Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Details of clinical phenotyping from the HERITAGE Family Study

Anthropometrics and Body Composition

Body weight, height, and waist and hip circumferences were measured according to standardized procedures, and body density was measured using the hydrostatic weighing technique as described.[1] The mean of the highest three (of ten) measurements was used to calculate percent body fat from body density by using the equation of Siri[2] for Caucasian men and of Lohman[3] for Caucasian women. Fat mass was derived by multiplying body weight by percent body fat. Adipose tissue accumulation and distribution (visceral and subcutaneous) were assessed by abdominal computed tomography at the level of the L4-L5 vertebrae, as previously described.[4,5]

Plasma Lipid, Lipoprotein, and Apolipoprotein Measurements

Fasting blood samples were obtained on two separate days both before and after completion of the exercise training program (about 24 and 72 hours after the last exercise session) and then averaged. Cholesterol and triglyceride (TG) levels were determined in plasma and in lipoprotein fractions using enzymatic methods with a Technicon RA-500 analyzer (Bayer Corp Inc).[6] The plasma very low-density lipoprotein fraction (VLDL; density <1.006 g/mL) was isolated by ultracentrifugation, and the high-density lipoprotein (HDL) fraction was obtained after precipitation of low-density lipoprotein (LDL) in the infranatant (density >1.006 g/mL) with heparin and $MnCl_2$. Apolipoprotein B (ApoB) and A1 (ApoA1) concentrations were measured in plasma by the rocket immunoelectrophoretic method of Laurell.[7] Cholesterol content of HDL_2 and HDL_3 subfractions was determined after further precipitation of HDL_2 with dextran sulfate.[8] Reproducibility of all lipid-lipoprotein measurements has been found to be excellent.[9]

Lipoprotein Subclass and Particle Size Measurements

Lipoprotein subclass and particle size analysis was performed in archived, fasting plasma samples before and after exercise training by nuclear magnetic resonance (NMR) spectroscopy at LipoScience, Inc (Raleigh, NC) using the LipoProfile-3 algorithm.[10] Concentrations of large, medium, and small, VLDL-P and HDL-P, large and small LDL-P, and intermediate-density lipoprotein particles (IDL-P), as well as weighted-average VLDL-P, LDL-P, and HDL-P sizes were obtained. A full description of these methods has been previously published.[11]

Intravenous glucose tolerance test and insulin and glucose homeostasis variables

An intravenous glucose tolerance test (IVGTT) was performed the morning after an overnight (12 h) fast before and

after the exercise training program according to the protocol proposed by Walton et al.[12] Details of the IVGTT protocol have been published.[13] IVGTT-derived variables were generated from MINMOD analysis (MINMOD Millennium, version 5.18), and included the insulin sensitivity index (S_i), glucose effectiveness (S_g), and the acute insulin response to glucose (AIR_g). The disposition index (D_i) is the product of S_i and AIR_g and represents a measure of overall glucose homeostasis and the ability of the β-cell to compensate for insulin resistance. Fasting plasma insulin was measured by radioimmunoassay after polyethylene glycol separation and glucose was assayed using a commercial kit (Diagnostic Chemicals). Post-training samples were obtained 24 hours after the last exercise training session.

eMethods 2. Metabolomic profiling of DMGV

Plasma aliquots of patient samples (10μL) were deproteinized, vortexed, and subjected to centrifugation, and the supernatants were transferred to autosampler vials with glass inserts for LC-MS analysis. Prepared samples were directly injected (10μL) onto an Agilent 1260 system coupled to a 4000-QTrap Quadrupole-Linear ion trap mass spectrometer (AB SCIEX) run in a scheduled Multiple Reaction Monitoring (sMRM) mode. Mass spectrometry data were analyzed using Sciex MultiQuant software. Peaks were manually reviewed for quality by two separate analysts in a blinded manner. In addition, samples were normalized to the average of the nearest neighbor bracketing pair of pooled plasma, which was injected every 20 samples to monitor and correct for temporal drift in mass spectrometry performance.

eReferences

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eTable 1. List of metabolic traits examined.

Lipids, Lipoproteins, Apolipoproteins	Glucose and Insulin traits	Anthropometrics and Body Composition
HDL cholesterol	Fasting plasma glucose	Body Mass Index
HDL triglycerides	Fasting plasma insulin	Waist circumference
LDL cholesterol	Fasting C-peptide	Abdominal visceral fat area
LDL triglycerides	Insulin sensitivity	Abdominal total fat area
VLDL cholesterol	Acute insulin response to glucose	Fat mass
VLDL triglycerides		Fat-free mass
Plasma triglycerides	Disposition index	Body fat percentage
Total plasma cholesterol	Glucose effectiveness	Hip Circumference
Apolipoprotein A1		
Apolipoprotein B		
VLDL apolipoprotein B		
LDL apolipoprotein B		
HDL2 cholesterol		
HDL3 cholesterol		
HDL Particles		
Large HDL Particles		
Medium HDL Particles		
Small HDL Particles		
HDL Size		
IDL Particles		
LDL Particles		
Large LDL Particles		
Small LDL Particles		
LDL Size		
VLDL & Chylomicron Triglyceride		
Large VLDL & Chylomicron Particles		
VLDL & Chylomicron Particles		
Medium VLDL Particles		
Small VLDL Particles		
VLDL Size		

eTable 2. Associations between baseline DMGV levels and metabolic traits, with and without adjustment for fasting insulin.

	DMGV		DMGV adj. insulin	
Phenotype	**beta**	**p-value**	**beta**	**p-value**
VLDL triglycerides in mmol / L	0.190	5.6E-4	0.151	2.2E-3
Large VLDL & Chylomicron Particles	0.189	1.2E-4	0.162	1.6E-3
VLDL Size	0.191	2.4E-4	0.156	4.5E-3
VLDL & Chylomicron Triglyceride (total)	0.167	6.9E-4	0.143	5.3E-3
Plasma triglycerides in mmol / L	0.152	0.001	0.111	0.024
HDL cholesterol in mmol / L	-0.148	0.002	-0.098	0.045
HDL3 cholesterol in mmol / L	-0.162	0.002	-0.110	0.049
LDL Size	-0.157	0.002	-0.128	0.018
Small LDL Particles (total)	0.140	0.003	0.112	0.023
VLDL cholesterol in mmol / L	0.144	0.003	0.105	0.037
Insulin sensitivity	-0.125	0.010	-0.042	0.386
Glucose effectiveness	0.141	0.012	0.118	0.046
Fasting plasma glucose, mmol / L	0.117	0.017	0.072	0.1554
Acute insulin response to glucose	0.124	0.022	0.046	0.385
VLDL apolipoprotein B in mmol / L	0.106	0.036	0.088	0.094

Cross sectional associations between baseline DMGV levels and metabolic traits in a linear regression model adjusted for age, sex, BMI, abdominal visceral fat (left column) plus additional adjustment for insulin (right column)

eTable 3. Changes in plasma DMGV levels after endurance exercise training in the HERITAGE cohort.

	T-score	P-value	Median Percent Change
All subjects	-3.78	1.8E-4	-9.4% (-31.1-18.9%)
Parents	-2.43	0.016	-5.1% (-31.1-18.5%)
Offspring	-2.90	0.041	-9.8% (-31.2- 21.0%)

Logarithmically transformed DMGV values were used in a paired Student's *t* test.

eTable 4. Associations between changes in DMGV levels and changes in metabolic traits after 20 weeks of exercise training.

	Model 1		Model 2		Model 3	
Clinical trait	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**
Large VLDL & Chylomicron Particles	0.28	6.7E-11	0.28	4.9E-11	0.29	2.1E-11
VLDL & Chylomicron Triglyceride (total)	0.28	1.1E-10	0.29	8.2E-11	0.29	3.8E-11
Fasting C-peptide	0.26	3.4E-08	0.26	2.7E-08	0.23	4.0E-07
VLDL cholesterol, mmol/L	0.25	4.2E-08	0.25	5.6E-08	0.25	3.7E-08
Plasma triglycerides, mmol/L	0.24	8.7E-08	0.24	1.8E-07	0.24	1.0E-07
VLDL triglycerides, mmol/L	0.24	1.0E-07	0.24	1.4E-07	0.25	7.1E-08
Medium HDL Particles	0.21	2.2E-06	0.19	1.1E-05	0.19	1.5E-05
Acute insulin response to glucose	0.21	2.4E-06	0.20	3.0E-06	0.20	6.3E-06
VLDL apolipoprotein B, mmol/L	0.19	5.2E-06	0.20	3.7E-06	0.21	5.0E-07
Fasting insulin	0.19	1.0E-05	0.19	8.5E-06	0.17	2.7E-05
HDL Particles (total)	0.20	1.0E-05	0.19	3.0E-05	0.18	5.1E-05
Fasting glucose, mmol/L	0.19	3.0E-05	0.19	2.1E-05	0.19	2.3E-05
Apolipoprotein B, mmol/L	0.18	1.1E-04	0.18	1.0E-04	0.16	4.7E-04
VLDL Size	0.16	1.7E-04	0.17	5.0E-05	0.17	5.9E-05
Small LDL Particles (total)	0.16	2.7E-04	0.16	2.4E-04	0.16	4.8E-04
Plasma total cholesterol, mmol/L	0.16	5.5E-04	0.16	5.2E-04	0.14	1.9E-03
Medium VLDL Particles	0.13	1.9E-03	0.14	8.9E-04	0.14	7.0E-04

Absolute changes in DMGV and each clinical trait. Effect sizes for each clinical trait are reported per standard deviation (SD) increment of baseline DMGV levels in a linear regression model. Model 1 adjusts for the baseline value of each clinical trait. Model 2 further adjusts for age and sex. Model 3 further adjusts for BMI and the change in BMI after exercise training.

eTable 5. Associations between baseline levels of DMGV and fasting insulin and HDL-trait changes after exercise training.

	Insulin	DMGV	DMGV adj. insulin
Apolipoprotein A1			
Beta	-0.005	-0.14	-0.14
P-value	0.92	5.1E-3	5.6E-3
Total HDL-P			
Beta	-0.05	-0.11	-0.10
P-value	0.30	0.03	0.08
Small HDL-P			
Beta	0.05	0.13	0.11
P-value	0.28	3.7E-3	0.04
Medium HDL-P			
Beta	-0.12	-0.19	-0.09
P-value	0.01	2.7E-5	0.11

Effect sizes for each clinical trait are reported per standard deviation increment of DMGV or insulin levels based on a generalized linear model adjusted for age, sex, BMI and changes in BMI after exercise training. “DMGV adj. insulin” includes insulin as a covariate.

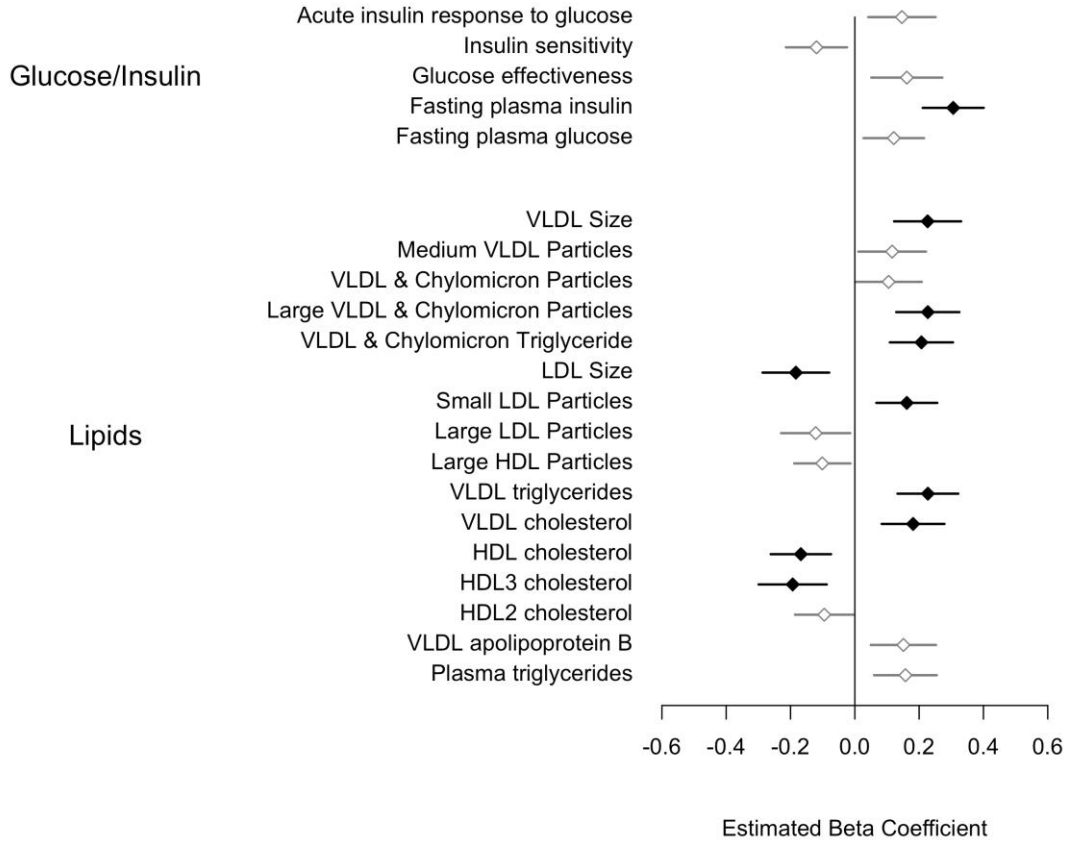
eTable 6. Changes in metabolic traits according to baseline DMGV levels.

	Model 1		Model 2		Model 3	
Clinical trait	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**
Medium HDL Particles	-0.20	5.4E-6	-0.19	3.4E-5	-0.19	2.7E-5
HDL Particles (total)	-0.18	7.2E-5	-0.15	2.3E-3	-0.11	0.03
Small HDL Particles	0.15	6.1E-4	0.14	2.5E-3	0.13	3.7E-3
Apolipoprotein A1, mmol/L	-0.14	1.9E-4	-0.14	2.3E-3	-0.14	5.1E-3
Insulin sensitivity (S_i)	-0.13	3.0E-3	-0.11	0.01	-0.04	0.36
IDL Particles	0.11	4.7E-3	0.07	0.09	0.05	0.28
HDL2 cholesterol, mmol/L	-0.11	0.03	-0.10	0.04	-0.10	0.05
Abdominal visceral fat area, cm2	0.11	0.03	0.11	0.03	0.02	0.70
Body weight, kg	0.11	0.04	0.12	0.02	0.01	0.53
HDL Size	-0.09	0.05	-0.07	0.16	-0.01	0.71
Fat-free mass, kg	0.08	0.14	0.13	0.01	0.06	0.26

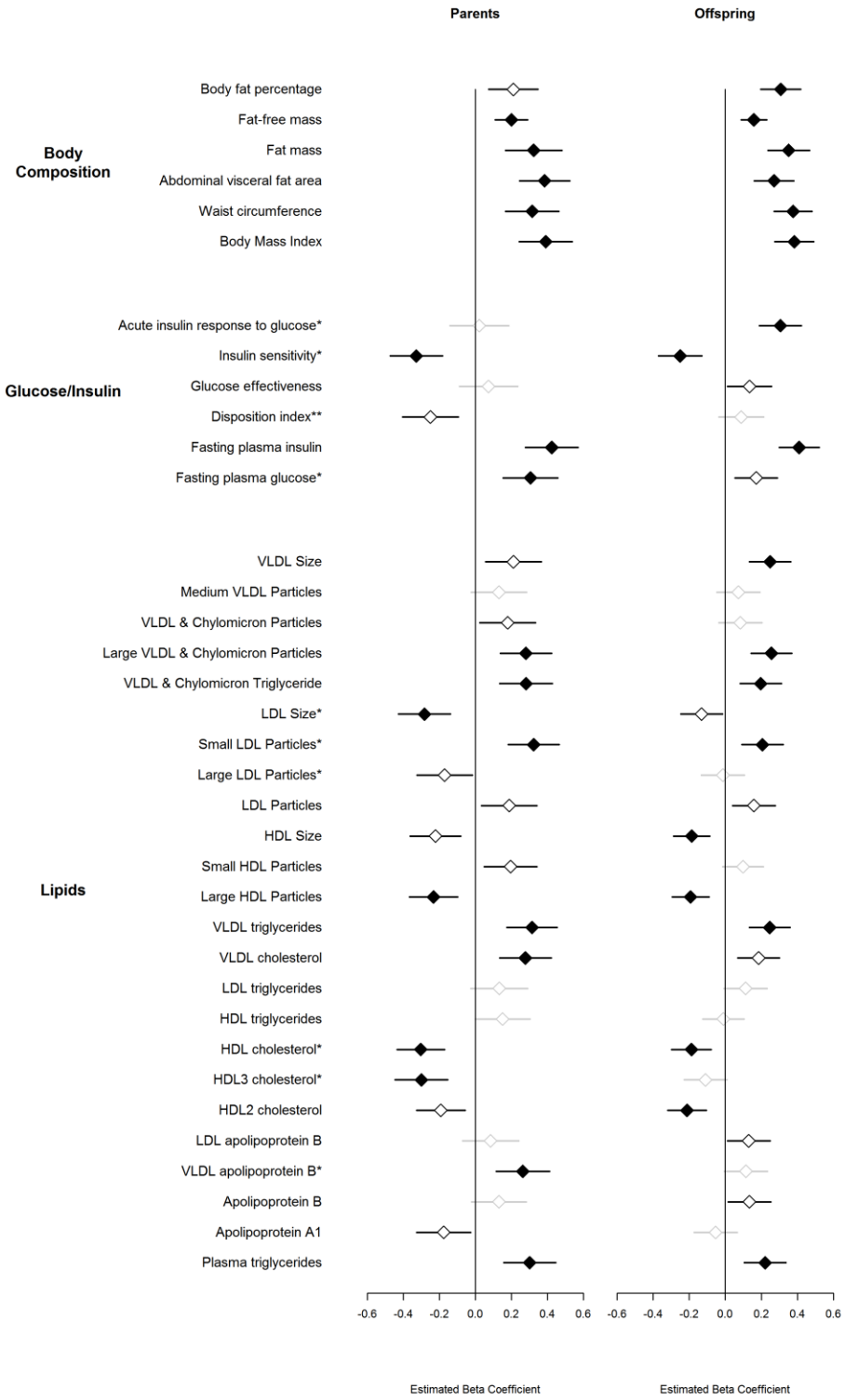
Absolute changes in each clinical trait. Effect sizes for each clinical trait are reported per standard deviation (SD) increment of baseline DMGV levels in a linear regression model. Model 1 adjusts for the baseline value of each clinical trait. Model 2 further adjusts for age and sex. Model 3 further adjusts for BMI and the change in BMI after exercise training.

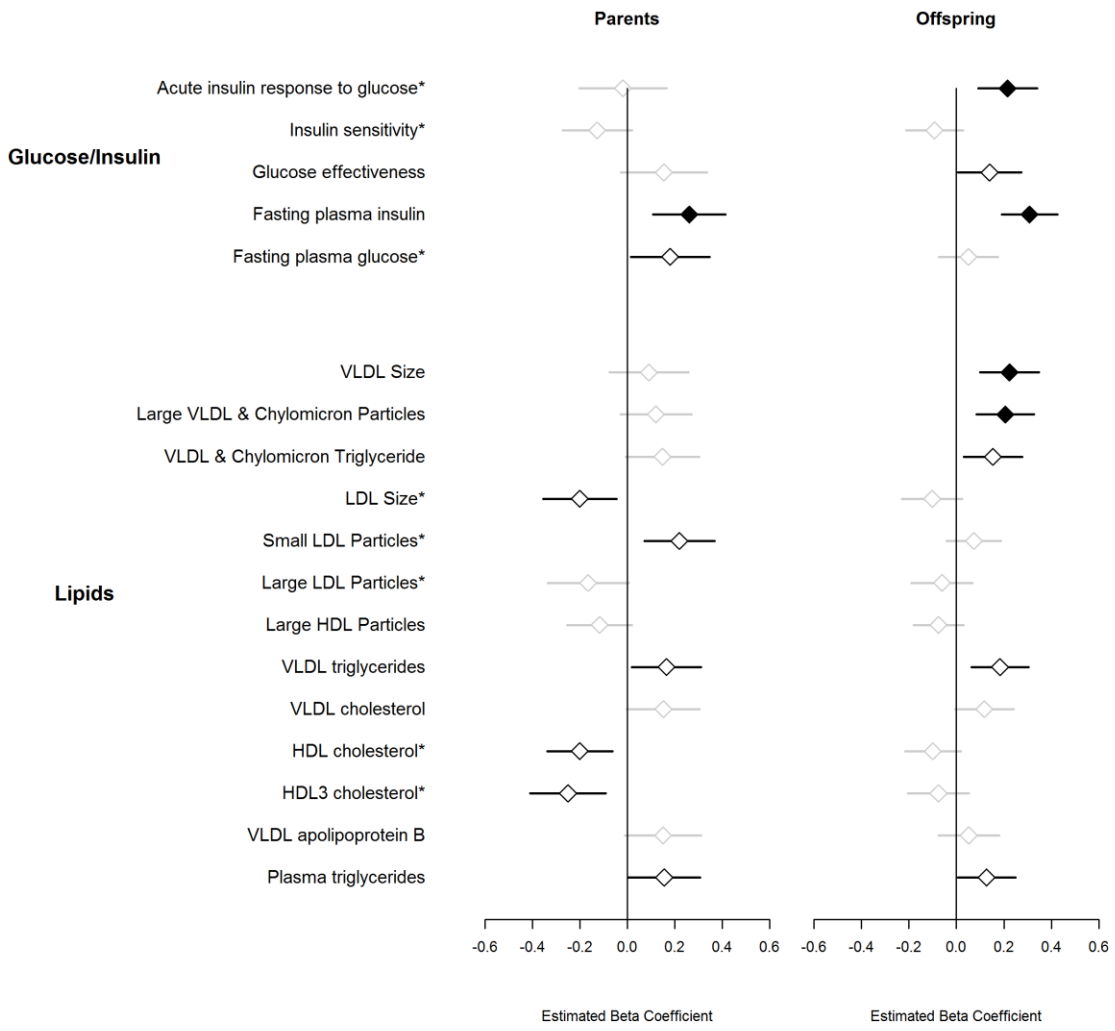
eFigure 1. Cross-sectional relationships between baseline DMGV levels and metabolic traits after adjustment for age, sex, BMI, and abdominal visceral fat.

Effect sizes for each clinical trait are reported per standard deviation (SD) increment of baseline DMGV levels in a linear regression model. Black bars with solid diamonds meet Bonferroni statistical significance (p<0.0011); grey bars with open diamonds meet nominal significance (p<0.05).



eFigure 2. Relationship between baseline DMGV levels and baseline metabolic traits among HERITAGE generations. Figure 2a is adjusted for age and sex. Figure 2b is further adjusted for BMI and abdominal visceral fat.

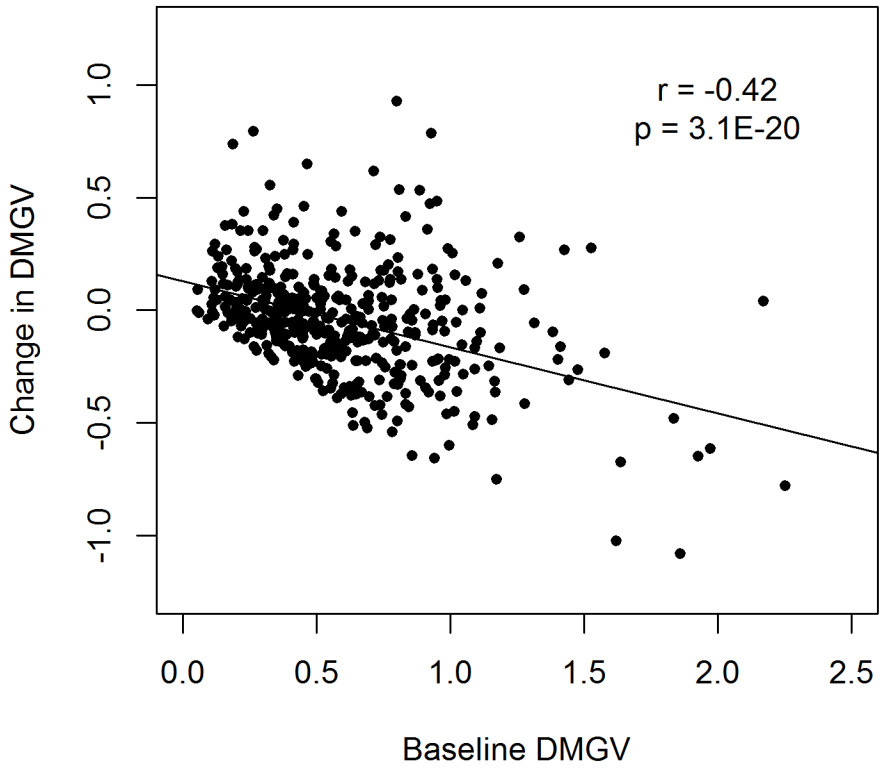




eFigure 2.

Effect sizes for each clinical trait are reported per standard deviation (SD) increment of baseline DMGV levels in a linear regression model. Black bars with solid diamonds meet Bonferroni statistical significance ($p<0.001$); grey bars with open diamonds meet nominal significance ($p<0.05$).

eFigure 3. Correlation between baseline DMGV levels and changes in DGMV levels after exercise training in the HERITAGE cohort.



Pearson's correlation analyses. DMGV levels were logarithmically transformed.