

# 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

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Submitted for publication August 2021.

Accepted for publication December 2021.

This review is dedicated to the late Jack H. Wilmore, PhD, and to the HERITAGE Project Directors: Jacques Gagnon, PhD (from 1992 to 1999), and Tuomo Rankinen, PhD (from 1999 to 2012).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site ([www.acsm-msse.org](http://www.acsm-msse.org)).

0195-9131/22/5405-0000/0

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DOI: 10.1249/MSS.0000000000002859

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

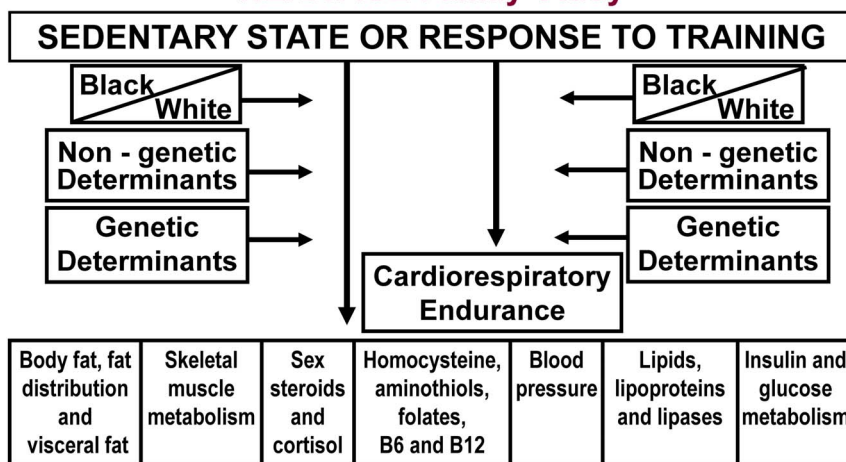
The aim of this article is to summarize the findings of the HERITAGE (*HE*alth, *RI*sk factors, exercise *TR*aining *ANd* *GE*netics) Family Study and their potential implications for cardiometabolic health and exercise performance. HERITAGE has contributed substantially to our understanding of adaptation 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

### The General Conceptual Model of the HERITAGE Family Study



**FIGURE 1**—The 1992 model integrating the various components of HERITAGE. Subjects were measured in the sedentary state and after the exercise program intervention. Cardiorespiratory fitness, exercise hemodynamics, and subsets of cardiometabolic and diabetic risk factors were monitored. Differences between Blacks and Whites were investigated. Studies on genetic and nongenetic determinants of human variability in the sedentary state and in response to endurance exercise training were pursued.

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 and then Indiana University, Laval University, University of Minnesota, and University of Texas at Austin), and four core laboratories (lipids, steroid hormones, glucose-insulin-related traits, and cell lines) located at Laval University. 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](http://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 posttraining 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	Baseline				Posttraining <sup>a</sup>			
	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

<sup>a</sup>Posttraining numbers include only the subjects who were classified as completers having completed at least 57 (95%) of the 60 exercise training sessions.

TABLE 2. List of the concomitant and behavioral variables available in the HERITAGE Family Study.

Phenotype	Baseline	Midtraining	Posttraining
Age, sex, and ethnicity	x		
Medical history: previous diseases and physical examination	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		
Menstrual cycle: age at menarche, menstrual status (premenopausal and postmenopausal), number of pregnancies, babies over 9 lb, estrogen, and PROG replacement	x	x	
Physical activity habits: leisure time index, work index, sports index	x		
EPAT: Score section I and section II	x	x	x
Food frequency questionnaire	x		
Anxiety levels	x		x

(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 by computed tomography (CT) (16). Scans were taken between the fourth and fifth lumbar vertebrae (L4–L5 space) (14). Subjects were examined in the supine position, with arms stretched above their heads. Total and visceral (AVF) abdominal fat areas (in cm<sup>2</sup>) 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 HDL<sub>2</sub> and HDL<sub>3</sub> 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<sup>-1</sup> 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-3 $\alpha$ , 17 $\beta$ -diol glucuronide, pregnenolone (PREG) 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 cardiorespiratory 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  $\dot{V}O_{2max}$ . The second test was a submaximal test with workloads of 50 W and then 60% of the power output (PO) at  $\dot{V}O_{2max}$ , 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  $\dot{V}O_{2max}$ , followed by a progressively increasing workload until exhaustion and the second estimate of  $\dot{V}O_{2max}$ . 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.



TABLE 3. List of phenotypes available in the HERITAGE Family Study.

Phenotype	Baseline		Posttraining	
	Test 1	Test 2	Test 3	Test 4
<b>Body fat and body composition</b>				
Weight, height, and BMI	x		x	
FM and fat-free mass (underwater weighing)	x		x	
<b>Regional fat distribution and visceral fat</b>				
CT total, subcutaneous, and visceral fat	x		x	
Sum of 8 skinfolds	x		x	
Waist and hip girths	x		x	
<b>BP</b>				
Resting	x	x	x (24 h)	x (72 h)
Exercise at 50 W and at 60% and 80% $\dot{V}O_{2max}$	x	x	x	x
<b>Insulin and glucose metabolism</b>				
IVGTT: Glucose, insulin, C-peptide	x		x	
Fasting: Glucose, insulin, C-peptide	x	x	x	x
<b>Lipids, lipoproteins, and lipases</b>				
Postheparin lipolytic activities: LPL and HL	x			x (72 h)
Fasting lipid, lipoprotein, apolipoprotein profile	x	x	x (24 h)	x (72 h)
<b>Steroids</b>				
Panel of androgens, estrogens plus SHBG	x	x	x (24 h)	x (72 h)
<b>Cardiorespiratory endurance</b>				
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, 60% $\dot{V}O_{2max}$ and 80% $\dot{V}O_{2max}$ , and max	x			x
<b>Additional phenotypes on subsamples</b>				
Leptin (resting and exercise)	x			x
Insulin-like growth factors 1 and 2	x		x	
Homocysteine, cysteine, glutathione, cysteinyl glycine, folates, vitamins B <sub>6</sub> , and B <sub>12</sub>	x			
LDL oxidation, TBARS, hemolysis, GSH-Px, $\alpha$ -tocopherol, $\beta$ -carotene, retinol, phospholipid fatty acids, Lp(a)	x		x	
Skeletal muscle fiber types, capillary density, enzymes	x		x (96 h)	
RMR	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  $\dot{V}O_{2max}$ . 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 50W followed by 8 min at the PO associated with 60% of  $\dot{V}O_{2max}$ . The last test repeated the conditions of the second test, followed by a workload corresponding to 80% of  $\dot{V}O_{2max}$ , and then increasing workloads until exhaustion was reached to provide a second estimate of  $\dot{V}O_{2max}$ . In the post-training period, these tests were performed at 24, 48, and 72 h following the last exercise training bout.

**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  $\geq 7$  METs for subjects 50 yr and older and  $\geq 8$  METs for those younger than 50 yr over at least the previous 3 months.

**Body mass index.** Body mass index (BMI) was  $<40 \text{ kg}\cdot\text{m}^{-2}$  because of metabolic abnormalities and difficulty in exercising that is associated with extreme obesity. However, a few subjects with a BMI of  $40 \text{ kg}\cdot\text{m}^{-2}$  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  $<160/100 \text{ mm Hg}$  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  $\dot{V}O_{2max}$ , 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%  $\dot{V}O_{2max}$ , determined from the initial maximal test, for 8 min at each PO to measure steady-state ventilation ( $\dot{V}E$ ),  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , respiratory exchange ratio (RER), systolic (SBP) and diastolic (DBP) BP, heart rate (HR), cardiac output ( $\dot{Q}$ ), and stroke volume (SV). During the third and final test, each participant repeated the submaximal steady-state exercise at 50 W

TABLE 4. Overview of the 20-wk training program in HERITAGE.

Weeks	Frequency (Sessions per Week)	Intensity (% $\dot{V}O_{2max}$ )	Duration (Minutes per Session) <sup>a</sup>
2	3	55	30
2	3	55	35
2	3	65	35
2	3	65	40
2	3	70	40
2	3	70	45
2	3	75	45
6	3	75	50

<sup>a</sup>Does not include 5-min warm-up or 3-min cool-down.

and 60%  $\dot{V}O_{2max}$  of the second test, after which the PO was increased to 80%  $\dot{V}O_{2max}$  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 at rest, during exercise at 50 W and at 60% and 80%  $\dot{V}O_{2max}$ , and immediately upon completion of the maximal test. Blood samples were analyzed for glucose, free fatty acids, lactate, and total proteins. Metabolic measurements and  $\dot{Q}$  ( $CO_2$  rebreathing technique) 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  $\dot{Q}$ .

### 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  $\dot{V}O_{2max}$  and began at the HR associated with 55% of baseline  $\dot{V}O_{2max}$  (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  $\dot{V}O_{2max}$  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) ( $100 \times SD/mean$ ) 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

TABLE 5. Reproducibility and technical error for selected traits as derived from the test–retest of the HERITAGE protocol at baseline and from a quality control substudy performed on subjects measured three times within 3 wk across the four clinical centers.

	Heritage Family Subjects (n Varies from 279 to 745)			Quality Control Substudy (n Varies from 55 to 60)		
	Intraclass Correlation	Technical Error	Coefficient of Variation (%)	Intraclass Correlation	Technical Error	Coefficient of Variation (%)
Body weight (kg)	1.00	0.1	0.2	1.0	0.7	0.2
HR 50 W (bpm)	0.89	6.1	4.4	0.79	6.5	6.1
$\dot{V}O_2$ at 60% max (mL $O_2$ ·min <sup>-1</sup> )	0.99	53.4	3.6	0.98	62.2	3.5
Cardiac output at 60% max (L·min <sup>-1</sup> )	0.93	0.8	5.9	0.95	0.7	4.5
Max HR (bpm)	0.88	5.4	2.9	0.88	3.7	2.0
$\dot{V}O_2$ max (mL·min <sup>-1</sup> )	0.95	119.9	5.1	0.96	136.9	4.7
Cholesterol (mmol·L <sup>-1</sup> )	0.95	0.21	4.7	0.93	0.24	6.0
HDL-Chol (mmol·L <sup>-1</sup> )	0.94	0.06	6.2	0.94	0.06	6.0
LPL (nmol·min <sup>-1</sup> ·mL <sup>-1</sup> )	0.95	8.1	15.4	0.95	7.6	14.7
TESTO (nmol·L <sup>-1</sup> )						
Male	0.95	1.9	13	0.95	1.8	11
Female	0.90	0.3	23	0.92	0.2	13
DHEAS (nmol·L <sup>-1</sup> )						
Male	0.96	955	16	0.94	868	13
Female	0.97	673	18	0.92	801	18
SHBG (nmol·L <sup>-1</sup> )						
Male	0.97	4.1	11	0.98	3.6	9
Female	0.97	11.7	15	0.99	11.9	12

Adapted from Wilmore et al. (26), Wilmore et al. (27), Skinner et al. (20), Despres et al. (18), and He et al (28).

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

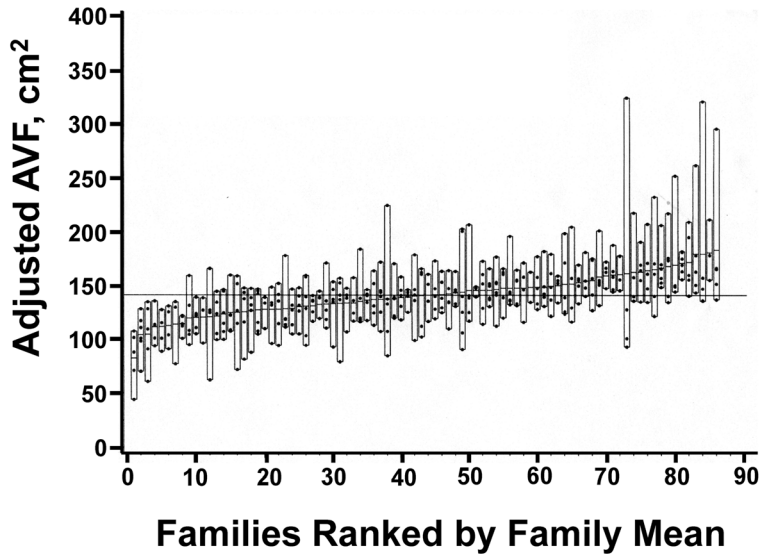
high degree of reproducibility was observed across all traits measured and at each of the clinical centers. For instance, intraclass correlations ranged from 0.95 to 0.99 for anthropometric measures and from 0.97 to 1.00 for body composition measures, all with low technical error variance; this suggests 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  $24.9 \pm 13.9 \text{ kg}\cdot\text{m}^{-2}$  in White women to  $27.9 \pm 6.0 \text{ kg}\cdot\text{m}^{-2}$  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  $\pm$  SE) in body weight ( $-0.2 \pm 0.1 \text{ kg}$ ). Decreases were also observed for the sum of skinfolds ( $-6.2 \pm 0.7 \text{ mm}$ ), waist girth ( $-0.9 \pm 0.1 \text{ cm}$ ), percent body fat ( $-0.8 \pm 0.1$ ), FM ( $-0.7 \pm 0.1 \text{ kg}$ ), abdominal subcutaneous fat ( $8.9 \pm 1.1 \text{ cm}^2$ ), AVF ( $-4.6 \pm 0.6 \text{ cm}^2$ ), and abdominal total fat ( $-13.5 \pm 1.4 \text{ cm}^2$ ) 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

## Familial aggregation of abdominal visceral fat in HERITAGE



**FIGURE 2**—CT measured AVF level at baseline adjusted for age, sex, and FM plotted by family rank among the families of European descent in HERITAGE. Dots within each bar represent individual values for each member of the given family. The dash within each bar is the mean AVF level of each family. For a given level of total adiposity, some families exhibit a mean adjusted AVF level of about 70 to 90 cm<sup>2</sup>, whereas others have AVF levels in the range of 120 to 150 cm<sup>2</sup>. Families are ranked by the mean-adjusted AVF level. A strong familial aggregation is observed with a heritability estimate of 47%. Adapted with permission from Rice et al. (32).

(31,32). 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  $\dot{V}O_{2max}$ , maximal and submaximal PO (at 60% and 80%  $\dot{V}O_{2max}$ ), and  $\dot{V}O_2$  at the ventilatory threshold ( $VO_2VT$ ). 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  $\dot{V}O_{2max}$  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).  $VO_2VT$  was also deemed satisfactorily reproducible with a test–retest correlation of 0.83 (35).

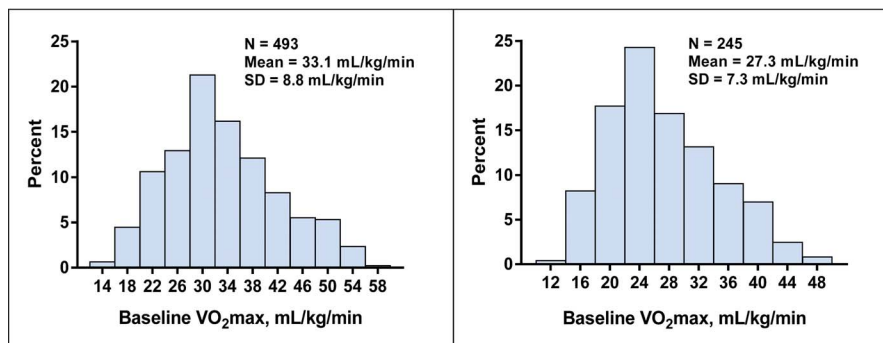
In a study on the accuracy of commonly used age-based prediction equations for maximal HR ( $HR_{max}$ ), Sarzynski et al. (36) compared intrinsic (baseline or pretraining)  $HR_{max}$  obtained in 762 subjects who underwent two separate maximal exercise tests and where  $\dot{V}O_{2max}$  was directly measured, and compared them with the Fox ( $220 - \text{age}$ ) (37) and Tanaka ( $208 - 0.7 \times \text{age}$ ) (38) formulas. It was observed that the measured  $HR_{max}$  was not highly correlated with estimated  $HR_{max}$  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  $HR_{max}$ .

**Baseline observations.** HERITAGE participants were quite representative of sedentary populations of relevant age groups. As depicted in Figure 3,  $\dot{V}O_{2max}$  (in milliliters of O<sub>2</sub> 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 cardiorespiratory fitness in the sedentary state was observed in each ethnic group, with a range of  $\dot{V}O_{2max}$  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



### Distribution of $\dot{V}O_{2\max}$ values at baseline in sedentary HERITAGE subjects



**FIGURE 3**—Distribution of  $\dot{V}O_{2\max}$  values at baseline in sedentary HERITAGE subjects.

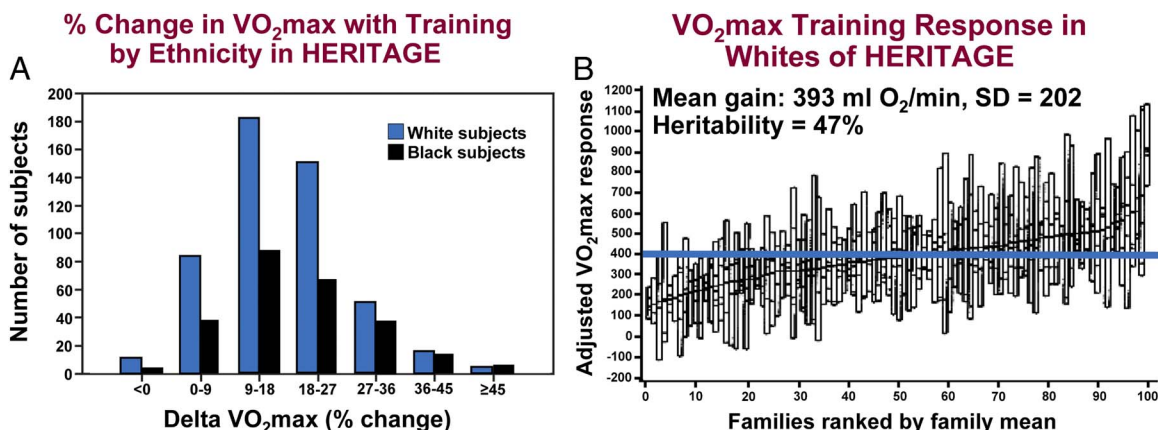
after training (39). The HR associated with 55%, 65%, 70%, and 75% of each subject's pretraining  $\dot{V}O_{2\max}$  was used to prescribe exercise intensity across the 20 wk of training. Using the linear relationship between HR,  $\dot{V}O_2$ , and PO, PO was predicted for each of the 60 training sessions 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  $\dot{V}O_{2\max}$  training response. Secondary analysis showed that the training program significantly increased mean  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  expressed in milliliters of  $O_2$ . (33).

The training program produced significant improvements in  $\dot{V}O_{2\max}$  (17%) and PO at 60%  $\dot{V}O_{2\max}$  (26 W or 28%) (40). There was wide interindividual variation in  $\dot{V}O_{2\max}$  response, with values ranging from -4.7% to +47.8% or -110 to +1100 mL  $O_2 \cdot \text{min}^{-1}$ , as illustrated in Figure 4A.

The effects of exercise training intensity relative to the VT on changes in PO (in watts) and  $VO_2VT$ , as well as changes

in  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  (individual's HR corresponding to 55% of baseline  $\dot{V}O_{2\max}$ ), there was substantial variability in the training intensity relative to VT. The range of participant's  $\dot{V}O_2$  at VT to  $\dot{V}O_{2\max}$  ( $VT\% \dot{V}O_{2\max} = VO_2VT/\dot{V}O_{2\max}$ ) 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  $VT\% \dot{V}O_{2\max}$  to the changes in  $VO_2VT$  and  $\dot{V}O_{2\max}$  after training, individuals who trained at higher intensities (relative to VT) had greater improvements in  $VO_2VT$  after training. Overall, training intensity relative to VT accounted for ~26% of the training effect on  $VO_2VT$ , 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  $\dot{V}O_{2\max}$  changes (35).

In a separate investigation, the changes in HR at a given percent of  $\dot{V}O_{2\max}$  with training were investigated across the full HERITAGE cohort and by age, sex, and ethnicity (42).



**FIGURE 4**—A, Distribution of the gains in  $\dot{V}O_{2\max}$  expressed as a percentage of baseline  $\dot{V}O_{2\max}$  level in the samples of Blacks and Whites. B, Familial aggregation of the age and sex adjusted  $\dot{V}O_{2\max}$  changes with the exercise program in the sample of Whites of European descent. Families are ordered by the mean gain for all family members. The  $F$  ratio from the ANOVA shows that there are 2.5 times more variance between families than within families ( $P = 0.0001$ ). The horizontal blue line represents the mean gain for the whole group. The heritability of the  $\dot{V}O_{2\max}$  training response reached 47%. Adapted with permission from Bouchard et al. (41).

TABLE 6. Maximal HR and  $\dot{V}O_{2max}$  training responses across HERITAGE categories of participants.

Group	n	Post Max HR (bpm)	$\Delta\dot{V}O_{2max}$ (mL O <sub>2</sub> )	$\Delta\dot{V}O_{2max}$ (%)
All	633	184 ± 13	389 ± 204	17 ± 9
Men	287	183 ± 13	444 ± 226	16 ± 8
Women	346	185 ± 12	344 ± 172	19 ± 10
Blacks	198	184 ± 13	366 ± 172	18 ± 10
Whites	435	185 ± 13	399 ± 217	17 ± 9
17–29 yr	315	191 ± 9	397 ± 216	17 ± 9
30–49 yr	200	181 ± 11	402 ± 189	19 ± 9
50–65 yr	118	172 ± 12	244 ± 192	18 ± 10

The 633 subjects retained for this table were completers with no missing data. Adapted with permission from Skinner et al. (43).

Although HR decreased at the same absolute intensity (PO in watts) with training, HR at the same relative intensity (% $\dot{V}O_{2max}$ ) 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 % $\dot{V}O_{2max}$  during a training program is unnecessary.

**Age, sex, and ethnicity.** The influence of age, sex, ethnicity, and initial fitness on the response of  $\dot{V}O_{2max}$  to training was examined in 435 Whites and 198 Blacks (287 men and 346 women) (43). As expected, the average  $\dot{V}O_{2max}$  in milliliters of O<sub>2</sub> 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  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$ . In the aggregate, age, sex, ethnicity, and initial fitness had limited influence on  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  explain about 11% of the variance in the response of  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  levels (i.e.,  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  (in milliliters per minute) adjusted for age, sex, body mass, FM, and fat-free mass (45). A significant spousal correlation in intrinsic  $\dot{V}O_{2max}$  ( $0.14 \leq r \leq 0.26$ ) was observed, consistent with results from prior studies (46,47). Importantly, the variability in  $\dot{V}O_{2max}$  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  $\dot{V}O_2$  ( $\dot{V}O_2$  at 60% and 80%  $\dot{V}O_{2max}$ ) and submaximal PO (PO at 60% and 80%  $\dot{V}O_{2max}$ ) 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  $\dot{V}O_{2max}$  to training was examined in 481 adults from 98 White families. There was 2.5 times more variance between families than within families for  $\dot{V}O_{2max}$  response to training (41). This pattern of familial clustering of  $\dot{V}O_{2max}$  trainability is illustrated in Figure 4B. The most parsimonious familial correlation model yielded a maximal heritability estimate of 47% for  $\dot{V}O_{2max}$  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%). 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  $\dot{V}O_2$  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%  $\dot{V}O_{2max}$  before and after the exercise program. Measurements included VE,  $\dot{V}O_2$ , VCO<sub>2</sub>, RER, SBP, DBP, HR,  $\dot{Q}$ , 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

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

Variable	Pretraining	Posttraining	Change	% Change
$\dot{V}O_2$ at 50 W (mL·min <sup>-1</sup> )	1029.7	994.9	-34.8	-3.2
HR 50 W (bpm)	120.2	108.8	-11.4	-9.5
SV 50 W (mL per beat)	95.9	99.8	3.9	4.1
Cardiac output 50 W (L·min <sup>-1</sup> )	11.3	10.7	-0.6	-5.31
a- $\dot{V}O_2$ diff 50 W (mL per 100 mL per beat)	9.2	9.4	0.2	2.2
$\dot{V}O_2$ at 60% $\dot{V}O_{2max}$ (mL·min <sup>-1</sup> )	1423.3	1625.9	202.6	15.3
HR 60% (bpm)	140.1	135.8	-4.3	-3.1
SV 60% (mL per beat)	98.6	109.2	10.6	10.8
Cardiac output 60% (L·min <sup>-1</sup> )	13.7	14.7	1.0	7.3
a- $\dot{V}O_2$ diff 60% (mL per 100 mL per beat)	10.3	10.9	0.6	5.8
$\dot{V}O_{2max}$ (mL·min <sup>-1</sup> )	2343.4	2725.9	382.6	17.4
Max HR (bpm)	184.9	184.3	-0.6	-0.3
Max cardiac output (L·min <sup>-1</sup> )	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.

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  $\dot{Q}$  at 60%  $\dot{V}O_{2max}$  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,  $\dot{Q}$ , and arteriovenous oxygen difference (a- $\dot{V}O_2$  diff) during submaximal and maximal exercise was also evaluated (61) (Table 7). Steady-state  $\dot{V}O_2$  at 50 W decreased only slightly (~3%) with training, likely the result of improved ergometer cycling efficiency. As expected,  $\dot{V}O_2$  improved considerably at maximal PO (17.4%) and at 60%  $\dot{V}O_2$  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%  $\dot{V}O_{2max}$ , with no change in HRmax. Slight increases in SV (4%) and a- $\dot{V}O_2$  diff (2%) were found at 50 W, which in combination with the lower HR led to a slight decrease (-5%) in  $\dot{Q}$  (-5%). During exercise at 60%  $\dot{V}O_{2max}$ , there were substantial increases in SV (11%),  $\dot{Q}$  (7%), and a- $\dot{V}O_2$  diff (6%) with training. Whereas HR at maximal exercise was unchanged, there were substantial gains in  $\dot{Q}$  (10%) and  $\dot{V}O_{2max}$  (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  $\dot{V}O_2$  and PO (60% or 80%  $\dot{V}O_{2max}$ ) 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 99 White families ( $n = 481$  participants) were investigated, and heritability estimates of HR at 50 W and 60%  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$ . In contrast, minimal heritability existed among DBP responses, SBP at 60%  $\dot{V}O_{2max}$ , 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  $\dot{V}O_{2max}$  had a 62% greater risk of future CHD. Another study found that



those with  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  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 Millennium 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 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, Sg, AIRg, and Kg ( $r = -0.11, -0.13, -0.24, \text{ and } -0.33$ , 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 Sg, 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<sup>-1</sup> or 100–125 mg·dL<sup>-1</sup>) 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<sup>-1</sup> 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

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 <sup>-1</sup> )	5.1 ± 0.6	5.2 ± 0.6*	5.0 ± 0.6	5.1 ± 0.6*	5.2 ± 0.6	5.3 ± 0.6	5.1 ± 0.6	5.3 ± 0.7*	5.1 ± 0.6	5.1 ± 0.6**
Fasting insulin (pmol·L <sup>-1</sup> )	70.7 ± 50.4	64.2 ± 39.3*	68.5 ± 50.3	61.5 ± 36.0*	73.2 ± 50.5	67.1 ± 42.6*	81.9 ± 67.7	70.5 ± 43.3*	66.1 ± 40.5	61.6 ± 37.4*
Si (10 <sup>-4</sup> min <sup>-1</sup> ·[μU/mL] <sup>-1</sup> ) <sup>a</sup>	3.9 ± 2.9	4.1 ± 2.7*	4.1 ± 2.9	4.2 ± 2.6	3.6 ± 2.8	4.1 ± 2.7*	2.8 ± 2.3	3.1 ± 2.1*	4.3 ± 2.9	4.6 ± 2.8*
AIRg (pmol·L <sup>-1</sup> × 10 min)	956 ± 1042	903 ± 933*	980 ± 1143	948 ± 1021	931 ± 924	855 ± 827*	1660 ± 1524	1570 ± 1348*	660 ± 524	623 ± 457*
Sg (min <sup>-1</sup> )	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 ± 0.01	0.02 ± 0.01*	0.02 ± 0.008	0.02 ± 0.01*
Di <sup>b</sup>	2713 ± 2411	2902 ± 2473*	2903 ± 2571	3053 ± 2714	2510 ± 2216	2741 ± 2177	3622 ± 3398	4015 ± 3348	2329 ± 1709	2435 ± 1804
Kg (%·min <sup>-1</sup> )	1.70 ± 0.6	1.75 ± 0.6*	1.80 ± 0.6	1.84 ± 0.6	1.58 ± 0.6	1.65 ± 0.6*	1.85 ± 0.7	1.95 ± 0.7*	1.63 ± 0.6	1.66 ± 0.6

Values shown as mean ± SD.

<sup>a</sup>Units are taken from the MINMOD program. To convert value to SI units (10<sup>-4</sup> min<sup>-1</sup> [pmol/mL]<sup>-1</sup>), multiply by 0.167.

<sup>b</sup>Di is calculated as Si generated from the MINMOD program (10<sup>-4</sup> min<sup>-1</sup> [μU/mL]<sup>-1</sup>) multiplied by AIRg (pmol·L<sup>-1</sup> × 10 min).

\* $P < 0.05$  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 levels 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 ( $60 \text{ IU} \cdot \text{kg}^{-1}$  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>2</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

TABLE 9. Plasma lipids, lipoproteins, and postheparin lipases in response to the HERITAGE exercise program by sex and ethnicity.

Variables	Total Participants (n = 675)		Women (n = 376)		Men (n = 299)		Blacks (n = 250)		Whites (n = 481)	
	Before	After	Before	After	Before	After	Before	After	Before	After
Total C <sup>a</sup>	171.0 ± 36.1	172.5 ± 35.8*	168.0 ± 33.6	169.0 ± 33.4	174.7 ± 39.9	173.9 ± 39.4**	165.9 ± 33.6	167.5 ± 35.7	174.3 ± 36.9	175.6 ± 36.1
TG <sup>a</sup>	110.1 ± 63.2	110.2 ± 67.2*	95.9 ± 48.7	95.3 ± 52.3	127.4 ± 74.1	121.5 ± 70.9***	92.5 ± 55.0	90.3 ± 58.5	122.1 ± 69.1	120.4 ± 69.9
LDL-C <sup>a</sup>	114.4 ± 31.5	114.6 ± 30.8	115.5 ± 29.4	111.1 ± 28.7	118.0 ± 33.7	117.1 ± 34.0	111.2 ± 29.4	111.8 ± 29.6	116.2 ± 31.8	116.2 ± 31.3
VLDL-C <sup>a</sup>	15.5 ± 12.6	15.4 ± 12.9	12.2 ± 8.2	12.1 ± 9.3	19.5 ± 15.6	18.4 ± 14.4	12.0 ± 11.1	11.6 ± 10.9	17.6 ± 13.2	17.2 ± 13.2
VLDL-TG <sup>a</sup>	69.5 ± 56.6	65.8 ± 56.0*	53.5 ± 39.0	52.2 ± 43.0	88.5 ± 67.5	82.0 ± 64.8**	47.8 ± 40.3	43.4 ± 37.7*	78.7 ± 59.0	76.2 ± 59.3
HDL-C <sup>a</sup>	41.1 ± 10.6	42.2 ± 11.0*	44.3 ± 10.2	45.7 ± 10.7*	37.1 ± 9.7	38.2 ± 10.2*	42.3 ± 12.3	43.4 ± 12.9*	40.3 ± 9.9	42.0 ± 10.5*
HDL <sub>2</sub> -C <sup>a</sup>	13.9 ± 7.6	14.7 ± 8.0*	16.4 ± 7.6	17.9 ± 8.0*	10.8 ± 6.3	11.5 ± 6.7***	14.3 ± 9.0	15.9 ± 9.7*	13.5 ± 6.9	14.4 ± 7.5****
HDL <sub>3</sub> -C <sup>a</sup>	27.2 ± 5.2	27.5 ± 5.0*	28.1 ± 5.5	27.8 ± 4.9	26.3 ± 5.0	26.7 ± 5.6*	28.0 ± 5.7	27.6 ± 5.3	26.8 ± 5.1	27.6 ± 5.0****
TC/HDL-C <sup>a</sup>	4.4 ± 1.3	4.3 ± 1.4*	4.0 ± 1.1	3.9 ± 1.1*	5.0 ± 1.6	4.8 ± 1.5***	4.2 ± 1.4	4.1 ± 1.4*	4.6 ± 1.5	4.4 ± 1.4*
ApoA-1 <sup>a</sup>	117.5 ± 16.7	119.8 ± 16.1*	119.7 ± 17.1	121.8 ± 16.8*	114.3 ± 15.8	117.0 ± 15.2*	115.5 ± 16.9	116.8 ± 16.0	118.2 ± 17.0	121.9 ± 16.4****
ApoB <sup>a</sup>	84.4 ± 23.7	85.1 ± 23.6	80.4 ± 21.8	81.2 ± 21.8	88.5 ± 25.2	88.1 ± 25.8**	80.9 ± 22.5	81.8 ± 22.7	86.3 ± 24.1	86.8 ± 23.8
LDL-ApoB <sup>a</sup>	76.7 ± 21.4	77.5 ± 21.4*	73.8 ± 20.1	74.3 ± 19.7	79.8 ± 22.8	79.7 ± 23.5	74.6 ± 20.7	75.4 ± 21.1	78.0 ± 21.6	78.5 ± 21.5
LPL <sup>b</sup>	61.3 ± 32.2	68.2 ± 29.0*	67.4 ± 33.7	72.2 ± 31.1*	54.2 ± 28.8	63.5 ± 25.4***	71.8 ± 34.2	76.5 ± 29.1*	56.6 ± 30.1	64.3 ± 28.1*
HL <sup>b</sup>	190.0 ± 74.0	179.4 ± 71.4*	158.2 ± 63.9	150.6 ± 62.5*	228.2 ± 68.1	213.4 ± 66.2***	152.8 ± 65.1	143.4 ± 63.9*	208.9 ± 70.7	196.5 ± 68.4*
HL-L <sup>-1</sup> PL <sup>b</sup>	5.3 ± 8.8	3.6 ± 3.4*	3.5 ± 3.9	2.8 ± 2.5*	7.5 ± 12.0	4.5 ± 4.1***	3.1 ± 3.3	2.3 ± 1.7*	6.4 ± 10.2	4.2 ± 3.9****

To convert mg·dL<sup>-1</sup> to mmol·L<sup>-1</sup>, divide cholesterol values by 38.7 and TG by 88.54.  
<sup>a</sup>mg·dL<sup>-1</sup>.

<sup>b</sup>nmol·mL<sup>-1</sup>·min<sup>-1</sup>.

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

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

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

Adapted from Leon et al. (84), Leon et al. (87), and unpublished data.

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 LPL activity in men (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/AII), was examined (82). Women had higher LpAI and lower LpAI/AII levels than men. As expected, LpAI was correlated with HDL<sub>2</sub>-C, whereas LpAI/AII was more closely associated with HDL<sub>3</sub>-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/AII 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 HDL<sub>2</sub>-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<sup>-1</sup> 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 HDL<sub>2</sub>-C subclass and not the smaller HDL<sub>3</sub>-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  $\dot{V}O_{2\max}$  (95). Weak but significant correlations were also found between change in submaximal HR at 50 W with change in HDL<sub>2</sub>-C ( $r = -0.17$ ; significant in men, women, 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 ( $\beta = 0.14$ ) and inversely associated with changes in medium and total HDL-P ( $\beta = -0.15$  and  $-0.19$ , respectively) and apoA-I ( $\beta = -0.14$ ).

**Ethnic and sex differences in plasma lipid, lipoprotein, and lipase activity responses to training.** No

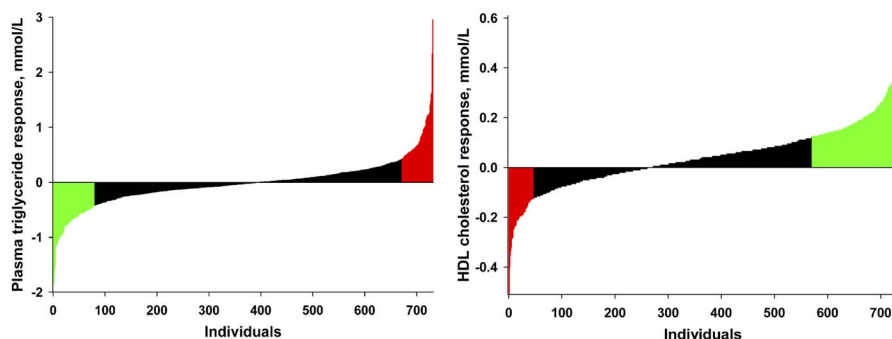
differences in the HDL-C response were noted by age, sex, or ethnicity (84,87). However, Blacks experienced greater increases in HDL<sub>2</sub>-C. Relative to apoA-I, there were greater increases in women than in men, in Blacks than in Whites, and in offspring than in parents (84). At the univariate level, absolute change in FM was correlated with change in HDL-C ( $r = -0.23$ ), HDL<sub>2</sub>-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 HDL<sub>2</sub>-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  $0.11 \text{ mmol}\cdot\text{L}^{-1}$ ) to a mean increase of 18% in quartile 4 (mean increase of  $0.18 \text{ mmol}\cdot\text{L}^{-1}$ ) of the HDL-C response distribution (87). Parallel changes in HDL<sub>2</sub>-C and HDL<sub>3</sub>-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

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



**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  $2 \times$  the technical error for each trait. To convert  $\text{mmol}\cdot\text{L}^{-1}$  of HDL-C to  $\text{mg}\cdot\text{dL}^{-1}$ , divide by 0.02586. To convert  $\text{mmol}\cdot\text{L}^{-1}$  of TG to  $\text{mg}\cdot\text{dL}^{-1}$ , 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  $\leq 0.12$  mmol·L<sup>-1</sup> or an increase of TG  $\geq 0.42$  mmol·L<sup>-1</sup>. 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 HDL<sub>2</sub>-C in Whites and a lack of heritability for change in LDL-C in Blacks. Heritability estimates ranged from 26% (Blacks) to 29% (Whites) for change in HDL-C 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, PREG, 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  $\geq 0.70$ , except for ALDO in women (0.40), CORT in men (0.55), and PREG 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

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 ± 111	394 ± 122*	349 ± 99	364 ± 113	383 ± 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	29.9 ± 20.8	29.5 ± 12.6	30.8 ± 13.5	28.2 ± 14.1	29.6 ± 22.8
E2	71.3 ± 44.1	72.3 ± 43.5	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
PREG	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	37.6 ± 15.7	38.5 ± 15.4	39.8 ± 16.7	39.4 ± 16.5
TESTO	15.0 ± 6.0	15.1 ± 5.6	16.2 ± 6	16.2 ± 5.9	14.7 ± 6	14.7 ± 5.5

Units are nmol·L<sup>-1</sup>, except for E2 (pmol·L<sup>-1</sup>).

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

\*\* $P < 0.05$  for difference in mean training response value 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.



TABLE 11. Steroid hormone levels at baseline and in response to the HERITAGE exercise program in women by menopause and hormonal drug status.

Variables	Premenopausal, No OC (n = 167)		Premenopausal, OC (n = 88)		Postmenopausal, No HRT (n = 24)		Postmenopausal, HRT (n = 22)	
	Before	After	Before	After	Before	After	Before	After
ADTG	101 ± 55	98 ± 55	82 ± 41	84 ± 43	66 ± 37	68 ± 41	61 ± 45	58 ± 42
ALDO	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
CORT	358 ± 134	414 ± 174	662 ± 275	683 ± 305	368 ± 129	401 ± 137	369 ± 129	427 ± 165
DELTA4	2.6 ± 1.5	2.6 ± 1.5	2.5 ± 1.6	2.8 ± 1.3	1.5 ± 0.8	1.5 ± 0.8	1.3 ± 0.6	1.3 ± 0.7
DHEA	14.8 ± 9.6	14.5 ± 10.2	16.3 ± 11.0	17.0 ± 9.7	7.9 ± 4.8	8.5 ± 7.0	6.8 ± 4.0	7.2 ± 5.5
DHEAE	8.4 ± 5.4	7.5 ± 4.8*	7.9 ± 5.5	8.2 ± 5.4	5.6 ± 3.7	5.6 ± 3.5	3.8 ± 2.0	4.5 ± 2.5
DHEAS	4023 ± 2212	3823 ± 2185*	4132 ± 2271	4087 ± 2186	1882 ± 1019	1930 ± 1061	1677 ± 830	1709 ± 1002
DHT	0.5 ± 0.2	0.6 ± 0.4	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.2
DIOLG	15.6 ± 8.1	16.1 ± 9.1	12.5 ± 5.8	13.4 ± 7.2*	12.1 ± 6.5	11.8 ± 5.5	9.4 ± 5.8	9.2 ± 5.8
E2	130 ± 106	137.1 ± 126	125 ± 221	105 ± 127	45 ± 55	36 ± 51	333 ± 389	325 ± 415
OHPRG	2.3 ± 1.7	2.7 ± 3.4	2.1 ± 1.4	2.1 ± 1.3	1.7 ± 0.9	1.6 ± 0.9	1.2 ± 0.8	1.2 ± 0.8
PREG	15.2 ± 9.0	14.5 ± 8.7	13.4 ± 9.4	14.3 ± 10.7	11.2 ± 6.3	10.9 ± 7.3	6.7 ± 3.0	7.3 ± 4.1
PROG	1.6 ± 0.8	1.5 ± 0.8	1.4 ± 0.7	1.4 ± 0.7	0.9 ± 0.6	0.8 ± 0.5	0.7 ± 0.5	0.7 ± 0.4
SHBG	66.1 ± 27.0	68.4 ± 34.3	113.3 ± 58.2	109 ± 55.2	65.9 ± 24.4	62.7 ± 25.6	118.6 ± 61.8	113.2 ± 59.0
TESTO	1.3 ± 0.5	1.3 ± 0.5	1.4 ± 0.6	1.4 ± 0.6	1.4 ± 1.2	1.1 ± 0.4	1.1 ± 0.5	1.0 ± 0.5

Units for all variables are nmol·L<sup>-1</sup>, except E2 (pmol·L<sup>-1</sup>).

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

OC, oral contraceptives.

Adapted from He et al. (28) 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 postheparin 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  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$ , except for the free androgen index (TESTO/SHBG × 100) which was lower across increasing baseline

$\dot{V}O_{2\max}$  quartiles. However, the overall strength of the associations between baseline hormones and  $\dot{V}O_{2\max}$  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 DIOLG 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 aldo-ketoreductase 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 PREG showed a decrease (Table 10). In premenopausal women not taking oral contraceptives, DHEAS decreased significantly with training, whereas DIOLG increased 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 cardio-respiratory fitness and body composition in response to training was examined in HERITAGE (28,102,103). Sex hormones were not related to the change in  $\dot{V}O_{2max}$ , 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 in 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 ( $11.0 \pm 7.2 \mu\text{mol}\cdot\text{L}^{-1}$ ), followed by White men ( $9.4 \pm 3.7 \mu\text{mol}\cdot\text{L}^{-1}$ ), Black women ( $8.5 \pm 4.2 \mu\text{mol}\cdot\text{L}^{-1}$ ), and White women ( $7.7 \pm 2.7 \mu\text{mol}\cdot\text{L}^{-1}$ ) (128). Although results from cross-sectional studies corroborate

TABLE 12. Baseline, training response, and familial aggregation of measures of oxidative stress in Whites of the HERITAGE Family Study.

Oxidative Stress Trait	Baseline				Training Response			
	n	Mean (SD)	Familial Aggregation		Mean (SD)	Training Effect (P Value)	Familial Aggregation	
			F	H <sup>2</sup>			F	H <sup>2</sup>
Plasma glutathione ( $\mu\text{mol}\cdot\text{L}^{-1}$ )	142	4.5 (1.5)	2.56	0.44	0.04 (1.8)	NS	3.20	0.46
Erythrocyte antioxidant enzyme GSH-Px ( $\text{U}\cdot\text{g}^{-1}$ hemoglobin erythrocyte)	109	57.2 (15.4)	6.09	1.00	-0.5	NS	NS	0.002
Resistance to hemolysis (C50-AAPH; $\text{mmol}\cdot\text{L}^{-1}$ )	106	75.9 (14.0)	3.06	0.36	3.6 (16.1)	0.02	2.08	0.35
LDL oxidation lag phase (min)	71	58.4 (23.3)	3.12	0.38	6.2 (38.9)	NS	2.97	0.84
LDL oxidation propagation rate ( $\text{nmol}\cdot\text{mg}^{-1}$ LDL $\cdot\text{min}^{-1}$ )	71	8.9 (2.6)	2.02	0.31	0.3 (4.0)	NS	2.12	0.44
Plasma TBARS ( $\text{nmol MDA}\cdot\text{mL}^{-1}$ )	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. H<sup>2</sup> 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  $\alpha$ -tocopherol, serum uric acid, and plasma phospholipid oleic acid (127). Baseline plasma  $\alpha$ -tocopherol,  $\beta$ -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 processes (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 ( $-3.5 \pm 7.0 \mu\text{mol}\cdot\text{L}^{-1}$ ) in subjects with elevated baseline tHcy ( $\geq 15 \mu\text{mol}\cdot\text{L}^{-1}$ ), whereas a significant increase ( $+0.3 \pm 1.5 \mu\text{mol}\cdot\text{L}^{-1}$ ) was observed in subjects with normal baseline levels of tHcy ( $< 15 \mu\text{mol}\cdot\text{L}^{-1}$ ) (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  $\alpha$ -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  $25 \text{ kg}\cdot\text{m}^{-2}$ . 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 Simoneau, who passed away shortly after the completion of data collection.

**Baseline observations.** Means and SDs for the proportion of type 1, 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 Simoneau 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), glyceraldehyde 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	
			P	F Ratio	P
% type 1	43.2 (11.8)	3.5 (9.4)	0.003	0.62	NS
% type 2 A	36.8 (8.9)	1.8 (9.9)	NS	1.81	0.057
% type 2B	20.0 (11.2)	-5.4 (10.3)	0.0001	1.13	NS
Type 1 caps	4.58 (0.81)	0.76 (0.72)	0.0001	1.65	0.09
Type 2A caps	4.40 (1.00)	0.63 (0.77)	0.0001	1.75	0.07
Type 2B caps	3.47 (1.00)	0.55 (0.68)	0.0001	0.99	NS
CK	389 (68)	27 (69)	0.0009	3.97	0.0001
PHOS	19.9 (4.9)	1.6 (3.3)	0.0001	2.14	0.017
HK	2.5 (0.5)	0.4 (0.5)	0.0001	4.01	0.0001
PFK	57.3 (13.9)	4.9 (10.6)	0.0002	1.85	0.043
GAPDH	422 (94)	7 (66)	NS	2.39	0.007
LPL	0.87 (0.27)	-0.01 (0.28)	NS	1.35	NS
CPT	0.12 (0.02)	0.05 (0.02)	0.0001	2.47	0.006
HADH	16.2 (3.0)	4.6 (3.0)	0.0001	2.13	0.017
CS	11.5 (2.1)	5.7 (2.5)	0.0001	2.73	0.002
COX	7.0 (1.7)	2.9 (2.5)	0.0001	2.07	0.021

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 *V*<sub>max</sub> of enzymes was expressed in micromoles of substrate per gram of wet weight tissue per minute. Total muscle LPL was expressed as nanomoles 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, glyceraldehyde phosphate dehydrogenase; LPL, lipoprotein lipase; CPT, carnitine palmitoyl 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).

(CPT), 3-beta-hydroxyacyl CoA dehydrogenase (HADH), citrate synthase (CS), and cytochrome *c* oxidase (COX) (143). Variability of the enzyme activities in the HERITAGE sample was slightly lower than in the larger cohort of younger sedentary adults (142). The more glycolytic type 2B fibers were characterized by a lower capillarity density than in muscle type 1 and 2A fibers.

**Effects of the training program.** As depicted in Table 13, there were increases in the proportion of type 1 fibers (about 3%) and decreases in type 2B fibers with no change in type 2A (138). The significance of these changes is unclear, as they are well within the confidence intervals of the fiber type assessment procedures (139). There were no changes in the surface areas of the various fiber types. The number of capillaries surrounding each fiber type increased with training, but the ratio of capillaries to fiber type areas decreased for all fiber types. Muscle enzyme activities were substantially and significantly increased after training, except for GAPDH and LPL.

**Familial aggregation profile.** The muscle biopsy data came from 19 nuclear families. Considering that there is a dearth of data on the genetic epidemiology of skeletal muscle phenotypes, HERITAGE offered an opportunity to test whether there was familial aggregation in the sedentary state and in response to training (138). There was no consistent evidence for familial

aggregation of fiber type distribution or fiber type areas in the sedentary state, with the exception of type 1 fibers. However, significant familial resemblance was observed for the number of capillaries and the number of capillaries per surface area around fiber types 1 and 2A. No evidence of family lines was detected in the observed variability of fiber type distributions, surface areas, and capillarity in response to training, although there were trends of familial aggregation for number of capillaries surrounding types 1 and 2A fibers. In contrast, strong familial aggregations for muscle enzyme activities (CK, PHOS, HK, PFK, CPT, HADH, CS, and COX) were observed at baseline (except GAPDH and LPL) and in response to the exercise program, with the exception of total muscle LPL (143). Studies with much larger sample sizes would be needed to provide more definitive quantification of the variability within and between families, along with relevant heritability estimates.

## TRAINING AND RESTING METABOLIC RATE

Research is equivocal on the effects of endurance training on RMR (144–152). Seventy-seven participants from the Texas clinical center (40 men) age 17 to 63 yr completed two RMR measurements by indirect calorimetry before training and one measurement each 24 and 72 h after the last exercise bout.

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

Resting Metabolic Rate	Pretraining			Posttraining		
	Trial 1	Trial 2	Mean	Trial 3: 24 h	Trial 4: 72 h	Mean
VO <sub>2</sub> (mL·min <sup>-1</sup> )						
Men	253 ± 34	249 ± 32	252 ± 33	255 ± 34	253 ± 34	255 ± 33
Women	194 ± 29	196 ± 29	196 ± 31	198 ± 33	200 ± 32	199 ± 31
Total	226 ± 43	224 ± 42	225 ± 42	227 ± 44	228 ± 42	228 ± 42
MJ·d <sup>-1</sup>						
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	6.56 ± 1.24	6.51 ± 1.21	6.54 ± 1.22	6.56 ± 1.26	6.61 ± 1.23	6.61 ± 1.23
kJ·kg <sup>-1</sup> FFM·d <sup>-1</sup>						
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	121.3 ± 13.0	121.3 ± 14.6	121.8 ± 13.8	121.3 ± 13.4	121.8 ± 15.1	121.8 ± 13.4

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  $\geq 0.90$ ) between the two pretests, including resting  $\dot{V}O_2$ , resting energy expenditure,  $HR_{sleep}$ , and  $HR_{RMR}$ .

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  $\dot{V}O_{2max}$  did not affect the results (Table 14). There was a large pretraining to posttraining increase in  $\dot{V}O_{2max}$  (17.9%) and small but significant differences in  $HR_{sleep}$ , (61.0–59.3 bpm),  $HR_{RMR}$  (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 by

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  $\dot{V}O_2$  and PO), hemodynamic-related traits (resting and submaximal exercise HR, SBP and DBP, and submaximal SV and  $\dot{Q}$ ), 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

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
<i>ACE</i>	Angiotensin I converting enzyme	B and R	R					(172,173)
<i>ADIPOQ</i>	Adiponectin, C1Q and collagen domain containing			B	B	B		(174)
<i>ADRA2A</i>	Adrenoreceptor alpha 2A			B				(175)
<i>ADRB2</i>	Adrenoreceptor beta 2			B and R				(176,177)
<i>ADRB3</i>	Adrenoreceptor beta 3			R				(177)
<i>AGRP</i>	Agouti related neuropeptide			B			Dietary intake	(178–180)
<i>AGT</i>	Angiotensinogen		B and R	B				(172,181)
<i>AMPD1</i>	Adenosine monophosphate deaminase 1	B and R	B and R					(182)
<i>ANG</i>	Angiogenin		B					(183)
<i>APOE</i>	Apolipoprotein E				B and R			(184,185)
<i>ATP1A2</i>	ATPase Na <sup>+</sup> /K <sup>+</sup> transporting subunit alpha 2	R						(186)
<i>BCHE</i>	Butyrylcholinesterase						Anxiety	(187)
<i>CETP</i>	Cholesteryl ester transfer protein				B and R			(185)
<i>CKM</i>	Creatine kinase, M type	B and R						(188)
<i>DRD2</i>	Dopamine receptor D2						PA level	(189)
<i>EDN1</i>	Endothelin 1		R					(190)
<i>FHL1</i>	Four and a half LIM domains 1					B and R		(191)
<i>FTO</i>	FTO alpha-ketoglutarate dependent dioxygenase			B and R				(192)
<i>GHRL</i>	Ghrelin and obestatin prepropeptide	B		B				(193)
<i>GNB3</i>	G protein subunit beta 3		R	R				(194)
<i>LEP</i>	Leptin					R		(195)
<i>LEPR</i>	Leptin receptor			B		R		(195,196)
<i>LIPC</i>	Lipase C, hepatic type				B	R		(197)
<i>LIPE</i>	Hormone-sensitive lipase			B				(198)
<i>LPL</i>	Lipoprotein lipase				B			(199)
<i>MC4R</i>	Melanocortin 4 receptor							(200)
<i>NOS3</i>	Nitric oxide synthase 3		R					(201)
<i>PON1</i>	Paraoxonase 1						Anxiety	(187)
<i>PPARG</i>	Peroxisome proliferator-activated receptor gamma	R			B and R	R		(202,203)
<i>PTPN1</i>	Protein tyrosine phosphatase nonreceptor type 1			B		B		(204)
<i>SLC4A5</i>	Solute carrier family 4 member 5	B	B					(205)
<i>TGFB1</i>	Transforming growth factor beta 1		B					(206)
<i>TNF</i>	Tumor necrosis factor						CRP B and R	(207)
<i>UCP3</i>	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  $\dot{V}O_{2max}$  to training adjusted for age, sex, BMI, and pretraining  $\dot{V}O_{2max}$ , but not baseline levels of  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  than C-allele carriers (202). Several candidate genes, *ADIPOQ*, *ADRA2A*, *ADRB2*, *AGRP*, *AGT*, *FTO*, *GHR*, *GNB3*, *LEPR*, *LIPE*, *PTPNI*, 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 female 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<sub>3</sub>-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*, *PPARG*, and *PTPNI*. 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 *PPARG* 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*, *TGFB1*). 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 angiogenin (*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  $\dot{V}O_{2\max}$  and its response to training led to the identification of four QTLs for baseline  $\dot{V}O_{2\max}$  and five QTLs for  $\dot{V}O_{2\max}$  response (210). Several other QTLs for traits related to cardiorespiratory 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.q34) led, respectively, to the identification of the titin (231), kinesin family member 5B (232), and cAMP responsive element binding protein 1 (233) genes as strong candidate genes for hemodynamic responses to training. A QTL for submaximal exercise capacity ( $\dot{V}O_2$  at 60%  $\dot{V}O_{2\max}$ ) 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  $\dot{V}O_{2\max}$  response to endurance training and identified a panel of 21 SNPs explaining about half of the variance in the trainability of  $\dot{V}O_{2\max}$  (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 ( $P = 3.79 \times 10^{-8}$ ) 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  $\sim 2.8$  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 ( $G \times E$ ) 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-CFDPI*,

*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.

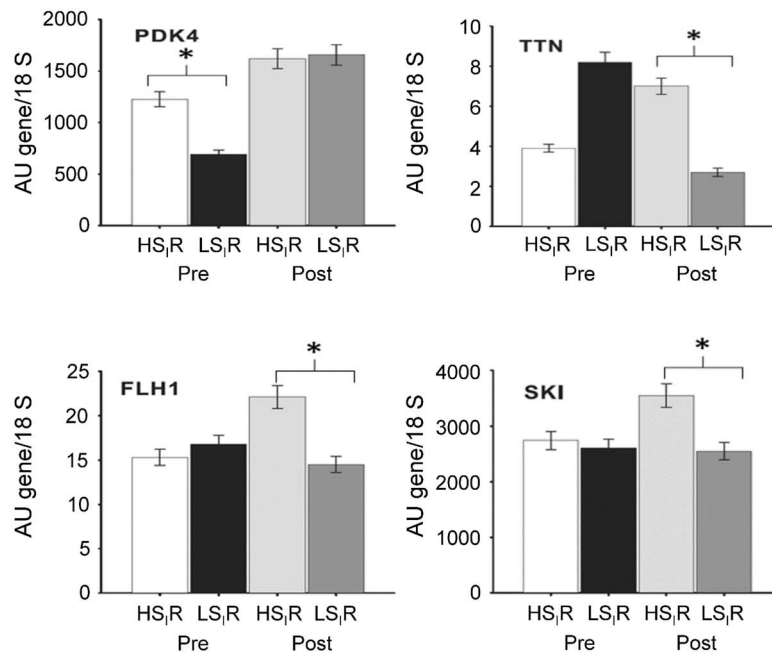
A first study examined muscle gene expression in 16 subjects discordant for the response of insulin sensitivity (Si) to the training program: 8 showing no changes (low responders) and 8 showing marked improvements (high responders; response ~4 times higher than that of low responders) (263). Differences 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 adenine 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  $\dot{V}O_{2max}$  due to endurance exercise training (265). A regression

### Muscle gene expression and insulin sensitivity response to the HERITAGE exercise program



**FIGURE 6**—Real time quantitative reverse polymerase chain reaction validation of gene expression levels for insulin sensitivity (Si) response to training identified in microarray experiments. HS<sub>1</sub>R, high Si responder; LS<sub>1</sub>R, low Si responder; PDK4, pyruvate dehydrogenase kinase 4; TTN, titin; SKI, V-Ski avian sarcoma viral oncogene homolog; FLH1, four-and-a-half LIM domain 1. Levels normalized over the corresponding 18S rRNA value. \**P* < 0.05 for difference in mean value between response groups. Adapted with permission from Teran-Garcia et al. (263).



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

### Muscle gene expression and plasma triglyceride changes in response to the HERITAGE exercise program

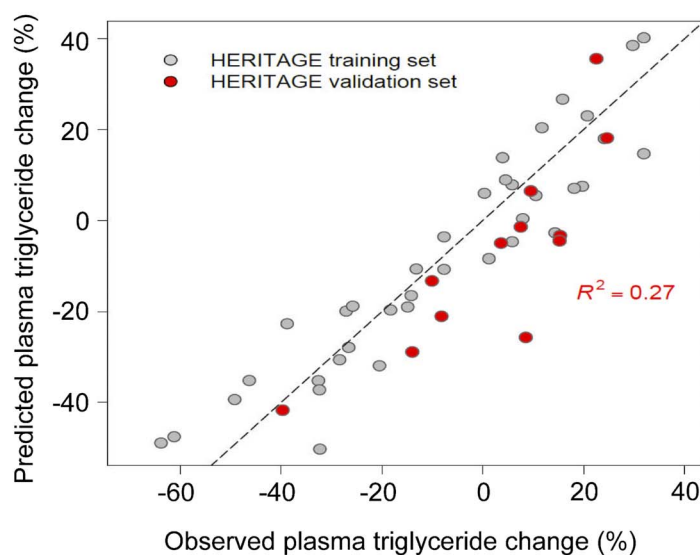
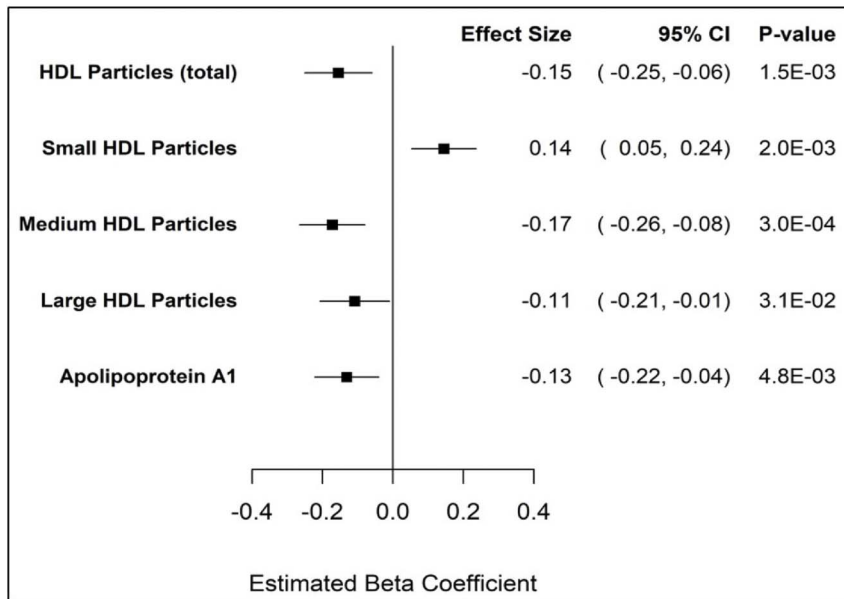


FIGURE 7—Performance of the vastus lateralis gene expression profile regression model derived from a training set ( $n = 37$ , gray dots) and a validation set ( $n = 12$ , red dots) for the prediction of exercise training-induced changes in TG in HERITAGE. Reproduced with permission from Sarzynski et al. (236).

## Baseline plasma DMGV and HDL-cholesterol changes with exercise training



**FIGURE 8**—Associations between baseline DMGV levels and HDL traits responsiveness to exercise training in HERITAGE Whites. Reproduced with permission from Robbins et al. (96).

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

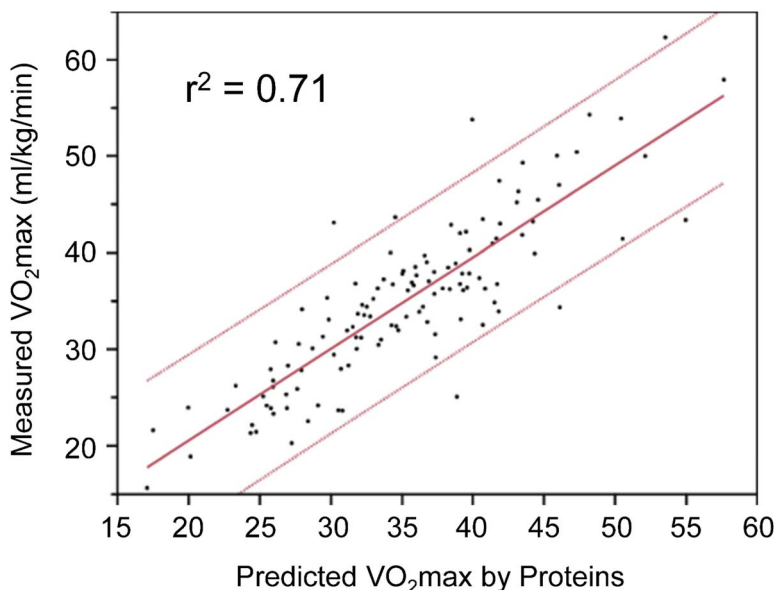
The most recent molecular profiling platform applied to HERITAGE has been proteomics. Specifically, to measure large-scale

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<sup>®</sup> (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  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$ , 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  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  was further refined in analyses that characterized proteins associated with baseline  $\dot{V}O_{2\max}$  and changes in  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  (Fig. 10, left panel), including 85 proteins that were positively associated and 62 proteins negatively associated. Proteins positively associated with baseline  $\dot{V}O_{2\max}$

## Prediction of $\dot{V}O_{2\max}$ from the plasma proteome in HERITAGE

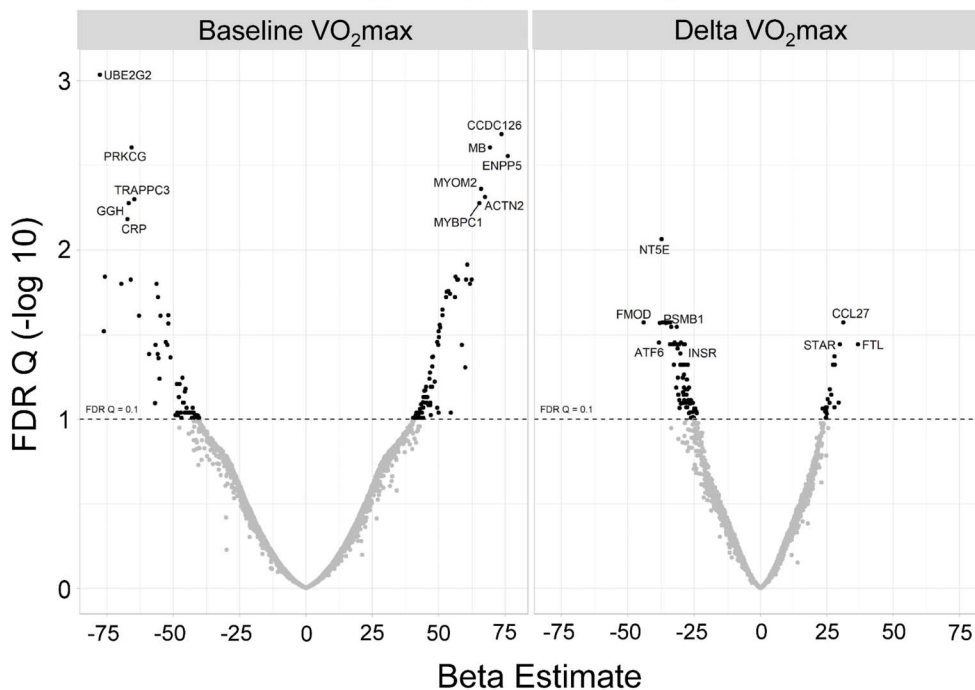


**FIGURE 9**—Prediction of measured  $\dot{V}O_{2\max}$  by 124 plasma protein levels in the validation data set of HERITAGE. Reproduced with permission from Williams et al. (271).

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  $\dot{V}O_{2\max}$ , including leptin, CRP, and insulin.

## Plasma proteome and baseline and training response $\dot{V}O_{2\max}$



**FIGURE 10**—Plasma proteins positively and negatively associated with baseline  $\dot{V}O_{2\max}$  (left panel) and change in  $\dot{V}O_{2\max}$  (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  $\dot{V}O_{2\max}$  with training in a linear regression model adjusted for age, sex, BMI, ethnicity, and the baseline level of  $\dot{V}O_{2\max}$  (Fig. 10, right panel). Minimal overlap was found between the proteins associated with baseline  $\dot{V}O_{2\max}$  versus change in  $\dot{V}O_{2\max}$ , as only five proteins (T132B, 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  $\dot{V}O_{2\max}$  traits (see the Cardiorespiratory Fitness section). Subsequently, analyses were aimed at determining the ability of plasma proteins to identify  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  responder. Lastly, the association of  $\dot{V}O_{2\max}$ -related proteins with mortality was examined in the Framingham Offspring Study. It was found that 12 proteins associated with baseline  $\dot{V}O_{2\max}$  and 9 proteins associated with change in  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  and trainability of  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  (hereafter called delta  $\dot{V}O_{2\max}$ ) was published (273). Using GWAS SNP associations to delta  $\dot{V}O_{2\max}$ , 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 found to be significantly associated with  $\dot{V}O_{2\max}$  trainability. Examining pathway overrepresentation among genes above a threshold of genetic association to delta  $\dot{V}O_{2\max}$  further identified a network of 35 genes, of which 31 were associated with delta  $\dot{V}O_{2\max}$  at  $P \leq 0.005$ . These network genes could be partitioned into pathways related to calcium signaling, nitric oxide signaling, and protein kinase A signaling.

A hypothesis-free gene prioritization tool was used to rank genes moderately associated with delta  $\dot{V}O_{2\max}$  in GWAS analysis. One group of genes (*PINX1*, *CD44*, *PARK2*, *RYR2*, *ADCY5*, and *SHANK2*) demonstrated consistently strong scores across all analyses, whereas a second group (*KCNQ5*, *GRIK4*, *RPTOR*, *ACVR1C*, and *ACSL1*) were strongly associated only in a subset. Several of the genes identified (*CAMTA1*, *RYR2*, *ACSL1*, *CD44*, *BIRC7/YTHDF1*) have been further replicated

(274). This bioinformatics exploration of the biology underlying  $\dot{V}O_{2\max}$  adaptation suggests that pathways related to calcium signaling, energy sensing and partitioning, mitochondrial biogenesis, angiogenesis, immune functions, and regulation of autophagy and apoptosis are some key mechanisms through which the physiological responses of  $\dot{V}O_{2\max}$  to training are mediated.

The bioinformatics explorations of the genetics and biology underlying  $\dot{V}O_{2\max}$  in the sedentary state (275) differed extensively in complexity and depth compared with the 2013 analysis of  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  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 $\dot{V}O_{2\max}$ bioinformatics: Discovery phase.

A pipeline consisting of seven steps was used to generate gene targets and hypotheses regarding the biology underlying  $\dot{V}O_{2\max}$ .

- Quantifying gene-level association to intrinsic  $\dot{V}O_{2\max}$ , 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*, *IGFN1*, and *CASQ2*.
- 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  $\dot{V}O_{2\max}$ .
- 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  $P \leq 1 \times 10^{-5}$ ). The top 3 most significant eQTL associations were observed for SNP rs2838815 regulating *ADARB1* gene expression in brain ( $P \leq 1 \times 10^{-18}$ ), a cluster of highly linked SNPs (clustered at *ATE1* 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  $\dot{V}O_{2\max}$ -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 *TMM8B*.
- e. Examining skeletal muscle gene expression profiles. Whole-genome expression profiling data were analyzed to identify genes that transcriptionally correlated with intrinsic  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$ -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  $\dot{V}O_{2\max}$ -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*, *IGFNI*, *NOTO*, *CASQ2*, *ARL6IP5*, *RAB11FIP5*, and *TMF1*.

#### Intrinsic $\dot{V}O_{2\max}$ 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 *Pradc1* knockout led to increased circulating glucose.
- i. Examining SNPs in candidate genes for associations to cardiovascular and muscle intermediate endophenotypes of intrinsic  $\dot{V}O_{2\max}$ . 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 HER-

ITAGE. 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% $\dot{V}O_{2\max}$ , SV at 60% $\dot{V}O_{2\max}$ , 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  $\dot{Q}$  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  $\dot{V}O_{2\max}$ . 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  $\dot{V}O_{2\max}$ , 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  $\dot{V}O_{2\max}$ . 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 sarcolemmal transmembrane 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  $\dot{V}O_{2\max}$ .

This effort based on a bioinformatics pipeline applied to  $\dot{V}O_{2max}$  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 substudies 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.

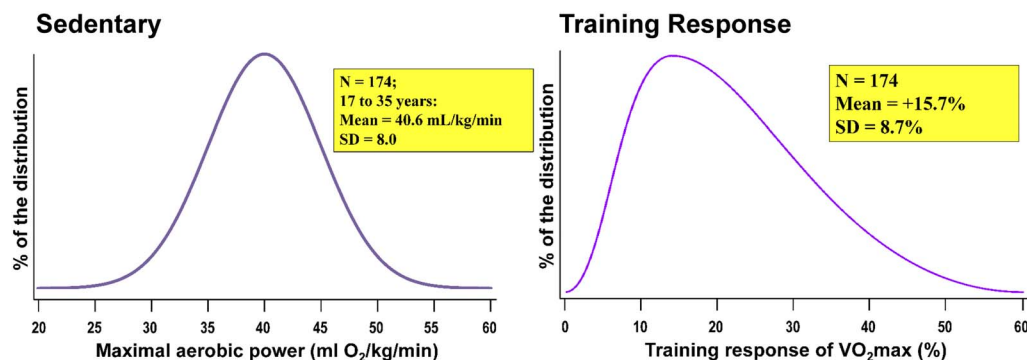
There are also limitations to the study, the most obvious of which being the lack of a control group. As mentioned previously, it was agreed 30 yr ago that a control group was not necessary to address the main aims of HERITAGE. Indeed, the

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  $\dot{V}O_{2max}$ .

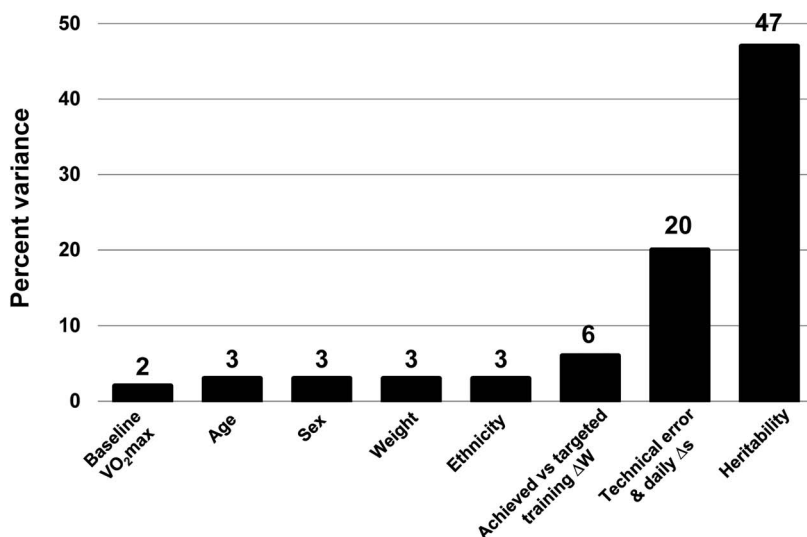
Individual differences in  $\dot{V}O_{2max}$  among sedentary adults were found to be large. An appreciation of their magnitude can be found in Figure 11. The left panel depicts the distribution of  $\dot{V}O_{2max}$  per kilogram of body weight per minute, based on two tests, within 1 wk, in a group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

## $\dot{V}O_{2max}$ in the sedentary state and in response to the HERITAGE endurance exercise program in young adult males



**FIGURE 11**—Individual differences in intrinsic  $\dot{V}O_{2max}$  (left panel) and its response to the standardized training program (right panel) in HERITAGE young adult males age 17 to 35 yr. Intrinsic  $\dot{V}O_{2max}$  is derived from two tests at baseline in the sedentary state. The gain in  $\dot{V}O_{2max}$  was calculated using the mean of two post training values minus the baseline level.

## Correlates of $\dot{V}O_{2\max}$ Training Response in HERITAGE



**FIGURE 12**—A summary of the correlates of the gains in  $\dot{V}O_{2\max}$  in response to the standardized and fully monitored 20-wk exercise program of HERITAGE. A maximum of 482 Whites and 259 Blacks were available for these calculations. Age ranged from 17 to 65 yr. The mean gain in  $\dot{V}O_{2\max}$  was 384 mL  $O_2$  (SD, 202). Reproduced with permission from Sarzynski et al. (33).

age 17 to 35 yr who were all physically inactive (mean of 40.6 mL  $O_2 \cdot kg^{-1} \cdot min^{-1}$ , 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  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  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

$\dot{V}O_{2\max}$ , 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  $\dot{V}O_{2\max}$  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  $\dot{V}O_{2\max}$  were all weakly associated with the gains in  $\dot{V}O_{2\max}$ , 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  $\dot{V}O_{2\max}$  training response. We estimated as discussed previously that the measurement error and the day-to-day variation in  $\dot{V}O_{2\max}$  explained about 20% of the variability in training response. Finally, as described earlier

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

	% Fat	AVF	Insulin	HDL-C	Small LDL-P	GlycA
$\dot{V}O_{2\max}$	-0.09	-0.05	0.03	0.06	-0.07	-0.05
% body fat		0.35*	0.16*	-0.07	0.08	0.03
AVF			0.23*	-0.07	0.10	0.08
Fasting insulin				-0.09	0.05	0.13*
HDL-C					-0.27*	-0.11
Small LDL-P						0.20*

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  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  trainability explain 87% of the variance, as detailed in Figure 12 (33).

One important question is whether the HERITAGE findings on  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  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  $\dot{V}O_{2max}$  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

### Expanded HERITAGE research working model

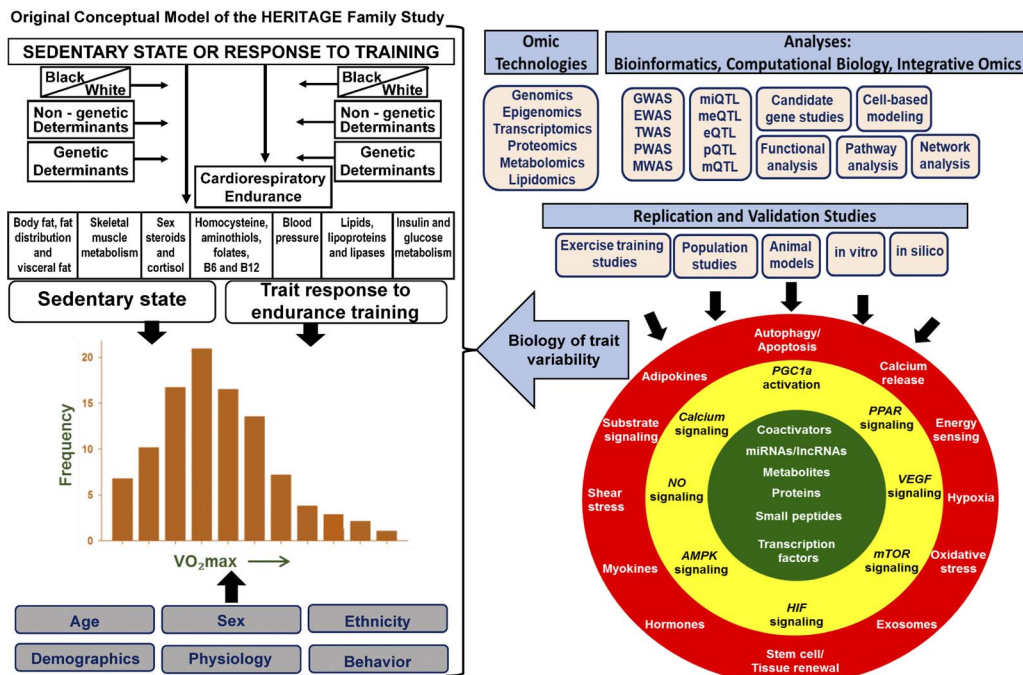


FIGURE 13—Schematic display of the original HERITAGE working model (left side of the figure) and the expanded research strategy deployed to investigate the biology and molecular determinants of exercise-related traits in the sedentary state and their responsiveness to an endurance training program meeting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



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 paths linking physical activity, fitness, and health.

Thanks are expressed to the HERITAGE families who participated in the study. The authors also want to express our gratitude to all colleagues, research collaborators, and technical personnel who were involved in the planning and execution of the study. They are listed in Supplementary Table S3 (<http://links.lww.com/MSS/C482>). The authors thank Melanie Peterson for her support in the preparation and editing of the manuscript.

The HERITAGE Family Study was initially funded by the National Heart, Lung and Blood Institute (NHLBI) through the following grants: HL45670 (C. Bouchard, PI) from 1992 to 2010, HL47317 (D. C. Rao, PI) from 1992 to 2010, HL47321 (J.H. Wilmore, PI, deceased) from 1992 to 2003, HL47323 (A. S. Leon, PI) from 1992 to 2003, and HL 47327 (J.S. Skinner, PI) from 1992 to 2003. Current funding for HERITAGE is provided to Dr. Sarzynski (R01HL146462) and to Drs. Gerszten and Sarzynski (R01NR019628). Dr. Bouchard is supported by the John W. Barton Sr. Chair in Genetics and Nutrition and by National Institutes of Health (NIH) P30GM118430. Dr. Clish is supported by NIH U24DK112340. Dr. Després is the Scientific Director of the International Chair on Cardiometabolic Risk supported by the Fondation de l'Université Laval, and he is supported by the Canadian Institutes of Health Research (FDN-167278). Dr. Gerszten is supported by NIH U54GM115428, R01DK081572, R01NR019628, and U24DK112340. Dr. Ghosh is supported by NIGMS U54GM104940 and P20GM103528 and the National Medical Research Council, Ministry of Health Singapore (WBS R913200076263). Dr. Robbins is supported by NHLBI K23HL150327-02 and John S. LaDue Memorial Fellowship in Cardiology at Harvard Medical School. Dr. Sarzynski is supported by NIH R01HL146462, R01NR019628, R01DK128057, and SC INBRE P20GM103499. Dr. Tchermof is Co-director of the Research Chair in Bariatric and Metabolic Surgery at Laval University. Dr. Tremblay is the holder of the Canada Research Chair in Environment and Energy Balance funded by the Natural Sciences and Engineering Research Council of Canada. Mr. Barber is supported by American Heart Association Predoctoral Fellowship Award 833917 and NIH SC INBRE P20 GM103499.

HERITAGE website: A website containing general information on the HERITAGE Family Study, including a listing of all associated publications, can be found at [www.HeritageFamilyStudy.com](http://www.HeritageFamilyStudy.com).

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## APPENDIX HERITAGE Family Study Review Paper

SUPPLEMENTARY TABLE S1. HERITAGE Consortium Steering Committee.

Claude Bouchard, PhD; Chairperson	Laval University (until 1999); later Pennington Biomedical Research Center
Jacques Gagnon, PhD; Project Director (1992–1999)	Laval University
Jean Paul Albert, MBA; Administrator (1992–1999)	Laval University
Tuomo Rankinen, PhD; Project Director (1999–2012)	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).

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.

<b>Laval University Consortium Coordinating Center and Clinical Center</b>	
PI: Claude Bouchard, PhD	
Local Project Coordinator: Marcelle Lareau, MSc	
Jean-Paul Albert, MBA	Benoit Lamarche, PhD
Manon Baril, BSc	Claude Leblanc, MSc
Jean Bergeron, MD	Gilles Lortie, MD, PhD
Come S. Bouchard, MSc	Isabel Mercier, MSc
Anne-Marie Bricault, MSc	François Michaud, BSc
Yvon Chagnon, PhD	Chantal Paré, BSc
Jean-Pierre Despres, PhD	Louis Pérusse, PhD
Diane Drolet, MSc	Denis Prud'homme, MD, MSc
Jacques Gagnon, PhD	Jean-Aime Simoneau, PhD
My-Anh Ho-Kim, MSc	Germain Thériault, MD
Karen Horth,	Angelo Tremblay, PhD
Louise Laberge,	John Weisnagel, MD, PhD
<b>Washington University Data Coordinating Center</b>	
PI: D. C. Rao, PhD	
Ping An, MD	Zhaohai Li, PhD
Ingrid B. Borecki, PhD	Stephen Mendel, PhD
Harry Cheng, MSc	Laura E. Mitchell, PhD
Gu Chi, PhD	Michael A. Province, PhD
Warwick Daw, PhD	Treva Rice, PhD
Habib El-Moalem, PhD	Kathryn A. Schallert, BSc
John O. Holloszy, MD	Kenneth B. Schechtman, PhD
Yuling Hong, MD	George P. Vogler, PhD
David J. Lerner, BSc	
<b>Laval University Core Laboratories</b>	
<i>Diabetes Research Unit</i>	
André Nadeau, MD, PhD; Director	<i>Lipid Research Center</i>
Yolande Montreuil, RN	Paul Lupien, MD, PhD; Director
Gilles Tancrede, MSc	Jean-Pierre Després, PhD
<i>DNA and Cell Line Unit</i>	Sital Moorjani, PhD
France T. Dionne, PhD; Director	<i>Steroid Laboratory</i>
Monique Chagnon, ART	Alain Bélanger, PhD; Director
Caroline Noel, BSc	Simon Caron, MSc
Marie-Christine Thibault, PhD	Fernand Labrie, MD, PhD
<b>University of Minnesota Clinical Center</b>	
PI: Arthur S. Leon, MD	
Local Project Coordinator: Ava J. Walker, PhD	
Marilyn Borkon,	Marcella Meyers, PhD
Fernando S. Branco, MD	James P. Norton, MSc
Erik J. Ekstrom, RD	Robert C. Serfass, PhD
Steve Gaskill, PhD	M. Katie Schmitz, MSc
William V. Mendoza, MD	

SUPPLEMENTARY TABLE S3. (Continued)

<b>Arizona State University Clinical Center (Until 1995)</b>	
<b>Indiana University Clinical Center (1996+)</b>	
PI: James S. Skinner, PhD	
Local Project Coordinator in Arizona: Kristine M. Wilmore, MA	
Local Project Coordinator in Indianapolis: Joanne Krasnoff, PhD	
Kelly Enders, RN	
Anna A. Jaskolska, PhD	
Artur J. Jaskolski, PhD	
<b>University of Texas at Austin Clinical Center</b>	
PI: Jack H. Wilmore, PhD	
Local Project Coordinator: Philip R. Stanforth, MSc	
Melissa Domenick, MA	
David W. Hayes, MD	
Paul J. Roach, MD	

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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)
Heterogeneity of training responses			
Large interindividual differences in phenotype responses to endurance training	$\dot{V}O_{2max}$ IVGTT traits	$\dot{V}O_{2max}$ responses ranged from no gain to $>1 \text{ L}\cdot\text{min}^{-1}$ (-5% to +48%). The proportion of subjects who had no change or decreases in IVGTT traits ranged from 42% to 55%.	(43,44,74)
No correlation between baseline $\dot{V}O_{2max}$ and its changes with training	$\dot{V}O_{2max}$	The correlation between baseline and delta $\dot{V}O_{2max}$ was 0.08, with baseline levels after adjustments explaining only 2% of the variance in delta $\dot{V}O_{2max}$ .	(33,43)
No aggregation of training responsiveness in individuals or subgroups	$\dot{V}O_{2max}$ $\dot{V}O_{2max}$ : % fat, AVF, insulin, HDL-C, small LDL, GlycA	There were low, average, and high $\dot{V}O_{2max}$ responses across ages, both sexes, both ethnic groups, and levels of initial $\dot{V}O_{2max}$ . Almost half of the cohort had at least one high response and one low response across seven cardiometabolic traits. There is a high degree of trait specificity in training responsiveness.	(43) (283)
Some unfavorable responses to endurance training	Fasting insulin; HDL-C; TG; SBP	Prevalence of potentially "adverse" response in HERITAGE ranged from 6% to 9% for the four traits.	(75)
Familial aggregation of baseline phenotype levels			
Within-subject variability	$\dot{V}O_{2max}$	Within-subject SD from measures repeated days and weeks apart range from 108 to 137 mL $O_2\cdot\text{min}^{-1}$ with CV of 4.1% to 5.0%.	(20,27,33,34), plus unpublished data
Significant familial resemblance for intrinsic $\dot{V}O_{2max}$	$\dot{V}O_{2max}$	2.7 times more variance between families than within families. Heritability of intrinsic $\dot{V}O_{2max}$ was 51%, with significant maternal heritability (29%).	(45)
Significant familial aggregation for intrinsic submaximal $\dot{V}O_2$	$\dot{V}O_2$ 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	SV and Q at 50 W and 60% $\dot{V}O_{2max}$	Maximal heritability estimates of 46% for SV and Q at 60% $\dot{V}O_{2max}$ and 41% and 42% at 50 W	(58)
Significant familial aggregation for visceral fat	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 muscle enzyme activities	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 markers of oxidative stress	LDL-ox, C50-AAPH, TBARS, glutathione	Heritability for oxidative stress traits ranged from 31% to 44%.	(127)
Familial aggregation of training response			
Significant familial aggregation for $\dot{V}O_{2max}$ training response	$\dot{V}O_{2max}$	Heritability was 47%, with maternal inheritance accounting for 28%.	(41)
Significant familial aggregation for submaximal $\dot{V}O_2$ training response	$\dot{V}O_2$ at 50 W, 60%, 80%	Heritability values ranged from 23% to 57% for the training response of submaximal measures of $\dot{V}O_2$ .	(48)
Significant familial aggregation of submaximal exercise BP and HR training responses	HR50, HR60%, SBP50	Heritabilities 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% $\dot{V}O_{2max}$	Heritabilities ranged from 24% to 38% for SV and Q at 50 W and 60% $\dot{V}O_{2max}$ .	(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  a- $\dot{V}O_2$ diff, SV, Q, and $\dot{V}O_2$ 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. Black men did not increase a- $\dot{V}O_2$ 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 $\dot{V}O_{250}$ .	(17), pp. 10–116 (61), MSSE, pp. 99–106
Significant sex interactions for insulin sensitivity response to training	Si	Insulin sensitivity increased by 10% in the total cohort, with the increase larger in men than women (16% vs 5%).	(74)
Significant ethnic and sex differences in lipid, lipoprotein, and lipase activity responses to training	ApoA-I, HDL2-C  LPL activity	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. LPL activity increased in all subgroups except Black men.	(84) (97)

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Novel or Impactful Findings	Trait(s)	Details	Citation(s)
Response of other traits to training Little influence of endurance training on markers of oxidative stress	LDL-ox, C50-AAPH, TBARS, antioxidants, and aminothiols	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 VLDL-P 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 Identification of QTLs for baseline $\dot{V}O_{2max}$ and for $\dot{V}O_{2max}$ response	$\dot{V}O_{2max}$	QTLs on 4q, 8q, 11p, and 14q were reported for baseline $\dot{V}O_{2max}$ . QTLs on 1p, 2p, 4q, 6p, and 11p were identified for change in $\dot{V}O_{2max}$ .	(210)
Dense mapping of 4 QTLs identified strong candidate genes for submaximal exercise capacity and hemodynamic responses to training	SV50, HR50	Titin, kinesin family member 5B, cAMP responsive element binding protein 1, <i>MPEP</i> , and <i>SGCG</i> genes were identified as strong candidates for changes in submaximal SV and HR and submaximal exercise capacity.	(211,231–233)
First GWAS of exercise response traits GWAS of $\dot{V}O_{2max}$ response to endurance training	$\dot{V}O_{2max}$	39 SNPs were associated at $P < 1.5 \times 10^{-4}$ , with a panel of 21 SNPs accounting for 49% of the variance in $\dot{V}O_{2max}$ trainability.	(234)
GWAS of submaximal HR response to training	HR50	40 SNPs associated at $P < 9.9 \times 10^{-5}$ , with top hit $8 \times 10^{-7}$ . 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 Muscle gene expression and SNP signatures predict $\dot{V}O_{2max}$ response to endurance training	$\dot{V}O_{2max}$	Genome-wide baseline muscle gene expression and validation identified a 29-RNA signature that predicted changes in $\dot{V}O_{2max}$ . Candidate genes from this predictor and the literature led to a 11-SNP signature that explained 23% of the variance in $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ and its trainability Plasma protein signature of cardiorespiratory fitness level	$\dot{V}O_{2max}$	Elastic net regression identified 115 proteins highly correlated ( $r^2 = 0.80$ ) with measured $\dot{V}O_{2max}$ , which was replicated in the validation data set, with an $r^2$ of 0.71 (Fig. 9).	(271)
Plasma proteins associated with intrinsic $\dot{V}O_{2max}$ and its trainability	$\dot{V}O_{2max}$	147 proteins were associated with baseline $\dot{V}O_{2max}$ , whereas 102 baseline proteins were associated with changes in $\dot{V}O_{2max}$ , with minimal overlap (only 5 proteins) between protein sets. A baseline 56-protein signature improved prediction of $\dot{V}O_{2max}$ response (AUC 0.84) compared with a model of only clinical variables (AUC 0.62).	(272)
Bioinformatics explorations of intrinsic $\dot{V}O_{2max}$ and its trainability Integrative pathway analysis of $\dot{V}O_{2max}$ response to training GWAS	$\dot{V}O_{2max}$	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 $\dot{V}O_{2max}$ to training are mediated.	(273)
Genetics and biology underlying intrinsic $\dot{V}O_{2max}$	$\dot{V}O_{2max}$	A bioinformatics pipeline applied to $\dot{V}O_{2max}$ data in the sedentary state suggests 4 loci related to cardiovascular physiology ( <i>ATE1</i> , <i>CASQ2</i> , <i>NOTO</i> , and <i>SGCG</i> ), four loci related to hematopoiesis ( <i>PICALM</i> , <i>SSB</i> , <i>CASQ2</i> , and <i>CA9</i> ), 4 loci related to skeletal muscle function ( <i>SGCG</i> , <i>DMRT2</i> , <i>ADARB1</i> , and <i>CASQ2</i> ), and 8 loci related to metabolism ( <i>ATE1</i> , <i>PICALM</i> , <i>RAB11FIP5</i> , <i>GBA2</i> , <i>SGCG</i> , <i>PRADC1</i> , <i>ARL6IP5</i> , and <i>CASQ2</i> ) 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	Fasting glucose, insulin, and lipids	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)