2$max in the sedentary state was best explained by a model in which maternal inheritance accounted for about 60% of the heritability level instead of the expected 50% (45).

Familial aggregation of three different measures of submaximal VO$_2$ (VO$_2$ at 60% and 80% VO$_2$max) and submaximal PO (PO at 60% and 80% VO$_2$max) was investigated in White participants at baseline (48). In analyses that adjusted for age, sex, and body mass, there were two to five times more variance between than within families for these baseline traits, with significant heritability estimates ranging from 48% to 74%, reflecting contributions from both genetic and shared environmental influences. These heritability estimates, as well as the individual patterns of familial resemblance (e.g., spouse, sibling, and parent–offspring correlations), were consistent with results from prior studies of submaximal performance measures from the Quebec Family Study (49) and family participants in the Canadian Fitness Survey (50).

The familial aggregation of the response of VO$_2$max to training was examined in 481 adults from 98 White families. There was 2.5 times more variance between families than within families for VO$_2$max response to training (41). This pattern of familial clustering of VO$_2$max trainability is illustrated in Figure 4B. The most parsimonious familial correlation model yielded a maximal heritability estimate of 47% for VO$_2$max response, with a maternal transmission accounting for slightly more than half of the estimate (about 28%). Significant familial aggregation was observed for VT traits, with heritability levels >50% for baseline measurements but more moderate levels for training responses (heritability ranging from 22% to 51%) (51). Heritability values ranged from 23% to 57% for the training response of other indicators of submaximal cardiorespiratory fitness (48). These findings are supported by findings from monozygotic twin studies showing significant within-pair correlations in training gains in submaximal exercise VO$_2$ and PO after training (7,52).

**EXERCISE AND HEMODYNAMICS.**

The data on exercise hemodynamics are derived from two maximal exercise tests performed at baseline, two maximal tests at 24 and 72 h after training, and submaximal exercise at 50 W and 60% VO$_2$max before and after the exercise program. Measurements included VE, VO$_2$, VCO$_2$, RER, SBP, DBP, HR, $O_2$, and SV, plus plasma glucose, free fatty acids, lactate, and total proteins. Cardiovascular, respiratory, and metabolic adaptations to submaximal exercise were moderately to highly reproducible, with CV values less than 9% and intraclass correlations greater than 0.80 (20,27). Reproducibility was generally better at higher PO. Except for SBP, DBP, and RER, cardiovascular, respiratory, and metabolic responses were also highly reproducible during maximal exercise, with intraclass correlations greater than 0.86 (20,27). Day-to-day variations were modest, and reproducibility of submaximal and maximal exercise values was comparable across the four clinical centers.

**Baseline familial aggregation studies.** A series of studies reported familial aggregation for intrinsic (baseline) cardiovascular variables and cardiorespiratory adaptation to training. The heritability of resting SBP and DBP among 482 White participants from 99 HERITAGE families was quantified (53). Heritability estimates for baseline SBP and DBP were 54% and 41%, respectively, and are comparable to those from prior studies (50,54,55) despite having lower CV values reflecting a more uniform (i.e., sedentary) HERITAGE sample combined...
with repeated baseline measurements. A subsequent investigation (56) showed substantially higher maximal heritability estimates for intrinsic resting SBP among African American families (68%), a population with limited existing data (57). Familial aggregation of exercise SV and \( Q \) at 60% VO\(_2\)max was significant with moderate heritability levels (46% for both) (58).

### Effects of the exercise program

Resting HR and BP responses to exercise training were investigated according to age, sex, and ethnicity. Small but consistent reductions in resting HR (-2.7 to -4.6 bpm) across subgroups were found (17). Prior studies have produced conflicting data about the effect of training on resting HR (59). In an ancillary study of HERITAGE, home-based HR monitoring was used to confirm the accuracy of resting HR recorded in the laboratory (17). Resting HR was measured on different days to determine intraindividual variability. Furthermore, and distinct from results of prior studies (60), there was no significant change in resting BP after training, which is likely explained by the lower baseline BP (118/68 mm Hg) of the participants. In contrast, SBP, DBP, and mean BP during submaximal exercise (at an absolute PO of 50 W) were generally lowered with training.

The response to training of SV, \( Q \), and arteriovenous oxygen difference (a-VO\(_2\) diff) during submaximal and maximal exercise was also evaluated (61) (Table 7). Steady-state VO\(_2\) at 50 W decreased only slightly (-3%) with training, likely the result of improved ergometer cycling efficiency. As expected, VO\(_2\) improved considerably at maximal PO (17.4%) and at 60% VO\(_2\)max (15.3%) with training. A significant decrease in HR was observed during exercise at 50 W (mean reduction of 11 bpm), but only a minor reduction of 4 bpm at 60% VO\(_2\)max, with no change in HRMax. Slight increases in SV (4%) and a-VO\(_2\) diff (2%) were found at 50 W, which in combination with the lower HR led to a slight decrease (-5%) in \( Q \) (-5%). During exercise at 60% VO\(_2\)max, there were substantial increases in SV (11%), \( Q \) (7%), and a-VO\(_2\) diff (6%) with training. Whereas HR at maximal exercise was unchanged, there were substantial gains in \( Q \) (10%) and VO\(_2\)max (17%). These observations are largely consistent with those from prior but smaller studies (62-65).

The role of menopause and hormone replacement on cardiovascular physiological traits was also examined (66). The response of submaximal and maximal exercise cardiovascular traits in premenopausal \( (n = 338) \) women, postmenopausal women on hormone replacement therapy (HRT; \( n = 28 \)), and postmenopausal women not on HRT \( (n = 29) \) were explored. In a novel finding, among all cardiovascular phenotypes examined, only baseline SBP during submaximal exercise (50 W) was significantly lower among premenopausal women than in postmenopausal women after adjustment for age. SBP during maximal but not submaximal exercise was significantly lower among postmenopausal women on HRT than those not taking HRT, a finding consistent with prior investigations (67,68). This may be due to estrogen’s vasodilatory effects at high exercise intensity. However, regular exercise-induced improvements in exercise hemodynamic traits among women are independent of age and menopausal or hormone replacement status.

### Familial aggregation of response to the exercise program

No familial aggregation was observed for resting BP or HR in response to training in the full HERITAGE cohort (53). However, comparing families who had elevated BP (>135 and/or 80 mm Hg, SBP or DBP, respectively) in this largely normotensive population with those who had normal BP, there was a genetic component (35%-44%) to the variance in training-induced resting HR and SBP changes. These findings are consistent with prior works (69) and further supported by a subsequent HERITAGE investigation (70), which found that SBP and HR responses to training were characterized by genetic contributions, but DBP responses seemed to be largely independent of family lines.

The familial aggregation of training-induced changes in cardiorespiratory hemodynamic traits was considered in a series of studies. Heritability estimates for the training-induced changes in submaximal exercise VO\(_2\) and PO (60% or 80% VO\(_2\)max) were significant (range of 23% to 57%), but tended to be lower than those for the baseline measures (48%-74%), as detailed previously (48). In contrast to baseline measurements, there was minimal correlation among spouses in training responses, suggesting that familial aggregation among these traits was largely due to genetic influences. The effects of the exercise program on HR and BP during submaximal exercise among 98 White families (n = 481 participants) were investigated, and heritability estimates of HR at 50 W and 60% VO\(_2\)max were 34% and 29%, respectively, and 22% for SBP at 50 W (71). These findings were independent of the effects of age, sex, BMI, tobacco use, and baseline VO\(_2\)max. In contrast, minimal heritability existed among DBP responses, SBP at 60% VO\(_2\)max, and all of the cardiovascular traits among Black participants (all families with a total of 257 participants). These findings were the first published analyses of familial aggregation of submaximal exercise BP and HR responses to an endurance exercise training program but have not been replicated yet.

The association of cardiorespiratory fitness with risk of future coronary heart disease (CHD) was examined using a revised Framingham Heart Study algorithm (72). Compared with the highest tertile, the lowest tertile of VO\(_2\)max had a 62% greater risk of future CHD. Another study found that

### TABLE 7. Changes with the exercise program in hemodynamic variables during submaximal and maximal exercise in HERITAGE participants.

| Variable                      | Pretraining | Posttraining | Change | % Change |
|-------------------------------|-------------|--------------|--------|----------|
| VO\(_2\) at 50 W (mL·min\(^{-1}\)) | 1029.7      | 994.9        | -34.8  | -3.2     |
| HR 50 W (bpm)                 | 102.0       | 108.8        | -6.4   | -6.3     |
| SV 50 W (mL per beat)         | 95.9        | 99.8         | 3.9    | 4.1      |
| Cardiac output 50 W (L·min\(^{-1}\)) | 11.3  | 10.7         | -0.6   | -5.3     |
| a-VO\(_2\) diff 50 W (mL per 100 mL per beat) | 9.2  | 9.4          | 0.2    | 2.2      |
| VO\(_2\) at 60% VO\(_2\)max (mL·min\(^{-1}\)) | 1423.3      | 1625.9       | 202.6  | 15.5     |
| HR 60% (bpm)                  | 140.1       | 135.8        | -4.3   | -3.1     |
| SV 60% (mL per beat)          | 96.6        | 109.2        | 10.6   | 10.8     |
| Cardiac output 60% (L·min\(^{-1}\)) | 13.7  | 14.7         | 1.0    | 7.3      |
| a-VO\(_2\) diff 60% (mL per 100 mL per beat) | 10.3  | 10.9         | 0.6    | 5.8      |
| VO\(_2\) diff (mL per 100 mL per beat) | 2343.4     | 2725.9       | 382.6  | 17.4     |
| Max HR (bpm)                  | 184.9       | 194.3        | 9.4    | 5.0      |
| Max cardiac output (L·min\(^{-1}\)) | 18.2      | 20.1         | 1.9    | 10.4     |

Based on >631 subjects with complete data. All training effects are significant at \( p < 0.05 \). Adapted from Wilmore et al. (61) and unpublished data.
those with VO₂max above the group median had lower levels of FM, AVF, and total abdominal fat than those below for a given BMI (30). These data suggest that lower total adiposity and abdominal fat is a mean by which higher VO₂max attenuates the health risks attributable to obesity.

EXERCISE, INSULIN, AND GLUCOSE METABOLISM

Fasting plasma glucose and insulin were assayed in the fasted state at baseline and 24 to 36 h after the last exercise training session. An IVGTT was performed before and 24 h after training with blood samples collected at 16 time points over 3 h to determine plasma glucose, insulin, and connecting peptide (C-peptide) concentrations, as described earlier (73). The protocol did not include an injection of insulin or tolbutamide. From the MINMOD Millenium software (19), the following traits were derived: Si, AIRg, Di, Kg, and Sg. The Si measures the ability of an increment in plasma insulin to enhance the net disappearance of glucose from plasma and is used as a measure of insulin sensitivity. The acute insulin response to glucose (AIRg) is defined as the integrated area under the insulin curve between 0 and 10 min of the IVGTT and represents a measurement of insulin response. The disposition index (Di) was calculated as Si multiplied by AIRg and measures the ability of the pancreatic beta cells to compensate for changes in insulin sensitivity. Kg is an estimate of the disappearance rate of plasma glucose calculated as the slope of glucose from 10 to 60 min postintravenous glucose injection. Glucose effectiveness (Sg) measures the ability of glucose, independent of change in plasma insulin, to increase glucose disposal and to suppress endogenous glucose output.

At baseline, mean Si was 61% higher in Whites than in Blacks and 12% higher in women than in men (74) (Table 8). Slight but clinically nonsignificant mean differences were seen in fasting glucose. Mean fasting insulin was comparable between men and women but was higher in Blacks than in Whites. The baseline AIRg was more than twice as high as in Blacks as in Whites. A positive correlation between Kg and glucose area below fasting glucose concentration was observed at baseline (r = 0.49, P < 0.001). Age was significantly correlated with baseline Si, Kg, AIRg, and Kg (r = −0.11, −0.13, −0.24, and −0.33, respectively; all P < 0.01).

**Effects of the exercise program.** Mean Si increased by 10% (P < 0.006) after the training program, along with a 3% (P < 0.001) decrease in AIRg, a nonsignificant 7% increase in Di, an index of glucose homeostasis and ability of beta cell to compensate for insulin resistance, a 11% (P < 0.002) increase in Kg, a 3% (P < 0.02) increase in Kg, and a 7% (P < 0.02) decrease in glucose area below fasting glucose concentration (74). Fasting plasma glucose was unchanged with training. However, among 55 subjects with impaired fasting glucose (5.6–7.0 mmol·L⁻¹ or 100–125 mg·dL⁻¹) at baseline, 24 became normoglycemic in response to the exercise program.

Interindividual variations in response to the exercise intervention were observed in IVGTT-derived measures (74). For instance, AIRg increased 7% in the quartile of subjects displaying the lowest baseline glucose tolerance, whereas it decreased 14% in the upper quartile. Such a diverging insulin response between the extreme quartiles of glucose tolerance warrants further investigation regarding its underlying biology. Another novel result was the significant reduction in area under the glucose curve below fasting levels (74). This suggests that training improves glucose regulation, not only by decreasing hyperglycemia but also by attenuating the risk of hypoglycemia.

In a secondary analysis, 38 HERITAGE Whites and 17 Blacks were found to have an increase in fasting insulin in response to the exercise program that was defined as adverse; that is, the increase was 24 pmol·L⁻¹ or more, which is twice the technical error for fasting insulin (75). Using the same definition of a fasting insulin adverse response, it was found across six exercise intervention studies, encompassing 1687 subjects, that the prevalence of an adverse fasting insulin response was 8.3%. Adverse responders were found in all six studies. This is a topic that has not received sufficient attention thus far and warrants further research because of its potential implications for public health and type 2 diabetes management.

A significant interaction (P = 0.02) with sex was found for the Si training response, with the mean increase in Si larger in men (15%) than in women (5%). The mean increase in Si was larger in Blacks (16%) than Whites (8%) and smaller in normal weight (6%) than in overweight (14%) and obese (15%) participants, but these differences were not statistically significant.

### TABLE 8. Measures of insulin and glucose homeostasis at baseline and posttraining in HERITAGE by sex and ethnicity.

| Variables | Total Participants (n ≥ 610) | Women (n ≥ 321) | Men (n ≥ 289) | Blacks (n ≥ 178) | Whites (n ≥ 432) |
|-----------|-----------------------------|----------------|--------------|----------------|-----------------|
|           | Before | After | Before | After | Before | After | Before | After | Before | After |
| Fasting glucose (mmol·L⁻¹) | 5.1 ± 0.6 | 5.2 ± 0.6* | 5.0 ± 0.6 | 5.1 ± 0.6* | 5.1 ± 0.6 | 5.3 ± 0.6 | 5.1 ± 0.6 | 5.3 ± 0.6* | 5.1 ± 0.6 | 5.1 ± 0.6** |
| Fasting insulin (pmol·L⁻¹) | 70.7 ± 50.4 | 64.2 ± 39.3* | 68.5 ± 50.3 | 61.5 ± 36.0* | 61.5 ± 36.0 | 67.1 ± 42.6* | 61.5 ± 36.0 | 67.1 ± 42.6* | 61.5 ± 36.0 | 67.1 ± 42.6* |
| Si (10⁻⁴ mmol·L⁻¹·μU·min) | 3.9 ± 2.9 | 4.1 ± 2.7* | 4.1 ± 2.9 | 4.2 ± 2.8 | 4.1 ± 2.9 | 4.2 ± 2.8 | 3.6 ± 2.6 | 4.1 ± 2.7* | 3.6 ± 2.6 | 4.1 ± 2.7* |
| AIRg (pmol·L⁻¹·10 min) | 956 ± 1042 | 903 ± 933* | 980 ± 1143 | 946 ± 1021 | 980 ± 1143 | 946 ± 1021 | 951 ± 924 | 855 ± 827* | 951 ± 924 | 855 ± 827* |
| Sg (min⁻¹) | 0.02 ± 0.01 | 0.02 ± 0.01* | 0.02 ± 0.01 | 0.02 ± 0.01* | 0.02 ± 0.01 | 0.02 ± 0.01* | 0.02 ± 0.009 | 0.02 ± 0.01* | 0.02 ± 0.009 | 0.02 ± 0.01* |
| Di (mmol·L⁻¹) | 2713 ± 2411 | 2902 ± 2473* | 2903 ± 2571 | 3053 ± 2714 | 2903 ± 2571 | 3053 ± 2714 | 2510 ± 2216 | 2741 ± 2177 | 2510 ± 2216 | 2741 ± 2177 |
| Kg (μg·kg⁻¹·min⁻¹) | 1.7 ± 0.6 | 1.8 ± 0.6* | 1.8 ± 0.6 | 1.8 ± 0.6* | 1.8 ± 0.6 | 1.8 ± 0.6* | 1.8 ± 0.7 | 1.9 ± 0.7* | 1.8 ± 0.7 | 1.9 ± 0.7* |

Values shown as mean ± SD.

*Units are taken from the MINMOD program. To convert value to SI units (10⁻⁴ mmol·L⁻¹·μU·min), multiply by 0.167.

**Di is calculated as Si generated from the MINMOD program (10⁻⁴ mmol·L⁻¹·μU·min) multiplied by AIRg (pmol·L⁻¹·10 min).

**P < 0.05 for difference between baseline and posttraining mean values.

**P = 0.05 for difference between baseline and posttraining mean values.

**P < 0.05 for difference in mean training response value between ethnic groups.

Si, insulin sensitivity index; AIRg, acute insulin response to glucose; Sg, glucose effectiveness; Di, disposition index; Kg, glucose disappearance rate

Adapted from Boule et al. (74) and unpublished data.
significant. Similarly, there was no difference in the Si response between 272 premenopausal and 40 postmenopausal women (74). No difference between Blacks and Whites was found for the AIRg response to training. At baseline, the IVGTT area under the glucose curve below fasting levels was inversely correlated with changes in Kg (r = −0.30, P < 0.001), suggesting that those who had the lowest glucose at the end of the IVGTT had smaller improvements in glucose tolerance. Changes in AIRg were weakly but positively associated (r = 0.09–0.13, P < 0.05), whereas changes in Si were not associated with changes in body mass, waist circumference, cardiorespiratory fitness, and plasma LDL-C, HDL cholesterol (HDL-C), or TG. These findings suggest that participants who had more weight loss had a greater reduction in insulin secretion, despite not having larger changes in insulin sensitivity (74).

After the 20-wk training program, fasting plasma insulin and glucose were measured 1 and 3 d after the last exercise bout (74). Fasting glucose in 547 participants slightly increased between 24 and 72 h after the last exercise bout, a response that was larger in Blacks than in Whites. Fasting insulin was decreased by 8% 24 h after the last exercise bout, which was comparable to the mean 10% increase in Si estimated from IVGTT. Moreover, fasting insulin returned to baseline levels after 72 h. This finding is consistent with those of previous studies, suggesting that improvements in insulin sensitivity and glucose tolerance were short-lived and became undetectable 60–72 h after the last exercise session (76–78).

**Familial aggregation of IVGTT-derived traits.** Significant familial resemblance and heritability coefficients were observed for the baseline level of the IVGTT-derived variables in both Blacks and Whites (79). After adjustment for age, sex, and BMI, the heritability estimates in Blacks and Whites, respectively, were 48% and 25% for Kg, 44% and 46% for AIRg, 38% and 44% for Si, and 32% and 24% for Di. Interestingly, Blacks had significantly higher heritability for overall glucose tolerance than Whites, but there was no ethnic difference in heritability estimates for insulin sensitivity or insulin secretion. No sex or generation differences were found in the familial correlations for any of the MINMOD traits (79). In the FUSION Study (a cohort of offspring and spouses of adults with type 2 diabetes), the reported heritability estimates for Di (23%) and AIRg (35%) were comparable to those from HERITAGE, whereas the heritability estimates for Si (28%), Sg (18%), and AIRg (35%) were somewhat lower in FUSION (80).

Changes in fasting insulin, Si, and Sg were characterized by significant familial aggregation in their response patterns. Specifically, familial aggregation analyses based on general linear models with adjusted data in MERLIN (81) showed that heritability estimates reached 8% for fasting insulin, 14% for Sg, and 16% for Si responses to exercise training (unpublished data). Changes induced by the exercise program in AIRg were not characterized by significant familial resemblance.

In summary, the large sample of participants in a well-controlled exercise regimen in HERITAGE provided a unique experimental context to study individual variations in the response of glucose and insulin metabolism to training. On average, training led to beneficial changes in fasting insulin levels, insulin sensitivity, and other glucose-insulin–related traits derived from an IVGTT. Fasting glucose and insulin measurements made 24 and 72 h after training indicate that, on average, the favorable changes are greatly attenuated 3 d after the last exercise session. A more beneficial response pattern was observed among participants who had the least favorable glucose-insulin profile at baseline.

**TRAINING, LIPID, LIPOPROTEINS, AND LIPASES**

Plasma lipid and lipoprotein levels were assayed from blood samples collected 2 d apart in the morning after a 12-h fast at baseline and, for most variables, 24 and 72 h after the last training session. Concentration values were adjusted for potential plasma volume changes resulting from the training program based on plasma total protein concentration difference between baseline and posttraining measurements. Plasma TC and TG levels were determined, and the following lipoprotein fractions were assayed by enzymatic methods: VLDL, HDL, and LDL. ApoB and ApoA-I concentrations were measured, and those of LDL-C, LDL-TG, and VLDL-ApoB were calculated. LPL and HL activities were measured in plasma obtained from 12-h fasted subjects, 10 min after intravenous injection of heparin (60 IU·kg<sup>−1</sup> of body mass). The procedures and assays used have been described in several publications (18,82–85). Reproducibility of assays and the day-to-day variation in plasma levels have been reported in detail (18). Briefly, the day-to-day variation in lipid and lipoprotein fractions was estimated to be small, with intraclass coefficients >0.87 for repeated measurements in 379 participants for all traits except HDL<sub>3</sub>-C (0.79). Similar levels of stability were observed based on the assays performed on three samples obtained within 3 wk in 60 subjects. Again, HDL<sub>3</sub>-C had the lowest coefficient of 0.71. CV values were 5% to 8% for all traits except TG (about 20%) and VLDL-C (about 30%). However, when the assays were repeated on split samples from the same blood draw, the intraclass coefficients were >0.93, with CV values <5%, except for HDL<sub>3</sub>-C (10%). Plasma post-HL and LPL were also reliably measured with split-sample repeatability coefficients >0.95.

**Baseline Observations**

The baseline plasma lipid, lipoprotein, and lipase activity profiles were compared between men and women and Blacks and Whites and associated with cardiometabolic risk factors. Sex differences were examined in a series of publications in Whites (18,82,86), whereas ethnic and sex differences were examined in two other articles (29,83). In general, men had less favorable profiles than women (18,83,86) and Blacks more favorable profiles than Whites (83) (Table 9). The ethnic differences in plasma HL and LPL activities are concordant with results from previous studies (88–90). The differences in plasma lipid profile between men and women were largely explained by differences in visceral adiposity and plasma LPL activity, whereas ethnicity
had only a minor contribution (29,83). Total body adiposity accounted for 26% to 36% of the variance in baseline blood lipids (29). Higher LPL activity was associated with a more favorable lipid–lipoprotein profile, whereas higher HL activity was associated with an unfavorable profile in both men and women (86). Fasting insulin was the only significant predictor of HDL-C in women (86). Fasting insulin was the only significant predictor of LPL activity in women (negative association), whereas menstrual status and fasting insulin (negative associations) and plasma sex hormone–binding globulin (SHBG) levels (positive association) were all independent predictors of LPL activity in women (86).

The distribution of the apolipoprotein composition of HDL, specifically the concentration of HDL containing apolipoprotein A-I only (LpAI) versus HDL with both apoA-I and apoA-II (LpAI/All), was examined (82). Women had higher LpAI and lower LpAI/All levels than men. As expected, LpAI was correlated with HDL2-C, whereas LpAI/All was more closely associated with HDL3-C levels in both sexes. In both sexes, higher LpAI levels were associated with higher HDL-C and apoA-I and lower TC/HDL-C ratio, whereas LpAI/All was positively correlated with TC, apoA-I, apoB, and insulin. LpAI levels were negatively correlated with body FM, waist circumference, and fasting levels of TG, apoB, and insulin in women only.

Familial Aggregation Studies at Baseline

Familial aggregation of baseline plasma lipid, lipoprotein, and lipase traits was assessed in 437 sedentary, White adults (171 parents, 266 offspring) from 86 families (85). Significant familial aggregation was observed for all age-adjusted phenotypes (TC, HDL-C, LDL-C, TG, LPL, HL), with heritability estimates of 62%, 83%, and 50% for TC, HDL-C, and LDL-C, respectively. For plasma TG levels and HL activity, the heritability estimates were 55% and 40%, respectively, but significant spouse correlations were found, suggesting common familial environment contributed to familial resemblance in addition to genetic factors. For plasma LPL activity, sex differences were found, with higher heritability in female pairs (76%) than in male pairs (30%) and opposite-sex pairs (44%).

Effects of the Training Program

The training responses of the lipid, lipoprotein, and lipase profile are summarized in Table 9. A 3.6% increase in plasma HDL-C was found in the total sample, mostly due to an increase in HDL2-C, with an associated increase of apoA-I (84). Significant reductions in plasma TG and VLDL-TG levels were observed in the 24-h samples only, reflecting a response to the last bout of exercise and not a training effect, per se. No changes were observed in TC, LDL-C, VLDL-C, and apoB. The findings for HDL-C are concordant with those from a review of 100 training studies that found changes in HDL-C ranged from 4% to 22% (91), as well as from a meta-analysis that found a modest net increase in HDL-C of 2.53 mg·dL⁻¹ across 25 randomized clinical trials (92).

Effects of training on the lipoprotein subclass profile

The investigation of the effects of training on lipids–lipoproteins was expanded by examining changes in the lipoprotein subclass profile measured by NMR (LabCorp, Morrisville, NC) (93). Overall, the concentration of large HDL (HDL-P) and large LDL (LDL-P) particles increased with training, while the concentration of small LDL-P and total, large, medium, and small VLDL-P and mean VLDL-P size decreased with training. These changes mirrored those found in a previous HERITAGE report, where the increase in HDL-C was mostly driven by increases in the larger HDL2-C subclass and not the smaller HDL3-C subclass (84). These results were expanded in a meta-analysis of 10 exercise interventions from six studies (including HERITAGE), which found significant changes in the following lipoprotein subclasses (independent of age, sex, ethnicity, baseline BMI, and baseline trait value): decreases in the concentration of large VLDL-P, small LDL-P, and medium HDL-P and mean
VLDL-P size, with significant increases in the concentration of large LDL-P and large HDL-P and mean LDL-P size (93). These results highlight that despite several studies showing no changes in some of the traditional measures of lipids—lipoproteins (e.g., TG and LDL-C did not change in HERITAGE), NMR subclass profiling revealed significant and favorable changes in several lipoprotein subclasses in response to training.

**Association of plasma lipid, lipoprotein, and lipase activity responses to training, with concomitant changes in cardiometabolic risk factors.** A study categorized men into four groups based on baseline TG (low/high) and HDL-C (low/high) concentrations and found that endurance training may be particularly beneficial in men with low HDL-C, elevated TG, and abdominal obesity (94). Greater fat loss, characterized by loss of FM, AVF, and abdominal subcutaneous fat, was associated with a better lipid response to training, characterized by an increase in HDL-C and decreases in TG, LDL-C, TC, and TC/HDL-C (29). In fact, a composite variate of change in body fat accounted for 21% of the variance in a composite variate of change in blood lipids in Blacks and 7–10% in Whites. Change in AVF was not as strongly associated with changes in lipids in Blacks as in Whites. Changes in lipid traits were not related to concomitant changes in VO2max (95). Weak but significant correlations were also found between change in submaximal HR at 50 W with change in HDL2-C (r = −0.17; significant in men, Whites) and LDL-C (r = 0.09, significant in men and Blacks) (40).

Although the predictors of lipoprotein subclass trait responses to training are largely unknown, a recent study (96) found that baseline levels of a circulating biomarker of liver fat called dimethylguanidino valeric acid (DMGV) were positively associated with changes in small HDL-P (β = 0.14) and inversely associated with changes in medium and total HDL-P (β = −0.15 and −0.19, respectively) and apoA-I (β = −0.14).

**Ethnic and sex differences in plasma lipid, lipoprotein, and lipase activity responses to training.** No differences in the HDL-C response were noted by age, sex, or ethnicity (84,87). However, Blacks experienced greater increases in HDL2-C. Relative to apoA-I, there were greater increases in women than in men, in Blacks than in Whites, and in offspring than in parents (84). At the univariate level, absolute change in FM was correlated with change in HDL-C (r = −0.23), HDL2-C (r = −0.17), and TC/HDL-C (r = 0.24) in men and with change in LDL-C (r = 0.22), TC (r = 0.19), and TC/HDL-C (r = 0.15) in women (95). Ethnicity was a significant predictor of changes in TG in both sexes.

Significant reductions in plasma HL activity and increases of LPL activity were observed in all sex and ethnic subgroups, except that the increase in LPL activity was not significant in Black men (97). Change in LPL activity was correlated with change in AVF (r = −0.17 for Whites and −0.26 for Blacks). In Whites, change in LPL activity was correlated with change in waist circumference (r = −0.18) and subcutaneous fat (r = −0.23), as well as change in TG (r = −0.20) and HDL-C and HDL2-C (r = 0.21 and 0.23). The finding of increased LPL activity associated with a decrease in TG reflects the hypothesis of an increased ability to clear TG-rich lipoproteins with regular exercise.

**Variability in plasma lipid and lipoprotein responses to training.** Although the mean increase in HDL-C was 3.6%, there was wide variability in responsiveness to training, ranging from a mean decrease of 9.3% in quartile 1 (mean reduction of 0.11 mmol·L−1) to a mean increase of 18% in quartile 4 (mean increase of 0.18 mmol·L−1) of the HDL-C response distribution (87). Parallel changes in HDL2-C and HDL3-C, apoA-I levels, and LPL activity were seen across quartiles of HDL-C response. A multivariate regression analysis that included baseline variables accounted for 15.5% of the variability in HDL-C response to training, with percent change in TG accounting for 8.8%, baseline HDL-C for 1.5%, baseline LPL activity for 1.2%, and baseline insulin for 1.0% (87).

The variability in lipid and lipoprotein responses to training was also highlighted in the previously mentioned study.

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**Changes with exercise training in plasma HDL-cholesterol and triglycerides in HERITAGE**

![Graphs](https://example.com/graph.png)

**FIGURE 5—Distribution of the response of HDL-C (left panel) and TG (right panel) to the HERITAGE exercise program with potential adverse responders highlighted in red, and the most favorable responders shown in green, both defined as plus or minus 2 × the technical error for each trait. To convert mmol·L−1 of HDL-C to mg·dL−1, divide by 0.02586. To convert mmol·L−1 of TG to mg·dL−1, divide by 0.01129. Adapted with permission from Bouchard et al. (75).**
examining the prevalence of potentially unfavorable responses to training across six studies (75). Adverse responses were defined as a decrease in HDL-C ≤ 0.12 mmol·L⁻¹ or an increase of TG ≥ 0.42 mmol·L⁻¹. Figure 5 depicts the changes in HDL-C and TG for all HERITAGE subjects, with the individual values highlighted in red representing those with unfavorable responses. The prevalence of adverse response for change in HDL-C was 6% in Whites and 8% in Blacks and 8% in both Blacks and Whites for change in TG. Across all six studies \( (n = 1687) \), the prevalence of adverse response was 13.3% for HDL-C and 10.3% for TG. The prevalence of unfavorable responses seemed to be similar across variable doses of training.

### Familial Aggregation Studies of Training Response

Familial aggregation was the strongest predictor of interindividual variation in plasma lipid responsiveness to training. For example, there was 1.8 times more variance between families than within families for HDL-C changes (44). Familial correlations for the training responses were significant for most lipid–lipoprotein variables, with heritability estimates ranging from 25% to 38% (98). Exceptions were for heritability levels near 60% for changes in apoB in Blacks and HDL2-C in Whites from 25% to 38% (98). Exceptions were for heritability levels ranging from 26% (Blacks) to 29% (Whites) for change in LDL-C in Blacks and from 29% (Whites) to 32% (Blacks) for change in TG. In Whites, the heritability estimate for training-induced changes in LPL activity was 15% (99).

### TRAINING, STEROIDS, AND OTHER HORMONES

Although mass spectrometry measurements are nowadays considered the gold standard for the quantification of plasma steroid levels (100), the assays used in HERITAGE were antibody based. However, the approach included prior extraction of steroids as well as high-pressure liquid chromatography separation before antibody measurements, and each step of the process was adequately controlled through calculation of recovery rates. Moreover, two blood samples were obtained at least 24 h apart at baseline, as well as two samples after training, one 24 and the other 72 h after the last exercise session. A large panel of steroid hormones or their derivatives were assayed (see Ukkola et al. (101) and He et al. (28)), including commonly measured hormones such as TESTO, E2, PROG, ALDO, and CORT. Steroid precursors such as DELTA4, PREGE, or the free, fatty-acid ester, and sulfated moieties of dehydroepiandrosterone (DHEA, DHEAE, DHEAS) were also examined, in addition to steroid metabolites such as DHT, ADTG, and androstenediol glucuronide (DIOLG). Circulating levels of the peptide steroid transporter SHBG were also examined.

Extensive quality control analyses were used to quantify the reproducibility of the steroid assays and document the day-to-day variability of hormone levels in the HERITAGE population. Moreover, blood samples were obtained in the early follicular phase of the menstrual cycle in premenopausal women before and after training. In brief, the reliability of repeated assays from split samples was highly significant, with intraclass coefficients generally >0.9 in 35 males and 25 females (28). Test-retest on 2 d apart in the whole cohort yielded coefficients of >0.8 in men and women, except for CORT (0.52) and E2 in males (0.59). All assays performed three times over 3 wk had intraclass coefficients of ≥0.70, except for ALDO in women (0.40), CORT in men (0.55), and PREGE in men (0.69).

**Baseline observations.** The baseline levels of all steroids and their response to training for the sample of men by ethnicity are displayed in Table 10. The steroid levels before and after training are shown in Table 11 for premenopausal women taking or not taking oral contraceptives, and for postmenopausal women with and without HRT.

Baseline levels of plasma steroids were examined in several publications (101,102,104). As summarized by Ukkola and colleagues (101), age was a predictor for most steroid hormones, with the strongest relationship observed for DHEAS \( (r = -0.39) \), whereas BMI was also associated with the

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**TABLE 10.** Steroid hormone levels at baseline and in response to the HERITAGE exercise program in men.

| Variables | Men \( (n = 267) \) | Blacks \( (n = 67) \) | Whites \( (n = 200) \) |
|-----------|----------------|----------------|----------------|
|           | Before | After | Before | After | Before | After |
| ADTG      | 156 ± 85 | 158 ± 91 | 160 ± 70 | 157 ± 67 | 155 ± 89 | 158 ± 98 |
| ALDO      | 0.26 ± 0.1 | 0.28 ± 0.2 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.3 ± 0.2 |
| CORT      | 375 ± 11 | 394 ± 12 | 349 ± 99 | 364 ± 113 | 393 ± 113 | 404 ± 123 |
| DELTA4    | 2.8 ± 1.4 | 2.7 ± 1.3 | 2.7 ± 1.1 | 2.4 ± 1 | 2.8 ± 1.5 | 2.9 ± 1.4*** |
| DHEA      | 15.6 ± 10.0 | 14.5 ± 9.1* | 14.7 ± 6.6 | 12.9 ± 5.7 | 15.9 ± 10.9 | 15 ± 10 |
| DHEAE     | 9.3 ± 5.7 | 8.7 ± 5.1 | 10.1 ± 4.7 | 8.1 ± 3.6 | 9 ± 6 | 8.9 ± 5.5** |
| DHEAS     | 5652 ± 3091 | 5593 ± 3032 | 5469 ± 2586 | 5065 ± 2247 | 5715 ± 3247 | 5770 ± 3238*** |
| DHT       | 2.7 ± 1.2 | 2.7 ± 1.1 | 3.2 ± 1.3 | 3.0 ± 1.3 | 2.6 ± 1.2*** | 2.6 ± 1.0 |
| DIOLG     | 28.5 ± 13.7 | 28.9 ± 20.8 | 29.5 ± 12.6 | 30.8 ± 13.6 | 28.2 ± 14.1 | 29.6 ± 22.8 |
| E2        | 71.3 ± 44.1 | 72.3 ± 43.6 | 79.3 ± 39.8 | 82.2 ± 50.8 | 68.6 ± 45.2 | 69.0 ± 40.3 |
| OHPROG    | 5.5 ± 2.8 | 5.5 ± 2.7 | 4.3 ± 2.2 | 4.5 ± 2.1 | 5.8 ± 2.9*** | 5.9 ± 2.9 |
| PREGE     | 16.6 ± 9.5 | 15.3 ± 8.8* | 18.8 ± 9.2 | 14.9 ± 6.8 | 15.8 ± 9.6 | 15.5 ± 9.4*** |
| PROG      | 1.66 ± 0.8 | 1.63 ± 0.9 | 1.80 ± 0.8 | 1.70 ± 0.8 | 1.60 ± 0.8 | 1.60 ± 0.9 |
| SHBG      | 39.2 ± 16.5 | 39.2 ± 16.2 | 37.8 ± 15.7 | 38.5 ± 15.4 | 39.8 ± 16.7 | 39.4 ± 16.5 |
| TESTO     | 15.0 ± 6.9 | 15.1 ± 6.8 | 16.2 ± 6 | 16.2 ± 5.9 | 14.7 ± 6 | 14.7 ± 5.5 |

Units are nmol·L⁻¹, except for E2 (pmol·L⁻¹). *P < 0.05 for difference between baseline and posttraining mean values. **P < 0.05 for differences between ethnic groups. ***P < 0.05 for differences between ethnic groups at baseline. Adapted from Ukkola et al. (101), He et al. (102), He et al. (103), and unpublished data.
variability of several measurements, including SHBG (r = 0.20) and TESTO (r = 0.12). When age and BMI were taken into account, ethnicity still contributed significantly to variations in CORT and DHT (for men and women) and in PROG for women. However, ethnicity had a much lower influence on plasma steroid levels than age and BMI. In addition, caloric and nutrient intakes, and smoking influenced steroid hormone variability, but their contributions were minor once age, BMI, and ethnicity were taken into account (101).

The well-documented decline in androgen levels with age observed in males (105,106) was also observed in men of HERITAGE. Interestingly, the concomitant age-related increase in total body FM explained an important part of the variation in steroid levels in men, particularly for TESTO levels (104). These results are consistent with those from studies showing an important contribution of adiposity indices to variations in steroid hormone levels in men (107), women (108) and in men and women of HERITAGE (102). In another HERITAGE report, baseline sex hormone and SHBG levels were examined in men and women with an emphasis on postpartum lipolytic enzyme activities (86). Activities of LPL and HL are known to be sensitive to androgens and estrogens, which likely explains part of the sex differences in blood lipids, particularly for HDL-C (109). TESTO was found to be an independent predictor of HL activity (r = 0.13), consistent with the known effects of androgens in decreasing HDL-C levels. In women, SHBG levels, which are regulated in part by androgens and estrogens, were correlated with HL activity (r = −0.39), independent of adiposity. This study showed that the sensitivity of lipase activities to sex hormones was confirmed in a large sample of men and women (86).

At baseline, increasing VO_{2max} was related to higher androgen levels (TESTO and DHT), as well as higher SHBG levels after adjustment for age and ethnicity (28). In women, no significant hormonal variations were observed as a function of VO_{2max}, except for the free androgen index (TESTO/SHBG × 100) which was lower across increasing baseline VO_{2max} quartiles. However, the overall strength of the associations between baseline hormones and VO_{2max} was relatively low.

**Familial aggregation at baseline.** Baseline hormone and SHBG levels were examined as a function of familial aggregation (110–114). The factors examined in these analyses were DHEA, DHEAE, and DHEAS (111,113), SHBG (110,112), and androgens and glucuronide conjugates (114). Regarding DHEAS, Whites showed a heritability estimate of 58% at baseline. Segregation analyses suggested a major Mendelian locus affecting baseline DHEAS accounting for about half of the trait variance (111). Ethnicity-related differences in familial aggregation patterns were also examined for DHEAS, and a comparable level of heritability of 66% was found (113).

Baseline levels of SHBG differed by sex, with higher levels in women, whereas SHBG levels were higher in fathers than in sons (111). Familial aggregation analyses of SHBG levels are consistent with the idea that multiple factors regulate the secretion of this hepatokine (115). A significant amount of the variance in SHBG was accounted for by TESTO, E2, age, BMI, and insulin. Nevertheless, the heritability estimate was 64%, independent of these factors. Analyses in Blacks revealed similar heritability levels (110).

CORT levels were significantly different between sex and generation groups at baseline in Whites, with higher values in females and offspring (116). In Blacks, lower baseline CORT levels were found in mothers than in fathers and higher levels in offspring and in daughters than in sons. The heritability of CORT was lower than for other steroids examined (116). Interestingly, familial cross-trait correlations and bivariate segregation analyses generated consistent results, suggesting common familial components underlying CORT and body fat covariation caused by common polygenic determinants (117). Such genetic pleiotropy was not found for the association of SHBG and adiposity indices (118). Evidence of genetic pleiotropy between CORT levels and adiposity phenotypes agrees with the well-known coregulation of adiposity, body fat distribution, and glucocorticoid homeostasis (119,120).
Finally, familial resemblance of circulating androgens and androgen metabolites at baseline was also examined (114). There were sex and generation differences at baseline, with parents having lower levels than offspring and males higher levels than females in both generations and ethnicities. This analysis again revealed high heritability estimates for most of the androgens and metabolites examined. For example, heritability levels in Whites reached 69% and 87% for TESTO and DHT, respectively, which were similar in Blacks (70% and 73%, respectively). The androgen metabolite DIOGL has been the focus of many studies in the field of obesity and body fat distribution (121,122). The relatively high heritability estimates obtained for this steroid (74% and 62% in Whites and Blacks, respectively) need to be considered in light of results from more recent twin studies indicating that differences in circulating DHT between lean and heavier co-twins are related to increased expression of androgen-metabolizing enzyme aldoketo reductase 1C2 in adipose tissue (123,124). Genome-wide linkage scans were performed in HERITAGE to identify loci influencing the various moieties of DHEA (125), as well as other steroids and SHBG (126). The results from these studies are of particular interest in the context of the growing body of evidence supporting the existence of an elaborate set of steroid-converting enzymes in human adipose tissues (122).

**Effects of the exercise program.** The training program had only marginal effects on fasting plasma levels of steroid hormones and SHBG, as evidenced by data in Tables 10 and 11. In males, only CORT had a significant increase with training, whereas DHEA and PREGE showed a decrease (Table 10). In premenopausal women not taking oral contraceptives, DHEAS increased, whereas DHEA and PREGE showed a decrease (Table 10). No significant change in any steroid was observed in postmenopausal women in those taking oral contraceptives (Table 11). No significant change in any steroid was observed in postmenopausal women with or without HRT, a finding that may have been impacted by the relatively small sample size of women in each group.

The impact of baseline steroid levels on changes in cardiorespiratory fitness and body composition in response to training was examined in HERITAGE (28,102,103). Sex hormones were not related to the change in V̇O₂max, suggesting that high baseline endogenous androgen levels do not predict higher responses to endurance exercise training in previously sedentary adults (28). No association was detected between baseline steroid hormone levels and changes in adiposity traits (102) or the improvements in resting and exercise BP (103). In another report, the impact of menopausal status and HRT on exercise hemodynamics during exercise was examined (66). Menopausal status was found to influence training adaptation of SBP, as menopausal women improved more. Age was a major covariate of variability in hemodynamic parameters (66). Postmenopausal women showed less improvement in SBP during submaximal exercise with the training program when taking HRT (66). Among other findings, no sex, generation, or ethnic differences were observed for mean change in SHBG with training (110). In Blacks, no differences between sexes and generations were observed for CORT training response.

**Familial aggregation of responses to the training program.** An analysis based on White families showed that changes in plasma DHEAS was characterized by a nonsignificant heritability level of 30% (113). The training response of SHBG level was also characterized by very low heritability estimates (110,112). Segregation analyses based on the White families provided evidence for a major locus effect influencing the CORT response to training; this was not detected in Blacks, perhaps because of the smaller sample size (116).

### TRAINING AND OXIDATIVE STRESS

Excess reactive oxygen species production is thought to contribute to the development of atherosclerosis and cardiovascular disease. Oxidative alterations of LDL particles are thought to play a critical role in atherosclerosis and inflammatory processes. Hemolysis induced by reactive oxygen species is an indicator of the overall antioxidant capacity. An ancillary study investigated these areas on a HERITAGE subsample at baseline and in response to training, with an emphasis on total homocysteine (tHcy), LDL oxidation, erythrocyte glutathione peroxidase activity (GSH-Px), retinol, and erythrocyte resistance to hemolysis (127).

**Baseline characteristics.** Significant sex and ethnic differences in tHcy were observed before training in the full cohort (n = 816). tHcy was highest in Black men (11.0 ± 7.2 μmol·L⁻¹), followed by White men (9.4 ± 3.7 μmol·L⁻¹), Black women (8.5 ± 4.2 μmol·L⁻¹), and White women (7.7 ± 2.7 μmol·L⁻¹) (128). Although results from cross-sectional studies corroborate

| Oxidative Stress Trait | n  | Mean (SD) | F   | H²  | Mean (SD) | Training Effect (P Value) | F   | H²  |
|-------------------------|----|-----------|-----|-----|-----------|---------------------------|-----|-----|
| Plasma glutathione (μmol·L⁻¹) | 142 | 4.5 (1.5) | 2.56 | 0.44 | 0.04 (1.8) | NS | 3.29 | 0.46 |
| Erythrocyte antioxidant enzyme GSH-Px (U·g⁻¹ hemoglobin erythrocyte) | 109 | 57.2 (15.4) | 6.09 | 1.00 | -0.5 | NS | NS | 0.002 |
| Resistance to hemolysis | 106 | 75.9 (14.0) | 3.06 | 0.63 | 3.6 (16.1) | 0.02 | 2.08 | 0.35 |
| (C50-AAPH; mmol·L⁻¹) | | | | | | | | |
| LDL oxidation lag phase (min) | 71 | 55.4 (23.3) | 3.12 | 0.38 | 6.2 (38.9) | NS | 2.97 | 0.84 |
| LDL oxidation propagation rate (mmol·mg⁻¹ LDL·min⁻¹) | 71 | 8.9 (2.6) | 2.02 | 0.31 | 0.3 (4.0) | NS | 2.12 | 0.44 |
| Plasma TBARS (nmol MDL·L⁻¹) | 138 | 1.9 (0.7) | 3.19 | 0.43 | -0.04 (0.9) | NS | 3.15 | 0.51 |

Mean values and training response P values have not been adjusted for any covariates. F ratio compares the between-family with the within-family variances and thus provides an estimate of familial resemblance. H² is the heritability coefficient. NS, not significant (P > 0.05).

Adapted with permission from Blache et al. (127) and unpublished data.
the finding of greater tHcy in males, evidence for ethnic differences is mixed, with some studies reporting White males having the highest tHcy levels (129) and others reporting no significant differences by ethnicity (130). Sex differences did not persist in measures of oxidative stress other than tHcy.

Other measures of oxidative stress were examined in a subset of 146 subjects from 28 two-generation, White, nuclear families from the Quebec clinical center (Table 12). A significant proportion (31.6%) of the variation in LDL oxidation lag time was accounted for by LDL-C, plasma α-tocopherol, serum uric acid, and plasma phospholipid oleic acid (127). Baseline plasma α-tocopherol, β-carotene, and retinol levels, as well as GSH-Px, were all significantly higher in parents than in children of either sex, whereas plasma glutathione was higher in children than in parents (127). The relationship between oxidative stress and aging is well established, particularly mitochondria oxidative stress commonly considered as one of the drivers of the aging process (131). Thus, the observed differences in baseline markers of oxidative stress across generations were expected and support an association between aging and elevated oxidative stress.

Effects of the training program. tHcy did not change in response to training. However, a subgroup analysis revealed differential responses by baseline tHcy levels and ethnicity. Exercise training resulted in a significant reduction of tHcy (−3.5 ± 7.0 μmol·L−1) in subjects with elevated baseline tHcy (≥15 μmol·L−1), whereas a significant increase (+0.3 ± 1.5 μmol·L−1) was observed in subjects with normal baseline levels of tHcy (<15 μmol·L−1) (128). Furthermore, training resulted in a nominal decrease in tHcy in Black males and a nominal increase in White males (P = 0.04 between Blacks and Whites) (128). In the ancillary study of Whites from the Quebec center, the only measure of oxidative stress that significantly changed with training was erythrocyte resistance to hemolysis, with no change in the other markers (Table 12). Interestingly, smoking status was a significant modifier of the beneficial erythrocyte response to training. Instead of beneficial effects of training, erythrocytes of smokers had a greater susceptibility to hemolysis after training (127). In sex-specific analyses, the percent increase in total glutathione was significant in both men and women, whereas erythrocyte resistance to hemolysis and plasma α-tocopherol was improved with training in women only (127).

Familial aggregation profile. There was significant familial aggregation of measures of oxidative stress at baseline and in response to training (Table 12). Specifically, LDL oxidation lag time and propagation rate, erythrocyte resistance to hemolysis, plasma thiobarbituric acid–reacting substance (TBARS), and plasma glutathione all exhibited significant familial aggregation at baseline and after training. Erythrocyte GSH-Px showed significant familial aggregation at baseline, but not in response to training (127). The baseline traits showed two to six times more variance between families than within families with maximal heritability estimates ranging from 31% to >90%, whereas maximal heritability estimates for training response traits ranged from 35% to 84%.

To conclude, subjects with elevated tHcy at baseline had significantly reduced tHcy levels with training. Although the clinical utility of tHcy for assessing CHD risk has been called into question (132), some evidence suggests that the observed reductions in tHcy may be enough to reduce CHD risk in those with hyperhomocysteinemia (133). The other beneficial effect of training was increased resistance to hemolysis of erythrocytes in women, which was significantly attenuated by smoking. Recently, distinct hematological patterns were found in smokers, and the differences were associated with greater risk of CHD in smokers (134). Similar results were reported in a cohort of over 100,000 Danish individuals where smoking was causally associated with increased blood leukocytes, neutrophils, lymphocytes, and monocytes. In addition, they suggest that smoking results in decreased erythrocyte life span due to increased hemolysis (135). Thus, cessation of smoking is likely necessary to improve erythrocyte fragility through training.

Overall, training only modestly improved markers of oxidative stress. These results were somewhat surprising, as regular exercise has been previously shown to decrease systemic oxidative stress and attenuate oxidative damage throughout the body (136,137). A potential reason for the apparent disagreement between the HERITAGE findings and those from other studies could be use of animal models in many studies examining training and oxidative stress that may not be generalizable to humans. Furthermore, training may reduce oxidative stress in older individuals only (136); thus, the HERITAGE cohort with its age inclusion limited to 65 yr may not represent the ideal target population for exercise interventions aimed at improving antioxidant defenses. Further research in diverse populations is needed to elucidate the impact of training on markers of oxidative stress.

EXERCISE AND SKELETAL MUSCLE BIOLOGY

Biopsies of the vastus lateralis muscle were obtained before and after training in a subset of 78 subjects from 19 White families (138). This subsample had a mean age of 33 yr and a mean BMI of 25 kg·m−2. Fiber type distribution, fiber areas, capillarity, and enzyme activities were assayed as described previously (139–141). This HERITAGE ancillary study was led by Dr. Jean-Aimé Simeneau, who passed away shortly after the completion of data collection.

Baseline observations. Means and SDs for the proportion of type I, 2A, and 2B fibers were close to the values previously reported from the same laboratory in samples of 203 adult females and 215 adult males ranging in age from 16 to 33 yr (142). The proportions of type 1 fibers were 43.2% (SD, 11.8) and 20.0% for type 2B (138). Mean fiber type areas were ~20% larger in the HERITAGE subsample than in the 10-yr younger cohort of 418 adults reported by Simeneau and Bouchard (142). Muscle enzyme activities were characterized by considerable heterogeneity, with CV values of 20% to 25% for creatine kinase (CK), phosphorylase (PHOS), hexokinase (HK), phosphofructokinase (PFK), glyceroldehyde phosphate dehydrogenase (GAPDH), carnitine palmitoyl transferase


TABLE 13. Effects of the endurance exercise program on vastus lateralis muscle fiber type, capillarity per fiber, and enzyme activities, and the familial aggregation of the training response.

| Muscle Trait                  | Pretraining | Training Response | Training Effect | Familial Aggregation of Training Response |
|------------------------------|-------------|-------------------|-----------------|------------------------------------------|
| % type 1                     | 42.2 (11.3) | 3.5 (9.4)         | 0.002           | NS                                       |
| % type 2 A                   | 36.8 (8.9)  | 1.8 (9.9)         | NS              | 1.81                                      |
| % type 2B                    | 20.0 (11.2) | -5.4 (10.3)       | 0.0001          | 1.13                                      |
| Type 1 caps                  | 4.5 (0.81)  | 0.76 (0.72)       | 0.0001          | 1.65                                      |
| Type 2A caps                 | 4.40 (1.00) | 0.63 (0.77)       | 0.0001          | 1.75                                      |
| Type 2B caps                 | 3.47 (1.00) | 0.55 (0.68)       | 0.0001          | 0.99                                      |
| CK                           | 389 (69)    | 27 (69)           | 0.0001          | 3.97                                      |
| PHOS                         | 19.9 (4.9)  | 1.6 (3.3)         | 0.0001          | 2.14                                      |
| HK                           | 2.5 (0.5)   | 0.4 (0.5)         | 0.0001          | 4.01                                      |
| PFK                          | 57.3 (13.9) | 4.9 (10.6)        | 0.0002          | 1.86                                      |
| GAPDH                        | 422 (94)    | 7 (66)            | NS              | 2.39                                      |
| LPL                          | 0.67 (0.27) | -0.01 (0.28)      | 0.0001          | 1.35                                      |
| CPT                          | 0.12 (0.02) | 0.05 (0.02)       | 0.0001          | 2.47                                      |
| HADH                         | 16.2 (3.0)  | 4.6 (3.0)         | 0.0001          | 2.13                                      |
| CS                           | 11.5 (2.1)  | 5.7 (2.5)         | 0.0001          | 2.73                                      |
| COX                          | 7.0 (1.7)   | 2.9 (2.5)         | 0.0001          | 2.07                                      |

Mean and (SD). F ratio of the between- to within-family variance components. Number of subjects ranged from 60 to 78 depending on the muscle trait. The Vmax of enzymes was expressed in micromoles of substrate per gram of wet weight tissue per minute. Total muscle LPL was expressed as nanomicro moles of FFA transformed per gram of wet weight tissue per minute. NS, not significant (P > 0.05); caps, number of capillaries per fiber; CK, creatine kinase; PHOS, phosphorylase; HK, hexokinase; PFK, phosphofructokinase; GAPDH, glyceroldehyde phosphate dehydrogenase; LPL, lipoprotein lipase; CPT, carnitine palmityl transferase; HADH, 3-beta-hydroxyacyl CoA dehydrogenase; CS, citrate synthase; COX, cytochrome c oxidase.

Modified from data reported in Rico-Sanz et al. (138) and Rankinen et al. (143).

TABLE 14. RMR before and after the HERITAGE exercise training program.

| Resting Metabolic Rate | Trial 1 | Pretraining | Trial 2 | Mean | Trial 3: 24 h | Posttraining | Mean |
|------------------------|--------|-------------|--------|------|--------------|--------------|------|
| VO2 (mL·min⁻¹)         |        |             |        |      |              |              |      |
| Men                    | 253 ± 54 | 249 ± 32    | 252 ± 33 | 255 ± 34 | 253 ± 34 | 255 ± 33 |
| Women                  | 194 ± 29 | 196 ± 29    | 196 ± 31 | 198 ± 33 | 200 ± 32 | 199 ± 31 |
| Total                  | 547 ± 43 | 546 ± 42    | 548 ± 43 | 553 ± 44 | 553 ± 44 | 552 ± 42 |
| MJ·d⁻¹                 |        |             |        |      |              |              |      |
| Men                    | 7.32 ± 0.97 | 7.25 ± 0.94  | 7.31 ± 0.95 | 7.37 ± 0.98 | 7.35 ± 0.98 | 7.38 ± 0.97 |
| Women                  | 5.62 ± 0.83 | 5.69 ± 0.91  | 5.70 ± 0.89 | 5.74 ± 0.95 | 5.80 ± 0.92 | 5.78 ± 0.89 |
| Total                  | 13.94 ± 1.79 | 13.94 ± 1.85 | 13.01 ± 1.84 | 13.11 ± 1.93 | 13.15 ± 1.90 | 13.16 ± 1.96 |
| KJ·kg⁻¹·FM·d⁻¹         |        |             |        |      |              |              |      |
| Men                    | 118.0 ± 11.7 | 117.6 ± 14.2 | 118.0 ± 12.6 | 117.1 ± 12.1 | 116.7 ± 14.6 | 117.2 ± 13.0 |
| Women                  | 125.9 ± 13.4 | 126.8 ± 14.2 | 127.2 ± 13.4 | 125.5 ± 13.4 | 128.9 ± 13.4 | 127.2 ± 12.1 |
| Total                  | 243.9 ± 13.0 | 244.4 ± 14.6 | 245.2 ± 15.8 | 242.6 ± 15.5 | 245.6 ± 17.0 | 244.4 ± 15.1 |

Mean ± SD. Maximum number of subjects: 40 men and 37 women. No significant effect of the training program was found on RMR expressed various ways. Reproduced with permission from Wilmore et al. (153).
Identical RMR testing procedures were used for each test (153). Reliability was high (intraclass correlation ≥ 0.90) between the two pretests, including resting VO$_2$, resting energy expenditure, HR$_{rest}$, and HR$_R$.

There was no change in RMR due to training at 24 or 72 h after training. Adjusting RMR values for age, sex, fat-free mass, and pretraining VO$_{2max}$ did not affect the results (Table 14). There was a large pretraining to posttraining increase in VO$_{2max}$ (17.9%) and small but significant differences in HR$_{rest}$ (61.0–59.3 bpm), HR$_R$ (60.7–58.7 bpm), percent body fat (29.2%–28.2%), FM (23.1–22.5 kg), and fat-free mass (54.3–55.0 kg) in this subsample.

The effect of aerobic training has continued to be a topic of interest, with most studies since the 1998 HERITAGE publication showing that aerobic training does not affect RMR (154–166). One study showed that White women did not experience a change in RMR after 6 months of training, whereas Black women saw a decrease (167). Another study showed an increase at 7 wk, but a return to pretraining levels after 14 wk of training (168). Three studies have shown an increase in RMR with aerobic training (169–171). There is no clear explanation for these apparent differences.

**CANDIDATE GENE AND GWAS STUDIES**

Genetic studies of exercise-related traits at the genome level in HERITAGE began in 1997 and have been characterized in several phases that closely matched advances in genomic technologies. The first wave of studies focused on candidate genes. It was followed by a series of reports based on an unbiased search of the genome using panels of microsatellite markers and linkage analytical strategies, which led to positional cloning and fine mapping of regions of interest. In a next phase, sets of candidate genes were uncovered by probing the variability in skeletal muscle gene expression, which were then submitted to further investigation. Finally, in more recent times, millions of single nucleotide polymorphisms (SNPs) covering the whole genome are being used in association studies of HERITAGE traits. A brief summary of the findings across these approaches follows.

**Candidate gene studies.** A total of 37 studies published between 1997 and 2010 tested associations between 34 candidate genes and a wide variety of traits measured at baseline and in response to training. The phenotypes investigated included cardiorespiratory fitness traits (maximal and submaximal VO$_2$ and PO$_2$), hemodynamic-related traits (resting and submaximal exercise HR, SBP and DBP, and submaximal VO$_2$ and PO$_2$), adiposity-related traits (BMI, sum of eight skinfold thicknesses, FM, percent body fat, and abdominal fat), plasma lipid and lipoprotein traits (TC, TG, LDL-C, HDL-C, and apolipoproteins), and insulin and glucose metabolism traits (fasting glucose, insulin and C-peptide levels, Si, AIRg, and Sg). In most studies, the association with baseline and response traits was assessed for at least one trait showing significant (P < 0.05) evidence of association with a variant in the gene locus. Gene symbols and names are from the National Center for Biotechnology Information.

**TABLE 15. Summary of candidate gene studies with baseline (B) and response (R) traits in HERITAGE.**

| Gene Symbol | Gene Name | CRF | Hemodynamic | Adiposity | Lipids and Lipoproteins | Insulin and Glucose | Other | References |
|-------------|-----------|-----|-------------|-----------|------------------------|-------------------|-------|------------|
| ACE         | Angiotensin I converting enzyme | B and R | R         |           |                        |                   |       | (172,173) |
| ADIPQ       | Adiponectin, C1Q and collagen domain containing | B | B         | B         |                        |                   |       | (174)     |
| ADRA2A      | Adrenoreceptor alpha 2A | B | B         |           |                        |                   |       | (175)     |
| ADRB2       | Adrenoreceptor beta 2 | B and R | R         |           |                        |                   |       | (176,177) |
| ADRB3       | Adrenoreceptor beta 3 | R |           |           |                        |                   |       | (177)     |
| AGT         | Angiotensinogen | B and R | B         |           |                        |                   |       | (172,181) |
| AMPD1       | Adenosine monophosphate deaminase 1 | B and R | B and R   |           |                        |                   |       | (182)     |
| ANG         | Angiogenin | B |           |           |                        |                   |       | (183)     |
| APOE        | Apolipoprotein E | B and R | B         |           |                        |                   |       | (184,185) |
| ATP1A2      | ATPase Na+/K* transporting subunit alpha 2 | R |           |           |                        |                   |       | (186)     |
| BCHE        | Butyrylcholinesterase | B | B         |           |                        |                   |       | (187)     |
| CEP         | Cholesteryl ester transfer protein | B and R | B         |           |                        |                   |       | (185)     |
| CKN         | Creatine kinase, M type | B and R | R         |           |                        |                   |       | (188)     |
| DDR2        | Dopamine receptor D2 | R |           |           |                        |                   |       | (189)     |
| EDN1        | Endostatin 1 | R |           |           |                        |                   |       | (190)     |
| FHL1        | Four and a half LIM domains 1 | B and R | R         |           |                        |                   |       | (191)     |
| FTO         | FTO alpha-ketoglutarate dependent dioxygenase | B and R | R         |           |                        |                   |       | (192)     |
| GHRL        | Ghrelin and obestatin prepropeptide | B | B         |           |                        |                   |       | (183)     |
| GNB3        | G protein subunit beta 3 | B | B         |           |                        |                   |       | (193)     |
| LEP         | Leptin | R |           |           |                        |                   |       | (194)     |
| LEPR        | Leptin receptor | B | R         |           |                        |                   |       | (195,196) |
| LIPC        | Lipase C, hepatic type | R |           |           |                        |                   |       | (197)     |
| LIPE        | Hormone-sensitive lipase | B | B         | R         |                        |                   |       | (198)     |
| LPL         | Lipoprotein lipase | B |           |           |                        |                   |       | (199)     |
| MC4R        | Melanocortin 4 receptor | R |           |           |                        |                   |       | (200)     |
| NOS3        | Nitric oxide synthase 3 | R |           |           |                        |                   |       | (201)     |
| PON1        | Paraoxonase 1 | R |           |           |                        |                   |       | (202)     |
| PPARC       | Peroxisome proliferator-activated receptor gamma | R | B and R   | R         |                        |                   |       | (203)     |
| PTP1N       | Protein tyrosine phosphatase nonreceptor type 1 | B | B         |           |                        |                   |       | (204)     |
| SLC4A4      | Solute carrier family 4 member 5 | B | B         |           |                        |                   |       | (205)     |
| TGFBI       | Transforming growth factor beta 1 | B |           |           |                        |                   |       | (206)     |
| TNF         | Tumor necrosis factor | R |           |           |                        |                   |       | (207)     |
| UCPO        | Uncoupling protein 3 | R |           |           |                        |                   |       | (208)     |

An entry indicates that at least one trait showed significant (P < 0.05) evidence of association with a variant in the gene locus. Gene symbols and names are from the National Center for Biotechnology Information.

CRF, cardiorespiratory fitness; PA, physical activity.
response traits was tested in Black and White subjects, and the associations tended to be ethnic specific. Table 15 summarizes the findings by indicating whether an association was reported at baseline (B) or in response to training (R) for any given phenotype in each trait category.

For cardiorespiratory fitness and hemodynamic-related traits, ACE, AGT, AMPD1, ANG, ATP1A2, CKM, GHRL, GNB3, NOS3, PPARG, and SLC4A5 showed evidence of association, with at least one phenotype measured at baseline or in response to training. For example, one of the first candidate gene studies showed that muscle-specific creatine kinase (CKM), a gene previously related to intrinsic cardiorespiratory fitness, was associated with response of VO2max to training adjusted for age, sex, BMI, and pretraining VO2max, but not baseline levels of VO2max in White subjects (188). A later study found that, in Black subjects, minor allele homozygotes (T/T) at PPARG rs2016520 showed smaller training-induced improvements in VO2max than C-allele carriers (202). Several candidate genes, ADIPQ, ADR4A2A, ADRB2, AGRP, AGT, FTO, GHR, GNB3, LEPR, LIPE, PTNP1, and UCP3, showed evidence of association with at least one adiposity-related trait. However, no association was found between polymorphisms in the ADRB3 and MC4R genes and adiposity. Minor allele homozygotes (A/A) at FTO rs8050136 were heavier and fatter in White men but not White women, whereas C allele carriers showed three times greater reductions in FM and percent fat than A/A homozygotes across all White subjects (192). These findings suggest that resistance to training-induced reductions in adiposity is one mechanism by which FTO is related to obesity.

For lipid and lipoprotein traits, evidence of association was found with these candidate genes: ADIPOQ, APOE, CETP, LIPC, LPL, and PPARG. The APOE polymorphism was associated with baseline lipoprotein levels, with the E2/3 genotype having the lowest and E3/4 and E4/4 genotypes the highest LDL-C levels in men and women of both ethnic groups. White female carriers of E3 had higher baseline HDL-C levels, and White male carriers of E2/3 and E3/3 showed larger increases in HDL-C in response to training than E4/4 carriers (184). A subsequent study showed that CETP genotypes and haplotypes were associated with HDL phenotypes, including that CETP rs1800775 A/A homozygotes had higher baseline HDL-C levels in both Blacks and Whites and greater increases in HDL-C and apoA-I with training in Whites only (185). Combined models found that the APOE and CETP rs1800775 genotypes accounted for 6%-9% of the variance in baseline and training-induced changes in apoA-I and HDL phenotypes. For traits related to insulin and glucose metabolism, evidence of association was observed with ADIPOQ, FHL1, LEP, LEPR, LIPC, PPARC, and PTNP1. For example, an association was observed between DNA sequence variation in the FHL1 gene and the response of fasting insulin and insulin sensitivity to training in White men (191). Evidence of a gene-training interaction was uncovered, with PPARC Ala carriers displaying greater improvements in glucose (Kg, Sg) and insulin (AIRg, Di) metabolism in response to training (203).

BP phenotypes were linked to several candidate genes (ACE, AGT, AMPD1, ANG, EDN1, GNB3, NOS3, SLC4A5, TGFβ1). The relationship between variants in angiotensinogen (AGT M235T, rs699) and angiotensin-converting enzyme (ACE insertion/deletion) and training-induced changes in resting and exercise BP was studied in 476 Whites from 99 families. It was found that men with the AGT MM and MT genotypes demonstrated improved DBP responses to training, whereas men with the TT genotype had minimal responses and, moreover, had significantly higher DBP responses during acute maximal exercise (172). In a sample that included both ethnic groups of HERITAGE, an angiotenogen (ANG) gene polymorphism was associated with both pretraining and posttraining resting and exercise SBP among Blacks, but not Whites (183). These findings extended previously known associations between ethnicity and resting BP to exercise BP responses. Subsequently, a polymorphism in the transforming growth factor-beta (TGF-β) gene was associated with SBP at rest and during acute exercise both before and after the exercise program among Whites, but not Blacks (206). Data from the Aerobics Center Longitudinal Study and HERITAGE were used to examine the association of endothelin 1 (EDN1) SNPs and BP phenotypes. In a case–control study design of incident hypertension, the investigators found that, although the allele and genotype frequencies of EDN1 tagging SNPs were not different between the two groups, there was a significant interaction between the EDN1 rs5370 genotype and baseline cardiorespiratory fitness levels on the future risk of hypertension. The EDN1 rs5370 risk allele (T) was also associated with attenuated responses in SBP and pulse pressure after training in HERITAGE White subjects, highlighting the capacity for physical activity and fitness status to potentially modify the influence of genes on cardiovascular outcomes (190).

Other studies found evidence of associations between genetic variation in BCHE and PON1 genes and anxiety scores (187), DRD2 gene and physical activity level (189), TNF gene and C-reactive protein (CRP) levels (207), and AGRP with total dietary and macronutrient intake (178).

**Genome-wide linkage studies.** Before the advent of GWAS to identify loci associated with complex traits, genome-wide linkage analysis (GWLA) was a common unbiased method used to identify regions of the genome (known as quantitative trait loci or QTLs) harboring genes that could influence complex traits. In HERITAGE, 23 GWLAs were undertaken to identify QTLs associated with traits measured at baseline or in response to training. For these linkage scans, up to 700 markers covering the 22 autosomes with a mean spacing of 4.1 Mb on the physical map were used. In these studies, the sibpair linkage method was used to test for linkage between the phenotype of interest and microsatellites, a class of highly polymorphic genetic markers consisting of variable copy number of repetitive DNA sequences found in the noncoding regions of the genome. Typically, multipoint linkage analysis was performed using a regression-based model, as implemented in MERLIN (81). Briefly, siblings who share a greater proportion of alleles identical-by-descent at the marker locus will also show a greater resemblance in the phenotype. The phenotypic resemblance of the siblings is linearly
regressed on the estimated proportion of alleles that the sibling pair shares identical-by-descent at each marker locus (209).

The first HERITAGE GWLA undertaken on baseline VO$_{2\text{max}}$ and its response to training led to the identification of four QTLs for baseline VO$_{2\text{max}}$ and five QTLs for VO$_{2\text{max}}$ response (210). Several other QTLs for traits related to cardiopulmonary fitness (211,212), hemodynamic phenotypes (213–218), adiposity (219,220), plasma lipids and lipoproteins (221,222), sex hormones and steroids (125,126), insulin and glucose metabolism (223–225), energy and macronutrient intake (226), CRP levels (227), and metabolic syndrome (228) have been identified. Two bivariate GWLAs were also undertaken to identify pleiotropic QTLs affecting adiposity and TG levels (229) and TG and HDL-C levels (230). Results suggested that one or more QTLs in the 19q13 region jointly influence TG levels and adiposity, with the APOE and LIPE (hormone-sensitive lipase) genes being strong candidates for the source of the pleiotropic QTL (229). Dense mapping of three QTLs associated with the changes in submaximal exercise (50 W) SV (2q31-q32 and 10p11.2) and HR (2q33.3q43) led, respectively, to the identification of the titin (231), kinesin family member 5B (232), and eAMP responsive element binding protein 1 (233) genes as strong candidate genes for hemodynamic responses to training. A QTL for submaximal exercise capacity (VO$_2$ at 60% VO$_{2\text{max}}$) on 13q12 was followed by dense mapping with more than 1800 SNPs over a 7.9-Mb region (211). Association analyses narrowed the target region to loci encoding MIPEP and SGCG.

**Genome-wide association studies.** To date, five GWAS based on HERITAGE data have been published. The first GWAS examined the association of 324,611 SNPs with VO$_{2\text{max}}$ response to endurance training and identified a panel of 21 SNPs explaining about half of the variance in the trainability of VO$_{2\text{max}}$ (234). A second GWAS revealed that nine SNPs accounted for the genetic variance of the submaximal exercise HR response to training (235). A third study performed genome-wide and transcriptome-wide profiling to identify genes associated with the TG response to training (236). The GWAS identified four SNPs accounting for the genetic variance of the TG response, whereas a molecular signature based on the baseline expression of 11 genes predicted 27% of TG changes in response to training. Association analyses of SNPs in or near these 11 predictor genes revealed that SNPs from only four genes were associated with TG changes. A composite score based on the top four SNPs, each from the genomic and transcriptomic analyses, was found to be the strongest predictor of the TG response to training in a model including over 50 clinical predictor variables (236). A fourth GWAS based on data from three different cohorts including HERITAGE identified 52 SNPs from 7 loci showing suggestive evidence of association ($P < 1.0 \times 10^{-6}$) with AVF and subcutaneous fat assessed by CT scan (237). A fifth GWAS revealed associations between SNPs and insulin sensitivity (Si). More specifically, the GWAS analysis identified one SNP significantly associated with baseline Si ($P = 3.79 \times 10^{-5}$) plus 7 SNPs with suggestive association ($P < 10^{-5}$), and 10 SNPs with suggestive association ($P < 10^{-5}$) with Si response to training. Interestingly, mouse knockouts in four of the genes associated with these SNPs showed phenotypes compatible with alterations with glucose metabolism (238).

**HERITAGE involvement in large GWAS consortia.** HERITAGE joined the GIANT (Genetic Investigation of ANthropometric Traits) and CHARGE consortia, international collaborative efforts aimed at identifying genetic loci associated with human body size, obesity, and behavioral and physiological traits. The primary focus of these large-scale projects has been meta-analysis of GWAS data. The GWAS data from HERITAGE Whites ($n \sim 483$) have been included in GWAS meta-analyses of obesity (239), height (240), BMI (241,242), and traits related to body shape/composition (waist circumference, waist-to-hip ratio, body fat percentage) (243–246). HERITAGE also contributed to GWAS meta-analyses of HR (247), plasma leptin (248), dietary macronutrient intake (249), and principal components of anthropometric traits (250).

These meta-analyses involved up to hundreds of thousands of individuals from dozens of studies, with genotype data on tens of thousands to millions of SNPs. For example, in one of the largest GWAS meta-analyses of BMI, 339,224 individuals were included from 80 studies in stage 1 and another 34 studies in stage 2 and found 97 BMI-associated loci ($P < 5 \times 10^{-8}$) (241). Conversely, a study of the influence of age and sex on genetic associations with body size and shape examined up to ~2.8 M SNPs in over 320,000 individuals (246). A number of these efforts have dealt with potential interactions with smoking, sleep, or educational attainment. One report investigated genome-wide lipid loci stratified by sleep duration (251), whereas another searched for loci related to resting BP and sleep (252). The smoking by genotype interactions on the risk of type 2 diabetes and fasting plasma glucose was considered in a recent study (253). Finally, the potential interactions with level of education were investigated for genome-wide loci associated with resting BP (254). This series of gene–environment interaction reports has successfully identified new genomic loci relevant to plasma lipids, blood glucose, and BP.

These GWAS meta-analyses are among the most cited articles in the fields of genetic epidemiology and obesity genetics and have contributed greatly to our understanding of the genetic architecture of anthropometric traits in adults.

**Genome-wide interaction studies.** Understanding the genetic and environmental factors and their interactions (G × E) underlying complex disease traits may provide insights into more effective intervention and therapeutic strategies. More recently, a major initiative was launched to pursue genome-wide interaction studies (GWIS) (255). Several GWIS reports identified important gene–lifestyle interactions for several cardiometabolic traits (243,246,256–262). The HERITAGE study investigators participated in most of these GWIS investigations, which contributed novel findings.

For example, several loci showing strong evidence for regulatory features supporting shared pathophysiology between cardiometabolic and addiction traits, such as smoking, were identified (262). GWIS reports have also highlighted a role in BP regulation for biological candidates such as modulators of vascular structure and function (CDKN1B, BCAR1-CFDP1, S23
PXDN, EEA1), ciliopathies (SDCCAG8, RPGRIP1L), telomere maintenance (TNKS, PINX1, AKTIP), and central dopaminergic signaling (MSRA, EBF2). Similarly, analyses of gene–smoking interactions in lipids have identified several new loci associated with lipids, some of which were detected only in smokers, thereby highlighting the importance of gene–behavior interactions (256). Despite some interesting findings, it is still unclear how prevalent such interactions are and what their full impact may be on the genetic and environmental architecture of complex traits. Only comprehensive and properly powered GWIS studies can address these uncertainties.

GENE EXPRESSION STUDIES

A subset of HERITAGE participants from the Quebec clinical center underwent *vastus lateralis* skeletal muscle biopsies to examine the association between the muscle gene expression profile and cardiometabolic phenotypes at baseline and in response to training.

A first study examined muscle gene expression in 16 subjects discordant for the response of insulin sensitivity (Si) to the training program: 8 showing no changes (low responders) and 8 showing marked improvements (high responders; response ~4 times higher than that of low responders) (263). Differences in gene expression before and after training were tested for 703 and 1166 transcripts, respectively, using pooled RNA from each group. A total of 38 transcripts were differentially expressed between response groups at both baseline and posttraining, with 22 having known functions: upregulated genes were related to cell adhesion/growth, signal transduction, and electron and adenosine transport, whereas downregulated genes were involved with oxidative phosphorylation, transcript regulation, and protein biosynthesis. Differential expression at either baseline or posttraining was confirmed by real-time quantitative reverse polymerase chain reaction in four of the five genes selected for validation (Fig. 6) (263). In a follow-up report on three SNPs at the *FHL1* locus, one of these four genes, it was found that allelic variants were associated with changes in fasting insulin, Si, Kg, and Di response to training in White men (191). Further analysis of the relationship between gene expression and Si was examined in a study where a transcriptional signature linked to the transcription factor MEF2A was found to be predictive of the response of Si both in HERITAGE and in an independent cohort (238).

HERITAGE gene expression data were also used in a study that identified an age-related gene expression signature in human muscle (264). Using a total of 97 Affymetrix U133 + 2 gene chips from two independent studies, including 52 from HERITAGE, 580 genes were identified whose expression levels were related to a continuum of age (18–78 yr). The muscle age-related genes were related to serotonin, DNA topoisomerase antagonism, and RXR activation.

In one study, a combination of genome-wide muscle gene expression with DNA sequence variation screening was used to identify genes associated with changes in VO\textsubscript{2max} due to endurance exercise training (265).
analysis combining SNPs associated with 29 skeletal muscle transcripts predicting changes in \( \dot{V}O_2 \)max and SNPs previously associated with the trainability of \( \dot{V}O_2 \)max led to the identification of a set of 11 genetic variants explaining 23% of the variance in \( \dot{V}O_2 \)max training response. Subsequently, regulatory molecules coordinating the complex network of ~800 genes found to be differentially regulated in response to training (i.e., training-responsive transcriptome) were examined by contrasting subjects who demonstrated the least versus the greatest improvement in \( \dot{V}O_2 \)max (low vs high responders) resulting in the identification of 86 genes (266). A panel of 3400 SNPs near these 86 genes were genotyped, and 24 were nominally associated with responder status \((P < 0.01)\) but not statistically significant after accounting for multiple testing: 8 of them belonged to the HLA cluster of genes on chromosome 6, whereas other significant SNPs were in loci coding for proteins largely involved in cell proliferation or tumor suppression (\( ASNS, DLC1, FABP5, NID1, PDZD2, SASH1, SPARC, \) and \( TRAM1 \) genes).

A data-driven, reverse-engineering approach was used to develop a genome-wide network model of muscle homeostasis in response to training (267). The analysis initially focused on identifying pairs of genes that were differentially coexpressed between pretraining and posttraining skeletal muscle in HERITAGE \((n = 52)\). The analysis identified a differential coexpression network, which the algorithm organized into 25 interconnected gene clusters. Further analysis of the inferred network revealed that genes found within core metabolic and translational pathways are a predominant feature of these networks. Interestingly, the eukaryotic translation initiation factor Eif6 was the most connected ribosome associated factor in the network leading to the hypothesis that Eif6 could be a part of an mTor-independent mechanism regulating metabolic adaptation. Analysis of the Eif6\(^{+/-}\) haploinsufficient mouse (50% reduction in eif6 expression) found different transcriptional profiles compared with wild-type mice (genes differentially expressed in the gastrocnemius: 2651 upregulated, 829 downregulated; soleus: 2482 upregulated, 1846 downregulated; tibialis anterior: 1361 upregulated, 958 downregulated; false discovery rate < 10%) and that the profile of the gastrocnemius muscle mirrored the predicted Eif6 signature found in HERITAGE both in functional profile and in direction of change. Further experiments of mitochondrial functionality and muscle performance in Eif6 heterozygote mice revealed alterations in electron transport chain dynamics, superoxide generation, and reduced exercise capacity, further supporting the role of this factor in regulating muscle energy homeostasis (267).

Finally, a study that utilized transcriptome-wide profiling to identify a baseline muscle gene expression signature of the response of TG to training was described previously (236). Briefly, after transcriptome-wide association analysis, a molecular signature based on the baseline expression of 11 genes predicted 27% of TG changes in response to exercise training (Fig. 7).

**METABOLOMIC STUDIES**

Targeted, liquid chromatography tandem mass spectrometry-based metabolomics was performed to profile 186 plasma metabolites in 441 White subjects before and after training. DMGV, a recently identified circulating biomarker of liver fat that predicts incident type 2 diabetes and decreases in body weight after weight loss surgery (268), was investigated to examine its relationship to cardiometabolic changes with training (96). In age- and sex-adjusted analyses, baseline DMGV levels were

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**FIGURE 7**—Performance of the vastus lateralis gene expression profile regression model derived from a training set \((n = 37, \text{gray dots})\) and a validation set \((n = 12, \text{red dots})\) for the prediction of exercise training-induced changes in TG in HERITAGE. Reproduced with permission from Sarzynski et al. (236).
positively correlated with adverse body mass and composition (including visceral fat), lipid and lipoproteins, and glucose and insulin homeostasis traits ($\beta$ coefficient ranges for associations meeting Bonferroni statistical significance: 0.17–0.46). After adjustment for both BMI and AVF, associations of DMGV levels with all glucose and insulin traits and 16 of 22 lipid metabolism traits remained highly significant (96). DMGV levels decreased with training (median change, $-9.4\%$), and changes in DMGV were positively correlated with changes of several lipid and glucose and insulin traits (e.g., total, medium, and large VLDL-P; VLDL-P size; TG; total and medium HDL-P; small LDL-P, TC; fasting insulin and glucose).

Baseline levels of DMGV were associated with attenuated improvements in HDL traits (inverse association with total and medium HDL particle concentration and apoA-I; positive association with small HDL particles) after training, even after adjusting for age, sex, baseline level of each outcome variable, and changes in BMI and visceral fat (96) (Fig. 8). Baseline DMGV levels were also inversely associated with changes in Si ($\beta = -0.13; P = 3.0 \times 10^{-3}$) and positively with changes in AVF ($\beta = 0.11; P = 0.03$) and body mass ($\beta = 0.11; P = 0.04$) in minimally adjusted models, but the associations were not significant in fully adjusted models. In summary, DMGV identified adverse metabolic traits in healthy adults of European descent in HERITAGE and was associated with attenuated improvements in lipid traits and insulin sensitivity with training.

**PROTEOMIC STUDIES**

The most recent molecular profiling platform applied to HERITAGE has been proteomics. Specifically, to measure large-scale circulating proteomic changes after endurance exercise training, baseline and posttraining plasma samples were analyzed using the Slow-Off rate Modified Aptamer (SOMAmer)-based platform, SomaScan® (SomaLogic, Inc., Boulder, CO). This platform utilizes affinity binding to quantify approximately 5000 distinct protein targets in circulation (269,270).

In a novel, proof-of-concept study utilizing SomaLogic proteomics data, the association of plasma proteins with cross-sectional V̇O$_{2\text{max}}$ was analyzed (271). An unpaired crossover sampling method (with 50% of samples from subjects at baseline and 50% at posttraining) was used to avoid correlation from pairs and to increase the observed range of V̇O$_{2\text{max}}$ values in the data set. An elastic net linear regression model was derived, refined, and validated on 80%, 10%, and 10% of participants, respectively. The derivation model (80%, or 523 subjects) identified 115 proteins that were highly correlated with measured V̇O$_{2\text{max}}$, with an $r^2$ of 0.80; this was replicated in the validation data set (10%, or $n = 62$), with an $r^2$ of 0.71 (Fig. 9). The top 3 proteins with the largest quantitative contribution to V̇O$_{2\text{max}}$ were leptin, C1QR1 (complement component C1Q receptor, part of immune system), and GGH (gamma glutaryl hydrolase, regulation of intracellular folate) (271).

The plasma proteomic profile of V̇O$_{2\text{max}}$ was further refined in analyses that characterized proteins associated with baseline V̇O$_{2\text{max}}$ and changes in V̇O$_{2\text{max}}$ in response to training in more than 650 subjects (272). After adjustment for age, sex, BMI, and ethnicity, 147 circulating proteins were associated with baseline V̇O$_{2\text{max}}$ (Fig. 10, left panel), including 85 proteins that were positively associated and 62 proteins negatively associated. Proteins positively associated with baseline V̇O$_{2\text{max}}$...
spanned specific organ systems and biologic processes including angiogenesis (e.g., extracellular matrix protein 1, anthrax toxin receptor 2), coagulation and hematopoiesis (e.g., complement decay-accelerating factor, tetranectin), and lipid metabolism (e.g., apolipoprotein F, lipase member K). Several well-known markers of metabolic dysregulation known to be positively associated with adiposity were inversely associated with baseline $\dot{V}O_{2\text{max}}$, including leptin, CRP, and insulin.

**FIGURE 9**—Prediction of measured $\dot{V}O_{2\text{max}}$ by 124 plasma protein levels in the validation data set of HERITAGE. Reproduced with permission from Williams et al. (271).

**FIGURE 10**—Plasma proteins positively and negatively associated with baseline $\dot{V}O_{2\text{max}}$ (left panel) and change in $\dot{V}O_{2\text{max}}$ (right panel) adjusted for age, sex, ethnicity, and BMI in HERITAGE. Reproduced with permission from Robbins et al. (272).
In addition, 102 baseline proteins were associated with changes in VO2max with training in a linear regression model adjusted for age, sex, BMI, ethnicity, and the baseline level of VO2max (Fig. 10, right panel). Minimal overlap was found between the proteins associated with baseline VO2max versus change in VO2max, as only five proteins (T12B2, ATF6A, CO9A1, insulin, and PIANP) were associated with both traits, proteomic findings that are consistent with clinical findings in HERITAGE demonstrating a lack of correlation between these two VO2max traits (see the Cardiorespiratory Fitness section). Subsequently, analyses were aimed at determining the ability of plasma proteins to identify VO2max gains (>15% change from baseline) to exercise training in comparison to clinical traits alone. A model based solely on clinical traits (age, sex, ethnicity, BMI) yielded an area under the curve of 0.62, whereas the addition of 56 proteins to the clinical trait model increased the area under the curve to 0.84, a substantial improvement in predicting who is likely to be a higher VO2max responder. Lastly, the association of VO2max-related proteins with mortality was examined in the Framingham Offspring Study. It was found that 12 proteins associated with baseline VO2max and 9 proteins associated with change in VO2max were associated with incident all-cause mortality (false discovery rate \( q < 0.1 \)) (272).

**BIOINFORMATICS EXPLORATIONS OF CARDIORESPIRATORY FITNESS TRAITS**

Here, the main findings from integrative bioinformatics explorations of two cardiorespiratory fitness traits representing intrinsic VO2max and trainability of VO2max are summarized. These two studies are separated in time by about 6 yr, and their methodologies reflect the evolving trends and capabilities of the bioinformatics field in general.

In 2013, an analysis examining the contributions of genes and biological pathways to variations in trainability of VO2max (hereafter called delta VO2max) was published (273). Using GWAS SNP associations to delta VO2max, significant contributions at the “gene set” or pathway level instead of individual SNP level were investigated. By considering association scores from all gene members of a gene set, pathways related to the broad categories of immune function, cardiomyopathy, extracellular matrix regulation, and metabolism (PPAR signaling, pantothenate, and CoA biosynthesis) were investigated. By considering association scores from all gene members of a gene set, pathways related to the broad categories of immune function, cardiomyopathy, extracellular matrix regulation, and metabolism (PPAR signaling, pantothenate, and CoA biosynthesis) were found to be significantly associated with VO2max trainability. Examining pathway overrepresentation among genes above a threshold of genetic association to delta VO2max further identified a network of 35 genes, of which 31 were associated with delta VO2max at \( P \leq 0.005 \). These network genes could be partitioned into pathways related to calcium signaling, nitrpic oxide signaling, and protein kinase A signaling.

A hypothesis-free gene prioritization tool was used to rank genes moderately associated with delta VO2max in GWAS analysis. One group of genes (PINX1, CD44, PARK2, RYR2, ADCA5, and SHANK2) demonstrated consistently strong scores across all analyses, whereas a second group (KCNO5, GRIK4, RPTOR, ACVR1C, and ACSL1) were strongly associated only in a subset. Several of the genes identified (CAMTA1, RYR2, ACSL1, CD44, BIRC7/THDF1) have been further replicated (274). This bioinformatics exploration of the biology underlying VO2max adaptation suggests that pathways related to calcium signaling, energy sensing and partitioning, mitochondrial biogenesis, angiogenesis, immune functions, and regulation of apoptosis and apoptosis are some key mechanisms through which the physiological responses of VO2max to training are mediated.

The bioinformatics explorations of the genetics and biology underlying VO2max in the sedentary state (275) differed extensively in complexity and depth compared with the 2013 analysis of VO2max trainability. In the 6-yr interim, there had been a profound expansion in databases and data analysis tools, especially access to large public data sets and the ability to formulate hypotheses about the function of noncoding SNPs, thereby greatly expanding the scope of understanding the putative roles of genetic variants. Using summary statistics from intrinsic VO2max GWAS as a starting point, the focus was first on the “discovery” of candidate genes and pathways, followed by a “validation” phase of candidate genes identified in the discovery phase in which knockout phenotypes and exercise cardiovascular and muscle physiology phenotypes were interrogated (275).

**Intrinsic VO2max bioinformatics: Discovery phase.**

A pipeline consisting of seven steps was used to generate gene targets and hypotheses regarding the biology underlying VO2max:

a. Quantifying gene-level association to intrinsic VO2max based on SNP data. The most likely causal genes at associated loci were prioritized. This exercise led to the identification of genes such as *PRADC1*, *TPM2*, *IGF1*, and *CASQ2*.

b. Effects of noncoding SNPs on promoter and enhancer binding sites in key tissues. A statistically significant enrichment for enhancer-associated SNPs was observed in tissues related to cardiorespiratory functions (aorta, right ventricle, skeletal muscle, myoblasts, and satellite cells). The analysis further revealed an increased overlap with active enhancers (H3K4me1 and H3K27ac marks) and active promoters (H3K4me3 and H3K9ac marks) in skeletal muscle-related samples, and increased levels of active enhancer associated H3K4me1-site overlaps in aorta. These findings provided insights into some possible roles of regulatory SNPs in influencing intrinsic VO2max.

c. Effects of noncoding SNPs on nearby gene expression (cis-eQTLs) in relevant tissues. A total of 216 SNPs was found to be significantly associated with cis-gene expression (eQTL) \( P \leq 1 \times 10^{-05} \). The top 3 most significant eQTL associations were observed for SNP rs2838815 regulating *ADARB1* gene expression in brain \( P \leq 1 \times 10^{-12} \), a cluster of highly linked SNPs (clustered at *ATEI* gene intron) regulating *NSMCE4A* gene expression in pancreas \( P < 1 \times 10^{-14} \), and another cluster of tightly linked SNPs affecting expression of the *PRADC1* gene \( P \leq 1 \times 10^{-12} \) in adipose tissue, as well as the heart and skeletal muscle. Analysis for joint effects, that is, genomic loci overlapping for both eQTL and histone binding sites, demonstrated the strongest overlaps in heart tissue, followed by the pancreas, skeletal muscle, and adipose tissue.
d. Enrichment of intrinsic VO$_{2\text{max}}$-associated candidate genes in tissue-specific functional networks. The functional connectivity of tissue-expressed genes to the set of GWAS-significant genes was examined. The top 5 tissues with high network connectivity included the placenta, heart, skeletal muscle, pancreas, and liver. Analysis of skeletal muscle specific subnetworks demonstrated a high degree of connectivity for the genes SSB, EED, PICALM, and TIMM8B.

e. Examining skeletal muscle gene expression profiles. Whole-genome expression profiling data were analyzed to identify genes that transcriptionally correlated with intrinsic VO$_{2\text{max}}$ levels. Forty-seven Affymetrix probes were significant. Among these, three genes (CASQ2, COX7A2L, PRADC1) were also identified in our other bioinformatic analyses.

f. Enrichment of intrinsic VO$_{2\text{max}}$-associated genes in biological pathways. Pathway enrichment analysis identified 34 pathways as significant. Hierarchical clustering of the significant pathways identified groups of pathways with shared genes, for example, pathways involved in calcium and inositol 1,4,5-trisphosphate signaling, and G-protein signaling. Additional pathway analysis further identified pathways related to insulin and PI3 signaling as significant.

g. Weighted ranking of candidate intrinsic VO$_{2\text{max}}$-associated genes identified in prior steps. To generate a priority list of candidate genes based on the findings from individual bioinformatics analysis, a weighted-ranking algorithm was developed. The top 10 genes identified through this exercise included ADARB1, PRADC1, TPM2, FBXO41, IGF1, NOTO, CASQ2, ARL6IP5, RAB11FIP5, and TMF1.

Intrinsic VO$_{2\text{max}}$ bioinformatics: Validation phase.
The prioritized genes were filtered against the evidence available from knockout mouse studies and from new data on intermediate cardiorespiratory fitness endophenotypes available in HERITAGE.

h. Examining the phenotypic effects of candidate gene knockouts in mouse models. Knockout mouse phenotype data from Mouse Genome Informatics were available for 21 of the 38 candidate genes identified through the bioinformatics analyses. Notably, knockout of the Casq2 gene affected all endophenotype categories (cardiovascular, hematopoietic, skeletal muscle, metabolism), with most observed phenotypes related to the cardiovascular system. Knockout of Picalm affected mostly hematopoietic traits. Sgcg knockout produced effects on cardiovascular and muscle phenotypes, whereas Pradcl knockout led to increased circulating glucose.

i. Examining SNPs in candidate genes for associations to cardiovascular and muscle intermediate endophenotypes of intrinsic VO$_{2\text{max}}$. Based on the results from the bioinformatics analyses, gene expression studies, and knockout mouse phenotypes, the next step was to investigate whether candidate genetic loci were associated with physiological and metabolic traits known to be relevant to intrinsic cardiorespiratory fitness and measured in HERITAGE. SNPs in genes such as ADRB1, ATE1, CASQ2, DMRT2, NOTO, and SGCG were found to be associated with exercise-related cardiovascular traits such as HR at 60%VO$_{2\text{max}}$, SV at 60%VO$_{2\text{max}}$, and SBP pressure at maximal exercise. Multiple SNPs of SGCg, DMRT2, ADARB1, and CASQ2 genes were nominally associated with muscle-related traits, including percent fiber type, capillary area per fiber, and muscle enzyme activities. As an example, CASQ2 SNP rs7523715 was associated with Q and HR during submaximal and maximal exercise, respectively, whereas CASQ2 SNP rs2999460 was associated with the percentage of type I and type 2B muscle fibers. CASQ2 gene expression was also positively associated with enzyme activities related to aerobic cellular respiration (cytochrome oxidase, CS, and HADH) and negatively associated with the rate-limiting enzyme of glycolysis (PFK).

Several candidate genes from the aforementioned integrative analyses provided new insights into the genetic regulation of intrinsic VO$_{2\text{max}}$. Of these, two genes—CASQ2 and SGCg—are highlighted hereinafter for illustrative purposes. The CASQ2 gene, encoding calsequestrin, a calcium-binding protein localized in the sarcoplasmic reticulum in cardiac and skeletal muscle cells, was highly ranked in the bioinformatics analysis, positively correlated in gene expression to intrinsic VO$_{2\text{max}}$, harbored SNPs positively associated with several muscle phenotype data from HERITAGE (e.g., greater proportion of type I and lower proportion of type IIb fibers), while also displaying knockout mouse phenotypes in all four classes of endophenotypes. The most notable effects were either cardiovascular (cardiac muscle contractility, increased heart mass, cardiac hypertrophy, sinus arrhythmia, etc.) or muscle-related (abnormal sarcoplasmic reticulum morphology, reduced muscle contractility). These findings in HERITAGE corroborate those from a study of high- and low-active mice in which CASQ1, the muscle form of CASQ2, was overexpressed in the soleus (primarily comprising type I fibers) of high-active mice compared with low-active (276). Moreover, transient knockdown of CASQ1 protein in high-active mice resulted in a significant decrease in physical activity. Rare variant mutations in CASQ2 (rs121434549, rs786205106, and rs121434550) have been implicated in catecholaminergic polymorphic ventricular tachycardia 2 (CPVT2) (277–279). These findings would suggest that common variants in CASQ2 also influence cardiac and skeletal muscle function relevant to intrinsic VO$_{2\text{max}}$. Although CASQ2 was predominantly associated with cardiovascular phenotypes, the largest number of muscle-related phenotypes was observed in sarcoglycan gamma (SGCG) knockout mouse models. Notably, SGCG is a sarcolemma membrane glycoprotein and a component of the sarcoglycan complex linking F-actin cytoskeleton and the extracellular matrix in muscle cells. Impairments in SGCG function result in early-onset recessive muscular dystrophy, especially limb–girdle muscular dystrophy, in humans (280,281). This study suggests a role for common variations in the SGCG gene in the regulation of intrinsic VO$_{2\text{max}}$.  

THE HERITAGE FAMILY STUDY: A REVIEW  

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This effort based on a bioinformatics pipeline applied to VO$_{2\text{max}}$ data in the sedentary state suggests four gene loci related to cardiovascular physiology (ATE1, CASQ2, NOTO, and SGCG), four loci related to hematopoiesis (PICALM, SSB, CASQ2, and CA9), four loci related to skeletal muscle function (SGCG, DMRT2, ADARB1, and CASQ2), and eight loci related to metabolism (ATE1, PICALM, RAB11FIP5, GBA2, SGCG, PRADC1, ARL6IP5, and CASQ2) as candidates for human variability in cardiorespiratory fitness.

**EPILOGUE**

The HERITAGE Family Study has been a remarkable story of scientific collaboration among multiple sites and a large number of scientists, fellows, and research associates over a period of 30 yr. As with all studies, HERITAGE has strengths and limitations. Strengths of the study include the relatively large sample size for an exercise training study, the inclusion of family members as opposed to singletons, and substantial samples of both sexes, different ages, and Blacks and Whites. An extensive panel of quality control measures were implemented to standardize testing and training procedures, staff training, and minute-by-minute monitoring of each training session for each participant with a level of compliance reaching >95%. These efforts included sub-studies to evaluate within-person variability over time in sedentary people, and whether there were between-site differences and drift in testing protocol over time by clinical center sites. There were extensive panels of behavioral, physiological, and metabolic phenotypes measured before and after training, with most traits being measured twice before and then 24 and 72 h after training, all complemented by a comprehensive data management system and an extensive biobanking resource.

There are also limitations to the study, the most obvious of which being the lack of a control group. As mentioned previously, it was agreed 30 yr ago that a control group was not necessary to address the main aims of HERITAGE. Indeed, the consensus was that the funding available would be better devoted to reaching a larger sample size of families and participants in both ethnic groups than to add a control group. The lack of a control group became a challenge when the HERITAGE resource was used in secondary analyses. However, a substantial panel of quality control measures were added to the protocol in to quantify the assay reproducibility (for blood-based assays), the reproducibility and technical error of physiological and metabolic measurements, and the within-person variability over time of these traits, plus estimates of between clinical center differences and drift over time in data, with the goal of compensating for the lack of a control group in secondary analyses. Other limitations include that only one standardized dose of exercise was tested, children and older adults were not incorporated into the study, and skeletal muscle samples were available on only a subsample of participants.

What we have learned has been reviewed in the preceding sections. The original aims of HERITAGE were to investigate the magnitude of the individual differences for exercise-related traits and their response levels with exposure to endurance training, to evaluate whether there was familial aggregation and compute the heritability level for each trait and their response to training, and to explore a set of candidate genes and unbiased genomic microsatellite markers associated with exercise traits and their trainability. As detailed previously, these aims have been thoroughly covered across the various phases of the HERITAGE research program. The most novel and impactful findings of the study are summarized in Supplementary Table S4 (Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C482). An illustration of the general trends in the reported findings is provided here with an emphasis on cardiorespiratory fitness, as evaluated by VO$_{2\text{max}}$.

Individual differences in VO$_{2\text{max}}$ among sedentary adults were found to be large. An appreciation of their magnitude can be found in Figure 11. The left panel depicts the distribution of VO$_{2\text{max}}$ per kilogram of body weight per minute, based on two tests, within 1 wk, in a group of 174 young adult males.

**VO$_{2\text{max}}$ in the sedentary state and in response to the HERITAGE endurance exercise program in young adult males**

FIGURE 11—Individual differences in intrinsic VO$_{2\text{max}}$ (left panel) and its response to the standardized training program (right panel) in HERITAGE young adult males age 17 to 35 yr. Intrinsic VO$_{2\text{max}}$ is derived from two tests at baseline in the sedentary state. The gain in VO$_{2\text{max}}$ was calculated using the mean of two post training values minus the baseline level.
age 17 to 35 yr who were all physically inactive (mean of 40.6 mL O₂·kg⁻¹·min⁻¹, SD, 8.0). There is a 3-fold range between the low and high values of “intrinsic” cardiorespiratory fitness. The same observation was made for women, older subjects, and both ethnic groups. The right panel illustrates the VO₂max training response in the same homogenous sample of young adult males. The mean gain reached 15.7%, with SD of 8.7%. Even though most tended to improve by about 10% to 20%, the range of responses is wide, with some experiencing no gain and others improving by 40% to 60%, as illustrated by the skewed distribution. Comparable variability in baseline levels and responses to training was observed for other traits, including submaximal exercise capacity, exercise HR and BP, visceral adiposity, insulin sensitivity, HDL-C, lipoprotein and hepatic lipases, and other exercise-related traits as was summarized in previous sections of this review.

The topic of familial aggregation and heritability has been addressed throughout this review. After adjustment for age, sex, body mass, and other appropriate concomitants, familial aggregation of maximal and submaximal indicators of cardiorespiratory fitness remained significant, with approximately 2.5 times more variance between families relative to within families. Familial aggregation for response traits persisted after adjustment for multiple covariates for the same traits. Heritability levels commonly reported in the scientific literature or traits such as body mass and body composition, fat distribution, visceral fat, BP, insulin and glucose traits, lipids and lipoproteins were generally replicated in HERITAGE sedentary state measurements. Intrinsic VO₂max was characterized by a heritability level of the order of 50% after adjustment for age, sex, body mass, and body composition. Globally, the heritability of the training response traits tends to be lower than the heritability in the sedentary state. One notable exception to this trend pertains to the trainability of VO₂max, whose heritability level reaches 45% to 50% when assessed in both the untrained and trained states.

Human heterogeneity in the response to a given dose of exercise over a given time period is deeply rooted in the biology of human adaptability, with genomic differences playing an important role. Using the changes in VO₂max in the HERITAGE cohort, we have attempted to quantify the correlates and apparent sources of response variability. Our findings have been summarized earlier and are depicted in Figure 12 (33). Age, sex, ethnicity, baseline body weight, and baseline VO₂max were all weakly associated with the gains in VO₂max, each accounting for 2% to 3% of the variance. The minute-by-minute fluctuations in achieved HR and PO compared with the prescribed HR and PO during each exercise session for each subject were computed. These differences between achieved versus prescribed were found to account for about 6% of the VO₂max training response. We estimated as discussed previously that the measurement error and the day-to-day variation in VO₂max explained about 20% of the variability in training response. Finally, as described earlier

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**TABLE 16. Correlation coefficients of exercise training responses among cardiometabolic traits in the HERITAGE Family Study.**

| Trait                  | % Fat | AVF  | Insulin | HDL-C | Small LDL-P | GlycA |
|------------------------|-------|------|---------|-------|-------------|-------|
| % Body fat             | -0.09 | -0.05| 0.03    | 0.06  | -0.07       | -0.05 |
| AVF                    | 0.35* | 0.18*| 0.07    | 0.08  | 0.03        |       |
| Fasting insulin        | 0.23  | -0.07| 0.04    | 0.10  | 0.08        |       |
| HDL-C                  | -0.09 | 0.05 | 0.13*   |       |             |       |
| Small LDL-P            | -0.27*| -0.11|         |       |             |       |

All listed traits represent the change in trait value in response to training (i.e., delta trait). Values are Pearson correlation coefficients and (P-value). Correlations adjusted for age, sex, ethnicity, and baseline value of the response traits. Multiple testing corrected threshold for statistical significance P < 0.007. Based on 564 subjects with complete data.

*P < 0.007 for statistical significance.

Fasting insulin, fasting plasma insulin; GlycA, plasma inflammatory marker; AVF, abdominal visceral fat; fasting plasma insulin; LDL-P, low-density lipoprotein particle, plasma inflammatory marker GlycA.

Adapted with permission from Barber et al. (283).
(Fig. 4), the genetic component of $\dot{V}O_{2\text{max}}$ trainability accounted for 47% of the variance, a heritability level compatible with estimates obtained in selection experiments performed in rats (282). Thus, in the aggregate, the sources of variation or correlates of $\dot{V}O_{2\text{max}}$ trainability explain 87% of the variance, as detailed in Figure 12 (33).

One important question is whether the HERITAGE findings on $\dot{V}O_{2\text{max}}$ trainability apply to the training-induced changes in other traits of interest. Individual differences in response level have been observed for a panel of seven cardiometabolic and cardiorespiratory fitness traits (44,283). Changes in $\dot{V}O_{2\text{max}}$ were shown to be independent of the changes in adiposity, visceral adipose tissue, insulin, lipid and lipoprotein, and inflammatory phenotype changes (Table 16). For instance, the correlation coefficients between the gains in $\dot{V}O_{2\text{max}}$ and the changes in cardiometabolic traits are less than 0.10. Similarly, the coefficients among several cardiometabolic trait changes are generally about 0.20 or less, except for the changes between percent body fat and AVF, which reached 0.35 (283). Thus, the response levels across traits do not aggregate in any given individual, indicating that there is a high degree of trait specificity in responsiveness to an endurance training program. One implication of these observations is that the profile of correlates and determinants for the training-induced gains in $\dot{V}O_{2\text{max}}$ is unlikely to be applicable to other response traits. It is also unlikely that the underlying biology of training responsiveness (as defined by molecular drivers, transcription factors, activators and repressors, small and large molecules, gene networks, pathways, and systems) will be the same for all response traits. Thus, each response trait or family of traits (e.g., a set of adiposity-related variables) may have its unique biological and molecular signature.

HERITAGE and the early training experiments performed with numerous pairs of sedentary monozygotic twins have successfully established that there are considerable individual differences in the response level to a given dose of exercise of cardiorespiratory fitness, physiological and cardiometabolic traits, and that genetic components account for substantial fractions of the response variability. Subsequently, HERITAGE was the first study to embark on a systematic search for the genomic variants associated with training response variability. Before the GWAS and omics era, multiple candidate genes were explored, and panels of microsatellite markers were used to perform unbiased screens of the genome taking advantage of the pairs of siblings and nuclear family structure of HERITAGE participants. One of the major lessons learned from these early genetic explorations was that some of the prevailing views during the first 15 yr or so of the HERITAGE research program were later found to be inadequate. The genetic component of complex, multifactorial traits, such as $\dot{V}O_{2\text{max}}$ trainability or the training response of any physiological and metabolic trait, could not be accounted for by a relatively small number of genes and genomic variants. Advances in technologies and high-throughput systems have allowed the recognition that such complex traits are commonly associated with large numbers of genomic variants, with each of them contributing only a tiny or small fraction of the genetic variance. Since then, HERITAGE resources

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**Expanded HERITAGE research working model**

![Expanded HERITAGE research working model](https://www.acsm-msse.org)

**FIGURE 13**—Schematic display of the original HERITAGE working model (left side of the figure) and the expanded research strategy deployed to investigate the biology and molecular determinants of exercise-related traits in the sedentary state and their responsiveness to an endurance training program meeting the requirements of the Physical Activity Guidelines.

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have been mobilized to incorporate genome-wide and molecular-wide (e.g., proteome and metabolome) screening approaches based on panels of millions of measured and imputed genomic markers, and thousands of muscle and plasma molecular markers.

This has made it possible to extend the original intent of the HERITAGE project to complex and multi-omics exploration of the biology of cardiovascular and diabetes related traits in the sedentary state and in response to endurance training. Displayed in Figure 13 is the contrast between the original HERITAGE plan versus the new and expanded effort currently in progress based on the contributions of several laboratories. The interest in both baseline exercise-related phenotypes in a sedentary cohort and in the variability of the response to training persists. However, the goal of trying to account for the biology of human variation based primarily on demographics, physiology, metabolism, behavior, and genetics has been greatly augmented. Because of the availability of the HERITAGE biobank, it has become possible to deploy other omics, including more extensive panels of genomic variants (about 18 million measured and imputed SNPs), muscle transcriptomic (in the subset with muscle biopsies), and plasma proteomic (a panel of 5000 proteins), metabolomic (a panel of about 3000 targeted and untargeted metabolites), and lipidomic profiles, as well as to increase our capability to define the biology and molecular foundation of exercise-related phenotypes. This whole new effort is supported by additional resources in computational biology and bioinformatics.

Physical activity level and cardiorespiratory fitness are both related to risk profiles, common morbidities, the risk of cardiovascular disease, diabetes and obesity, and all-cause and cardiovascular mortality. Physical inactivity has been shown to have an impact on all-cause mortality comparable to that of smoking and obesity, and is estimated to be responsible for more than 5 million deaths worldwide (284). Forty years ago, it was shown for the first time that cardiorespiratory fitness was associated with mortality (285). Since then, many studies have confirmed that low cardiorespiratory fitness level was associated with a higher rate of all-cause and cardiovascular mortality, even after adjustment for other known risk factors (286).

However, in spite of a large number of observational studies, it is still not clear whether the observed favorable effect of cardiorespiratory fitness depends on the intrinsic level observed in the sedentary state or if it is mainly driven by the low to moderate improvements commonly seen with exposures to physical activity (287). The expanded research program of HERITAGE cannot provide direct evidence in favor of or against either hypothesis, but it does provide highly relevant information on the molecular transducers and highly complex biology of cardiorespiratory fitness and common cardiometabolic traits in the causal pathways linking physical activity, fitness, and health.

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### APPENDIX

**HERITAGE Family Study Review Paper**

#### SUPPLEMENTARY TABLE S1. HERITAGE Consortium Steering Committee.

| Name                            | Institution                                      |
|---------------------------------|--------------------------------------------------|
| Claude Bouchard, PhD            | Laval University (until 1999); later Pennington Biomedical Research Center |
| Jacques Gagnon, PhD             | Laval University                                  |
| Jean Paul Albert, MBA           | Laval University                                  |
| Tuomo Rankinen, PhD             | Pennington Biomedical Research Center             |
| Arthur S. Leon, MD              | University of Minnesota                           |
| D. C. Rao, PhD                  | Washington University                            |
| James S. Skinner, PhD           | Arizona State University (until 1995); Indiana University (1996+) |
| Jack H. Wilmore, PhD            | The University of Texas at Austin (until 1997), later Texas A&M University, then back to The University of Texas at Austin after 2003 |

#### SUPPLEMENTARY TABLE S2. HERITAGE Consortium Advisory Board (1992–2008).

| Name                            | Institution                                      |
|---------------------------------|--------------------------------------------------|
| Elizabeth Barrett-Connor, MD    | University of California, San Diego              |
| Jean Davignon, MD               | Clinical Research Institute of Montreal           |
| E. Randy Eichner, MD (1992–2002)| University of Oklahoma                           |
| Robert C. Elston, PhD           | Louisiana State University and Case Western Reserve University |
| William L. Haskell, PhD         | Stanford University                               |
| Rudy Leibel, MD (2002–2008)    | Columbia University                               |
| Eric Ravussin, PhD (2002–2008)  | Pennington Biomedical Research Center             |

#### SUPPLEMENTARY TABLE S3. HERITAGE coordinating centers, clinical centers, and core laboratories: list of personnel.

**Laval University Consortium Coordinating Center and Clinical Center**

| PI: Claude Bouchard, PhD          | Benoit Larmande, PhD                            |
| Local Project Coordinator: Marcelle Lareau, MSc | Claude Leblanc, MSc |
| Jean-Paul Albert, MBA             | Isabel Mercier, MSc                             |
| Monar Baril, BSc                  | Chantal Pari, BSc                               |
| Jean Bergeron, MD                 | Louis Pérusse, PhD                              |
| Come S. Bouchard, MSc             | Denis Prud’homme, MD, MSc                       |
| Anne-Marie Bricault, MSc          | Jean-Aime Simonneau, PhD                        |
| Yvon Chagnon, PhD                 | My-Anh Ho-Kim, MSc                              |
| Jean-Pierre Despres, PhD          | Karen Horth, MD                                 |
| Diane Drolet, MSc                 | Louise Laberge, MD                              |
| Jacques Gagnon, PhD               | University of Minnesota                         |
| My-Anh Ho-Kim, MSc                | Laval University Data Coordinating Center       |
| Karen Horth, MD                   | Ping An, MD                                     |
| Louise Laberge, MSc               | Ingrid B. Borecki, PhD                          |
|                                   | Harry Cheng, MSc                                |
|                                   | Gu Chi, PhD                                     |
|                                   | Warwick Dav, PhD                                |
|                                   | Habib El-Moalem, PhD                            |
|                                   | John O. Hollosy, MD                             |
|                                   | Yuling Hong, MD                                 |
|                                   | David J. Lerner, BSc                            |

**Laval University Core Laboratories**

**Diabetes Research Unit**

| André Nadeau, MD, PhD, Director   | Lipid Research Center                           |
| Paul Lupien, MD, PhD, Director   | Paul Lupien, MD, PhD, Director                  |
| Jean-Pierre Despré, MD           | Sital Moorjani, PhD                             |
| Gilles Tancrede, MSc             | Steroid Laboratory                              |
| DNA and Cell Line Unit           | Alain Bélanger, PhD, Director                   |
| France T. Dionne, PhD, Director  | Simon Caron, MSc                                |
| Monique Chagnon, ART             | Caroline Noel, BSc                              |
| Carolin Noel, BSc                | Marie-Christine Thibault, PhD                   |
| University of Minnesota Clinical Center | medicine & science in sports & exercise | University of Minnesota Clinical Center | medicine & science in sports & exercise |
| PI: Arthur S. Leon, MD           | Marcella Meyers, PhD                            |
| Local Project Coordinator: Ava J. Walker, PhD | James P. Norton, MSc |
| Marilyn Borken,                  | Erika J. Ekstrom, MD                            |
| Fernando S. Branco, MD           | Robert C. Serfass, PhD                          |
| Steve Gaskill, PhD               | M. Katie Schmitz, MSc                           |
| William V. Mendoza, MD           | The University of Texas at Austin (until 1997), later Texas A&M University, then back to The University of Texas at Austin after 2003 |

Continued next page
## SUPPLEMENTARY TABLE S4. Listing of some of the most novel or impactful findings from the HERITAGE Family Study from 1995 to 2021.

| Novel or Impactful Findings | Trait(s) | Details | Citation(s) |
|-----------------------------|----------|---------|-------------|
| Familial aggregation of baseline phenotype levels | | | |
| Familial aggregation of baseline phenotype levels | $V_{O2\max}$ | Within-subject SD from measures repeated days and weeks apart range from 108 to 137 mL O$_2$·min$^{-1}$ with CV of 4.1% to 5.0%. | (20,27,33,34), plus unpublished data |
| Significant familial resemblance for intrinsic $V_{O2\max}$ | | 2.7 times more variance between families than within families. Heritability of intrinsic $V_{O2\max}$ was 51%, with significant maternal heritability (29%). | (45) |
| Significant familial aggregation for $V_{O2}$ at 50 W, 60%, 80% | | Maximal heritability estimates ranged from 48% to 70% and 29% to 48% for maternal heritability. | (48) |
| Significant familial aggregation for intrinsic submaximal Q and SV | | Maximal heritability estimates of 46% for SV and Q at 60% $V_{O2\max}$ and 41% and 42% at 50 W | (58) |
| Significant familial aggregation for AVF | | Heritability of 47% to 48%, independent of total body fat | (32) |
| Higher heritability of resting BP in Black subjects | | Resting SBP and DBP Heritabilities of SBP and DBP were 68% and 56% in Black subjects vs 43% and 24% in White subjects. | (56) |
| Significant familial aggregation for markers of oxidative stress | CK, PHOS, HK, PFK, GAPDH, LPL, CPT, HADH, CS, COX | Strong familial aggregation for activities of baseline muscle enzymes related to PCr, glycolytic, and oxidative metabolism. | (138,143) |
| Significant familial aggregation for LDL-ox, C50-AAPH, TBARS, glutathione | | Hertility for oxidative stress traits ranged from 31% to 44%. | (127) |
| Familial aggregation of training response | | | |
| Significant familial aggregation for $V_{O2\max}$ training response | $V_{O2\max}$ | Heritability was 47%, with maternal inheritance accounting for 28%. | (41) |
| Significant familial aggregation for submaximal $V_{O2}$ training response | $V_{O2}$ at 50 W, 60%, 80% | Heritability values ranged from 23% to 57% for the training response of submaximal measures of $V_{O2}$. | (48) |
| Significant familial aggregation of submaximal exercise BP and HR training responses | HR50, HR60%, SBP50 | Hertitabilities for HR50, HR60%, and SBP50 responses were 34%, 29%, and 22%. Heritability for DBP traits and all HR and BP traits were lower in Black subjects. | (71) |
| Significant familial aggregation for submaximal Q & SV training responses | SV & Q at 50 W and 60% $V_{O2\max}$ | Heritabilities ranged from 24% to 38% for SV and Q at 50 W and 60% $V_{O2\max}$. | (58) |
| Significant familial aggregation of interindividual variation in plasma lipid responsiveness to training | TC, TG, LDL-C, apoB, HDL-C, HDL2-C, HDL3-C, apoA-I | Heritability ranged from 25% to 38% for lipid response traits. Exceptions were for heritability levels near 60% for changes in apoB in Blacks and HDL2-C in Whites and a lack of heritability for change in LDL-C in Black subjects. | (98) |
| Significant familial aggregation for training response of markers of oxidative stress | LDL-ox, C50-AAPH, TBARS, glutathione | Heritability for oxidative stress training response traits ranged from 35% to 84%. | (127) |
| Significant familial aggregation for training response of muscle enzyme activities | CK, PHOS, HK, PFK, GAPDH, CPT, HADH, CS, COX | Strong familial aggregation was found for training response of muscle enzymes related to PCr, glycolytic, and oxidative metabolism. | (138,143) |
| Ethnic and sex differences in responses to training | | | |
| Training responses of submaximal exercise measures of hemodynamic traits differed by sex and ethnic groups | HR, SBP, DBP at 50 W | Submaximal HR, SBP, and DBP decreased with training, with greater reductions in women compared with men and in Black and older subjects compared with White and younger subjects. | (17), pp. 10–116 |
| Significant sex interactions for insulin sensitivity response to training | $a$-$V_{O2}$ diff, SV, Q, and $V_{O2}$ at 50 W | Black men did not increase $a$-$V_{O2}$ diff at 50 W. Thus, on average, Black men had greater increases in SV50 and smaller decreases in Q50 compared with White men to achieve similar VO250. | (61), MSSE, pp. 99–106 |
| Significant sex differences in lipid, lipoprotein, and lipase activity responses to training | ApoA-I, HDL2-C | ApoA-I increased more in women than men, in Black than White subjects, and in offspring than in parents. Black subjects experienced greater increases in HDL2-C compared with White subjects. | (84) |
| | LPL activity | LPL activity increased in all subgroups except Black men. | (97) |
| Novel or Impactful Findings | Trait(s) | Details | Citation(s) |
|-----------------------------|----------|---------|-------------|
| Response of other traits to training | LDL-Cx, C50-AAPH, TBARS, antioxidants, and aminoacids | Only erythrocyte resistance to hemolysis significantly changed with training, which interacted with smoking status (smokers did not experience beneficial effects of training on erythrocytes), and was significant in women only. | (127) |
| No change in RMR with training. | RMR via indirect calorimetry | Sample size (N = 77) was larger than previous studies. There was no change in RMR at 24 or 72 h after training. | (153) |
| Favorable changes in clinically relevant lipoprotein subfractions in response to training | NMR-based lipoprotein subfractions | Large HDL-P and LDL-P increased, whereas small LDL-P and all VLDL subfractions and VLDP-L size decreased with training. These findings were not captured with traditional lipid profiling (i.e., TG and LDL-C did not change in total sample). | (93) |
| Genome-wide linkage studies of baseline and response phenotypes | VO2max | QTLs on 4q, 8q, 11p, and 14q were reported for baseline VO2max. QTLs on 1p, 2p, 4q, 6p, and 11p were identified for change in VO2max. | (210) |
| First GWAS of exercise response traits | VO2max | 39 SNPs were associated at P < 1.5 x 10^{-4}, with a panel of 21 SNPs accounting for 49% of the variance in VO2max trainability. | (234) |
| GWAS of submaximal HR response to training | HR50 | 40 SNPs associated at P < 9.9 x 10^{-5}, with top hit 8 x 10^{-6}. Nine SNPs accounted for the genetic variance of the submaximal exercise HR response to training. | (235) |
| Molecular signatures of exercise response derived from integrative analyses of genomic and transcriptomic profiles | VO2max | Genome-wide baseline muscle gene expression and validation identified a 29-RNA signature that predicted changes in VO2max. Candidate genes from this predictor and the literature led to a 11-SNP signature that explained 23% of the variance in VO2max trainability. | (265) |
| Combined genome-wide and transcriptome-wide analysis identifies SNPs associated with TG response to training | TG | GWAS identified 4 SNPs accounting for the genetic variance of TG response, whereas molecular signature based on the baseline expression of 11 genes predicted 27% of TG changes in response to training. An 8-SNP score comprising 4 SNPs each from transcriptomics and GWAS was the strongest predictor of TG training response. | (236) |
| GWAS and transcriptional signature of insulin sensitivity response to training | Si | Integrative analysis of functional genomic and transcriptomic profiles identified combined variation in genes linked to cholinergic, calcium, and chemokine signaling associated with Si training response. MEF2A transcription factor was the most significant candidate driving the transcriptional signature associated with Si, strengthening the relevance of calcium signaling in exercise training-mediated Si response. | (238) |
| Proteomic signatures of VO2max and its trainability | VO2max | Elastic net regression identified 115 proteins highly correlated (r² = 0.88) with measured VO2max, which was replicated in the validation data set, with an r² of 0.71 (Fig. 9). | (271) |
| Plasma proteins associated with intrinsic VO2max and its trainability | VO2max | 147 proteins were associated with baseline VO2max, whereas 102 baseline proteins were associated with changes in VO2max, with minimal overlap (only 5 proteins) between protein sets. A baseline 56-protein signature improved prediction of VO2max response (AUC 0.84) compared with a model of only clinical variables (AUC 0.62). | (272) |
| Bioinformatics explorations of intrinsic VO2max and its trainability response to training GWAS | VO2max | Using GWAS results followed by candidate gene prioritization and pathway analysis, pathways related to calcium signaling, energy sensing and partitioning, mitochondrial biogenesis, angiogenesis, immune functions, and regulation of autophagy and apoptosis were identified as key mechanisms through which the physiological responses of VO2max to training are mediated. | (273) |
| Genetics and biology underlying intrinsic VO2max | VO2max | A bioinformatics pipeline applied to VO2max data in the sedentary state suggests 4 loci related to cardiovascular physiology (ATE1, CASQ2, NOTO, and SGCG), four loci related to hematopoiesis (PIKCALM, SSB, CASQ2, and CA9), 4 loci related to skeletal muscle function (SGCG, DMR21, ADAR91, and CASQ2), and 8 loci related to metabolism (ATE1, PIKCALM, RAB11FIP5, GBA2, SGCG, PRAD1C1, ARGLIPS, and CASQ2) as candidates for human variability in cardiorespiratory fitness among sedentary adults. | (275) |
| Metabolomic biomarker of training responsiveness | DMGV is a biomarker of metabolic response to endurance training | Baseline levels of plasma DMGV associated with lack of improvement in HDL traits. DMGV levels decreased with training and were positively correlated with several lipid, glucose, and insulin traits | (96) |