

# Total Body Fat and Abdominal Visceral Fat Response to Exercise Training in the HERITAGE Family Study: Evidence for Major Locus But No Multifactorial Effects

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The familial etiology of the response in total fat mass (FM) and abdominal visceral fat (AVF) to 20 weeks of exercise training was investigated in families participating in the HERITAGE Family Study. AVF (measured by computed tomographic scanning) and FM (measured by underwater weighing techniques) were assessed at baseline (in a sedentary state) and after 20 weeks of exercise training. The response AVF ( $AVF_{\Delta}$ ) and response FM ( $FM_{\Delta}$ ) were computed as the simple delta values (posttraining – baseline) and adjusted for the effects of sex, generation, and a polynomial in age using multiple regression analysis. To index the AVF response independently of the response in FM and the initial level of visceral fat, the  $AVF_{\Delta}$  was also adjusted for age and baseline AVF ( $AVF_B$ ) and  $FM_{\Delta}$ . Familial correlation analysis was used to investigate the multifactorial familial effects (polygenic and/or familial environmental), and segregation analysis was used to search for major gene effects. For the age-adjusted  $AVF_{\Delta}$ , a putative recessive locus accounting for 18% of the variance ( $q^2 = 1\%$ ) was detected. Adjusting  $AVF_{\Delta}$  for  $AVF_B$  and  $FM_{\Delta}$  slightly increased the percentage of variance accounted for (to 26%,  $q^2 = 3\%$ ) but did not radically alter the pattern of the parameter estimates. For  $FM_{\Delta}$ , a putative dominant locus accounting for 31% of the variance ( $q^2 = 49\%$ ) was noted. In conclusion, the results were consistent across methods in suggesting that there is little evidence of a multifactorial heritability for either  $AVF_{\Delta}$  or  $FM_{\Delta}$ . Rather, the familial etiology of the response to exercise training appears to be primarily due to putative major genes (a recessive locus for  $AVF_{\Delta}$  and a dominant locus for  $FM_{\Delta}$ ). In addition, a pleiotropic/oligogenic system underlying these variables was inferred. That is, the putative loci for  $FM_{\Delta}$  and/or  $AVF_B$  also may impact the  $AVF_{\Delta}$ , with an additional independent major locus effect on  $AVF_{\Delta}$  after the former influences have been removed.

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IT IS WELL RECOGNIZED that obesity is an independent risk factor for cardiovascular disease and type 2 diabetes in both sexes,<sup>1,4</sup> and abdominal visceral fat (AVF) is the depot that may convey an extra risk in obese people.<sup>5-7</sup> One implication of these studies is that fat reduction in the AVF area could be helpful in controlling some of the health risks associated with obesity. Obesity is a complex trait and arises, in part, when there is an excess of energy intake compared with energy expenditure. Consequently, dietary restrictions and exercise (ie, inducing negative energy balance) are common weight-loss interventions. Reviews of this topic<sup>8,9</sup> suggest that dietary restrictions (regardless of exercise conditions) lead to greater weight reduction than exercise without dietary restrictions, presumably because individuals tend to eat more to compensate for the negative energy balance. Although exercise-induced mean changes in weight are generally less dramatic, there is a wide

range of intersubject variability that is beyond measurement error and generally nonrandom.<sup>10</sup>

The interindividual variability in response to weight reduction is likely caused in part by genetic predisposition. For example, susceptibility genes may influence energy requirements, muscle metabolism, fuel utilization, and even food preferences. Thus, there is likely a complex system of genetic factors underlying the response to weight-reduction techniques. However, only a few studies have investigated these underlying familial factors. In a long-term experiment, seven pairs of monozygotic (MZ) twins exercised twice daily for 9 of 10 days over a period of 93 days, to expend 244 MJ over the energy cost of weight maintenance while consuming the baseline amount of calories.<sup>11</sup> The reduction in AVF was striking ( $-29 \text{ cm}^2$ ) and was related to twinship. Subjects with the same genotype (within-MZ comparisons) were significantly more alike in their response than those with different genotypes (between-MZ comparisons) despite the large individual differences in the response, suggesting a genetic heritability for the response to long-term training accompanied by negative energy balance.

The HERITAGE Family Study<sup>12</sup> is the first large-scale study designed, in part, to investigate the genetic factors underlying the response to endurance exercise training on various physiological and biochemical traits. These families were sedentary at the onset of the study (baseline), and were exercise-trained for 20 weeks under supervision so that each individual was exercising at a heart rate associated with 75% maximal oxygen consumption ( $\dot{V}O_{2\text{max}}$ ) for 50 minutes during the last 6 weeks of training. Energy intake was required to be constant for each individual during the entire study. At baseline and again after training (post-visit), AVF (computed tomographic scan) and total fat mass ([FM] underwater weighing) were measured. Previous HERITAGE studies found that the adiposity measurements were highly reproducible, with intraclass correlations of

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.97 to 1.00,<sup>13</sup> and the exercise protocol produced statistically significant reductions in adiposity.<sup>14</sup> Moreover, males had a significantly greater reduction in AVF (6.4 cm<sup>2</sup>) than females (3.1 cm<sup>2</sup>), with a similar pattern for FM (males lost significantly more [0.9 kg] than females [0.5 kg]).

The primary purpose of the current study was to determine if there is a familial etiology for AVF and FM responses to exercise training in the HERITAGE families. The response (AVF<sub>Δ</sub> and FM<sub>Δ</sub>) was computed as the simple delta (post-training baseline) and was adjusted for age, sex, and generation. In addition, AVF<sub>Δ</sub> was further adjusted for baseline AVF (|AVF<sub>B</sub>) to correct for the initial level of abdominal adiposity) and for FM<sub>Δ</sub> (to correct for the overall level of adiposity). For each of the adiposity responses, familial correlations were used to estimate the multifactorial heritability (ie, additive effects, including both polygenic and familial environmental factors) and segregation analysis was used to investigate major gene contributions.

## SUBJECTS AND METHODS

### Sample and Study Design

The total sample with complete data for both the baseline and post-exercise training visits consisted of 440 caucasian individuals from 98 nuclear families (209 males and 231 females). Families of African-American descent were also recruited and tested in the HERITAGE study, but their results are not reported here. The criteria for participation in the HERITAGE study have been described elsewhere.<sup>12</sup> Briefly, the parents were younger than 66 years of age and offspring were older than 17 years. All participants were sedentary at baseline, defined as no regular physical activity over the previous 6 months. Regular physical activity included activity lasting 30 minutes or more and involving an energy expenditure of at least 7 METS (1 MET = 3.5 mL O<sub>2</sub> uptake per kg body weight per min) for subjects 50 years or older and at least 8 METS for subjects younger than 50 years. With a few exceptions approved by a physician, the body mass index (BMI) was 40 kg/m<sup>2</sup> or less, systolic blood pressure (SBP) was 159 mm Hg or less, and diastolic blood pressure (DBP) was 99 mm Hg or less. Individuals with a BMI greater than 40 kg/m<sup>2</sup> were included with sufficient clinical justification by a physician on a case-by-case basis. In general, subjects were required to be in good physical health as certified by a physician in order to complete the 20-week training program.

Subjects were asked to keep their energy intake constant throughout the training period. At baseline, subjects completed dietary questionnaires<sup>15,16</sup> and were instructed to maintain the same diet throughout the protocol. Adherence was monitored during the training, and analyses of the repeated dietary measures revealed no significant changes (Walker AJ, Thompson PA, Gagnon J, et al. submitted, 1999).

Individuals were assessed on a battery of physiological and biochemical tests at baseline. Each participant then trained on a cycle ergometer three times per week for 20 weeks. The intensity and/or duration of training were adjusted for each individual every 2 weeks so that the participant was working at a heart rate associated with 75%  $\dot{V}O_{2max}$  for 50 minutes during the last 6 weeks of training. The power output was adjusted automatically to the heart rate response by a built-in computer program on the cycle ergometer during all training sessions. Training sessions were supervised on-site, and adherence to the protocol was strictly monitored at the four participating laboratories.<sup>12</sup> After completion of the 20-week exercise training, participants were again assessed using the same battery of physiological and biochemical tests (post-training measures).

### Measures

Computed tomography scanning was used to measure AVF. Participants were examined in the supine position with their arms stretched above the head,<sup>17</sup> and the abdominal scan was obtained between the L4 and L5 vertebrae. The attenuation interval used to quantify the area of adipose tissue was -190 to -30 Hounsfield units. The AVF area was defined by drawing a line within the muscle wall surrounding the abdominal cavity. Underwater weighing was performed to determine FM,<sup>18</sup> with adjustment for residual lung volume by the oxygen dilution method<sup>19,20</sup> or the helium dilution technique.<sup>21,22</sup> AVF<sub>Δ</sub> and FM<sub>Δ</sub> were computed as the simple difference between post-training and baseline measurements.

### Data Adjustments

Age can affect the mean value of traits, and the variance of traits may differ with age (heteroscedasticity). Consequently, the effects of age (and other concomitant variables such as sex) on the mean and the variance are usually removed prior to familial analysis. Here, the effects of sex, generation, and age were removed from AVF<sub>Δ</sub> and FM<sub>Δ</sub> using a stepwise multiple regression procedure.<sup>23</sup> In summary, each response variable was regressed on a cubic polynomial in age separately within each of four sex-by-generation groups (fathers, mothers, sons, and daughters) to investigate age effects on the mean values. Only terms that were significant at the 5% level were retained. The resulting squared residual from this mean regression was similarly regressed on another polynomial in age to investigate age effects on the variance (heteroscedasticity). The analysis variable (or phenotype) was computed as the residual from the mean regression divided by the square root of the standard deviation of the predicted score from the variance regression, and a final standardization step ensured a mean of 0 and a standard deviation of 1.

In addition to these age adjustments, AVF<sub>Δ</sub> was adjusted for a polynomial in age, AVF<sub>B</sub>, and FM<sub>Δ</sub> using the same procedure just described. In summary, there were three analysis variables: age-adjusted AVF<sub>Δ</sub>, age-FM<sub>Δ</sub>-AVF<sub>B</sub>-adjusted AVF<sub>Δ</sub>, and age-adjusted FM<sub>Δ</sub>.

### Familial Correlations

A simple method for deriving an estimate of the additive effect (or multifactorial heritability) is the familial correlation model. The correlation method yields a generalized heritability (ie, combined genetic and familial environmental sources), requires relatively few assumptions, and can be applied to simple family structures such as nuclear families. The assumption underlying the correlation model is that the effects of genes and environments are linear and additive. Since parent-offspring and sibling pairs share (on average) half of their genes, either correlation may be assumed to estimate half of the additive familial effect (multifactorial heritability). This estimate includes any additive effects due to polygenes, major genes, and shared environmental factors. An additional assumption applies to the spouse correlation. If there is random mating for the trait, then spouses share only familial environments and not genes. Therefore, significant spouse resemblance suggests that at least some portion of the heritability is due to familial environments, while nonsignificant spouse resemblance implies that the heritability is primarily genetic in origin. In contrast, if there is assortative mating (ie, spouses select each other based on a commonality of the trait or a correlated trait), then the spouse correlation also can reflect genetic similarity, if the trait is under genetic influence.

The familial correlation model used in the HERITAGE study is based on four types of individuals (fathers [f], mothers [m], sons [s], and daughters [d]) which yield eight correlations within three familial groups (one spouse [ $r_{fm}$ ], four parent-offspring [ $r_{fs}$ ,  $r_{fd}$ ,  $r_{ms}$ , and  $r_{md}$ ], and three sibling [ $r_{ss}$ ,  $r_{dd}$ , and  $r_{sd}$ ]). The computer program SEGPATH<sup>24</sup> was used to estimate familial correlations by the maximum-likelihood method, which yields a logarithmic likelihood ( $-2 \ln L$ ) goodness-of-fit

statistic for each model. The familial correlations were fit directly to the family data under the assumption that the phenotypes in a family follow a multivariate normal distribution.

A general model (all eight correlations estimated) and several reduced models investigating certain hypotheses were estimated. Each hypothesis was tested using the likelihood ratio test (LRT), which is the difference in  $-2 \ln L$  between the general model and the reduced model. The LRT is distributed as a  $\chi^2$  with the degrees of freedom (df) representing the difference in the number of parameters estimated in the general and reduced models. A significant  $\chi^2$  test suggests that the model reduction does not fit the data, while a nonsignificant  $\chi^2$  test implies that the reduced model provides an acceptable fit. The hypotheses included tests for an environmental model by equating all eight correlations ( $r_{im} = r_{is} = r_{id} = r_{ms} = r_{md} = r_{ss} = r_{dd} = r_{sd}$ ,  $df = 7$ ) and for no familial resemblance at all ( $r_{im} = r_{is} = r_{id} = r_{ms} = r_{md} = r_{ss} = r_{dd} = r_{sd} = 0$ ,  $df = 8$ ). The significance of correlations was also tested by familial group: no spouse correlation ( $r_{im} = 0$ ,  $df = 1$ ), no parent-offspring correlations ( $r_{is} = r_{id} = r_{ms} = r_{md} = 0$ ,  $df = 4$ ), and no sibling correlations ( $r_{ss} = r_{dd} = r_{sd}$ ,  $df = 3$ ). Nonrejected hypotheses were combined to determine the most parsimonious model.

### Commingle and Segregation Models

The familial correlation model indexes, in part, the additive genetic effects due to polygenes, ie, many genes that each have a small effect and are measurable only in the aggregate. On the other hand, there may be one or a few genes that have major effects. While polygenic effects are additive and usually result in a normal distribution, major genes can be nonadditive and yield multiple distributions. Commingle and segregation analysis is used to investigate whether there are major genes.

Commingle analysis is used to characterize the distribution of a variable as a single normal component or as a mixture of two or three normal components while allowing for residual skewness. The method described by MacLean et al<sup>25</sup> and implemented in the computer program SKUMIX<sup>26</sup> was used. A mixture of up to three distributions in Hardy-Weinberg proportions can be fit, optionally including the power transformation parameter ( $p$ ). The effect of the power transformation is to reduce skewness under a hypothesis that posits a certain number of components. Competing hypotheses of skewness and commingle can be evaluated.

There are five parameters in the SKUMIX model in addition to  $p$ .  $E$  is the common variance in each component,  $u$  is the overall mean,  $q$  determines the relative proportion ( $q^2$ ) of the component distribution with the highest mean,  $t$  is the displacement between the two extreme component means, and  $d$  is the relative position of the mean of the middle component. Parameters are estimated by the maximum-likelihood method, and tests of nested hypotheses are performed using the LRT. The Akaike<sup>27</sup> information criterion [ $AIC$ ] =  $-2 \ln L$  plus twice the number of estimated parameters; the "best" model is the one with the smallest AIC) is used to compare the fit among non-nested models.

While a mixture of components is compatible with a major gene influence, it is not considered *prima facie* evidence of it, since commingle can arise from other sources such as shared environmental factors. A segregation analysis tests whether the trait is being transmitted from parents to offspring according to Mendelian expectations. Segregation analysis was performed using the unified mixed model as implemented in the computer program POINTER.<sup>25,26,28,29</sup> The model assumes that a phenotype is composed of the independent and additive contributions from a major gene effect, a heritable multifactorial background, and a unique environmental component (residual). The major effect is assumed to result from the segregation at a single locus with two alleles (ie,  $A$  and  $a$ ). The  $A$  allele is defined as decreasing the quantitative phenotype. There are 10 parameters in the model, including the overall variance ( $V$ ), overall mean ( $u$ ), major locus gene frequency

( $q$ , frequency of the  $a$  allele), displacement between the two homozygous means ( $t$ ), relative position of the heterozygous mean, or dominance ( $d$ ), and multifactorial heritability in offspring ( $H$ ) and in parents ( $HZ$ ). Note that  $u$ ,  $d$ ,  $t$ , and  $q$  represent the same parameters outlined in the commingle analysis. The transmission pattern of the major gene from parents to offspring can be tested in the unified mixed model to verify that the gene is segregating according to Mendelian expectations.<sup>28</sup> The transmission pattern is characterized by three parameters:  $\tau_1$  is the probability that an  $AA$  individual transmits allele  $A$  to the offspring,  $\tau_2$  is the probability that  $Aa$  transmits allele  $A$ , and  $\tau_3$  is the probability that  $aa$  transmits allele  $A$ . Under Mendelian transmission,  $\tau_1 = 1$ ,  $\tau_2 = 1/2$ , and  $\tau_3 = 0$ . No transmission of the major effect is obtained when the three  $\tau$ s are equal.

To infer the existence of a major gene, three conditions are usually required<sup>28</sup>: (1) the no-major-effect hypothesis ( $d = t = q = 0$ ) is rejected, (2) the hypothesis of Mendelian transmission is not rejected, and (3) the no-transmission-of-the-major-effect model (equal  $\tau$ s) is rejected. All parameters are estimated using maximum-likelihood methods, and competing models are tested for significance using the LRT and the AIC.

## RESULTS

### Sample Statistics

The mean  $\pm$  SD for baseline (subscript B) and response (subscript  $\Delta$ ) AVF and FM are listed in Table 1, as well as the BMI, SBP, and DBP. The sample characteristics in terms of the frequency distribution of the BMI, SBP, and DBP are also depicted in Fig 1. About 10% of the HERITAGE sample is obese (BMI > 32 kg/m<sup>2</sup>),<sup>30</sup> and mild hypertension (140/90 mm

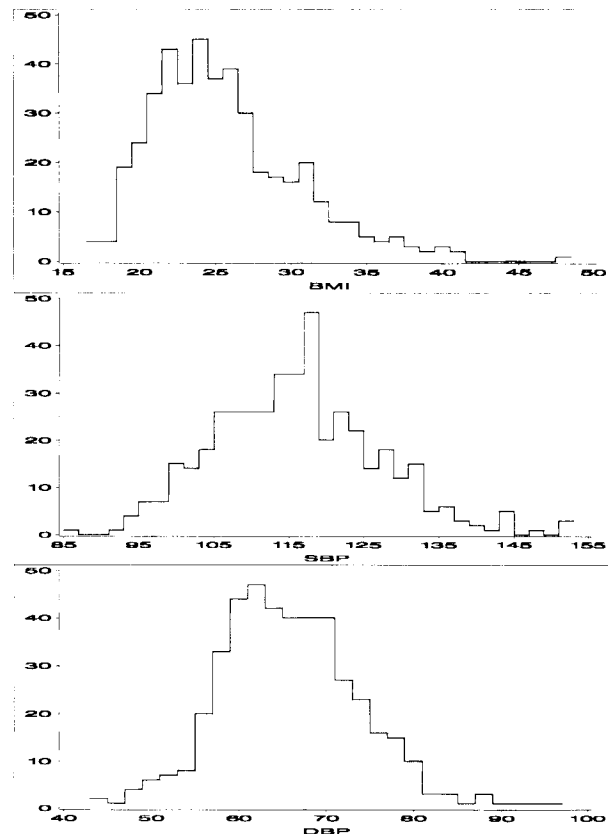


Fig 1. Sample characteristics for baseline BMI, SBP, and DBP.

**Table 1. Sample Statistics**

Variable	Mean ± SD	Range	Mean ± SD	Range
	Fathers (n = 83)		Mothers (n = 80)	
Age <sub>B</sub>	53.5 ± 5.1	45.6-64.3	52.1 ± 5.0	42.4-65.1
BMI <sub>B</sub>	28.2 ± 4.5	20.6-41.5	27.2 ± 5.0	18.5-47.5
SBP <sub>B</sub>	121.4 ± 13.1*	96.3-151.7	116.6 ± 12.3	85.7-152.5
DBP <sub>B</sub>	72.4 ± 8.7*	50.0-95.7	67.3 ± 6.9	50.7-84.2
AVF <sub>B</sub>	155.0 ± 61.5*	55.2-380.3	116.7 ± 61.1	31.6-327.6
AVF <sub>Δ</sub>	-9.3 ± 21.8	-76.0-39.0	-6.5 ± 18.2	-60.0-37.0
% Δ	12.8		12.7	
FM <sub>B</sub>	24.5 ± 9.2*	7.6-58.2	26.9 ± 10.6	10.2-62.1
FM <sub>Δ</sub>	-0.7 ± 1.7	-4.4-4.7	-0.7 ± 1.7	-5.2-6.5
% Δ	7.1		5.2	
	Sons (n = 126)		Daughters (n = 151)	
Age <sub>B</sub>	25.4 ± 6.1	17.0-40.3	25.6 ± 6.5	17.2-40.9
BMI <sub>B</sub>	25.4 ± 4.4*†	17.3-41.3	23.6 ± 4.5‡	17.0-39.4
SBP <sub>B</sub>	118.8 ± 8.4*†	93.0-137.3	110.4 ± 7.9‡	91.7-126.8
DBP <sub>B</sub>	65.3 ± 7.4*†	44.5-83.3	61.7 ± 6.3‡	43.5-79.3
AVF <sub>B</sub>	76.5 ± 39.5*†	15.9-220.0	52.4 ± 29.9‡	13.1-175.0
AVF <sub>Δ</sub>	-5.9 ± 15.0*†	-65.4-52.0	-1.8 ± 9.2‡	-31.0-33.0
% Δ	15.9		14.3	
FM <sub>B</sub>	17.2 ± 10.4	0.3-52.8	18.1 ± 10.2‡	3.3-58.9
FM <sub>Δ</sub>	-1.0 ± 2.0*†	-12.5-4.1	-0.5 ± 1.9	-6.6-4.0
% Δ	11.7		8.7	

NOTE. Units of measure are as follows: BMI, kg/m<sup>2</sup>; SBP, mm Hg; DBP, mm Hg; AVF, cm<sup>2</sup>; FM, kg.

\*Based on a comparison of the standard errors (SEs), there are significant sex differences.

†Based on a comparison of SEs, there are significant generation differences in males.

‡Based on a comparison of SEs, there are significant generation differences in females.

Hg)<sup>31</sup> occurs between the 95th and 99th percentile, although no subjects exhibit stage 2 or stage 3 hypertension (ie, >140 mm Hg for SBP and/or 90 mm Hg for DBP).

The general pattern for these mean values (Table 1) is for males to have a higher BP, BMI, and AVF and a lower FM than females, and also for the parental values to be higher than those in the offspring. The statistical significance of the sex and generation group differences was judged by a comparison of standard errors (mean ± 2 SE). Using this criterion (Table 1), there were significant sex differences for SBP<sub>B</sub>, DBP<sub>B</sub>, and AVF<sub>B</sub> (both generations), BMI<sub>B</sub> (offspring only), and FM<sub>B</sub> (parents only). There were significant generation differences in both sexes for all baseline measures, except that for FM<sub>B</sub>, the generation difference was significant only in females. For the response measures, only the offspring exhibited significant sex differences (both AVF<sub>Δ</sub> and FM<sub>Δ</sub>). Generation differences were noted in both sexes for AVF<sub>Δ</sub>, but only in the males for FM<sub>Δ</sub>. Finally, the mean responses for AVF<sub>Δ</sub> and FM<sub>Δ</sub> were significantly different from zero in all four groups (ie, there is a mean response).

*Data Adjustments*

The regression analysis revealed few age effects on these variables. For AVF<sub>Δ</sub>, a quadratic age term accounted for 5.3% of the mean variation in sons, but not in any of the other three sex-by-generation (fathers, mothers, and daughters) groups. In addition, a linear age term accounted for 6.4% of the variance

due to heteroscedasticity only in the mothers. Similarly, for FM<sub>Δ</sub>, a cubic term in age accounted for 6% of the variance in sons, with no mean age effects in any of the other groups. However, AVF<sub>B</sub> and FM<sub>Δ</sub> were significant covariates of AVF<sub>Δ</sub>, accounting for 19% (fathers), 19.8% (mothers), 29.5% (sons), and 24.2% (daughters) of the variance. In addition, there were significant heteroscedastic effects of AVF<sub>B</sub> (6.4% in mothers and 6.0% in sons) and age<sup>3</sup> (6.1% in daughters).

*Familial Correlations*

Results of the hypothesis tests are listed in Table 2 in terms of P values. For age-adjusted AVF<sub>Δ</sub>, an environmental hypothesis (reduced model 1, P = .083) fit the data, as did the no-familial-resemblance hypothesis (reduced model 5, P = .062). The latter was chosen as the most parsimonious model. For the age-AVF<sub>B</sub>-FM<sub>Δ</sub>-adjusted AVF<sub>Δ</sub> measure, none of the reduced hypotheses were rejected, and reduced model 5 (no familial resemblance) was chosen as the most parsimonious. For the age-adjusted FM<sub>Δ</sub>, the only rejected hypothesis was for no spouse resemblance (model 4), and the most parsimonious model was the combination of no sibling and no parent-offspring correlations (models 2 and 3).

Table 3 lists the correlations under the general models. Under the most parsimonious models, all of these correlations are zero, except that the spouse correlation for FM<sub>Δ</sub> is significant (.32 ± .12). We note that while some of the correlations appear fairly large (eg, fm = .20 ± .11 for age-adjusted AVF<sub>Δ</sub>), the likelihood ratio test and the standard error comparison both suggest that they are not significantly different from zero in this sample. These correlations provide little support for multifactorial familial resemblance, except for a possible environmental effect between spouses for the response in total fat.

**Table 2. Familial Correlation Model Hypothesis Tests (P)**

Reduced Model	Age-Adjusted AVF <sub>Δ</sub>	Age-AVF <sub>B</sub> -FM <sub>Δ</sub> -Adjusted AVF <sub>Δ</sub>	Age-Adjusted FM <sub>Δ</sub>
<b>1. Environmental</b>			
(r <sub>fm</sub> = r <sub>fs</sub> = r <sub>fd</sub> = r <sub>ms</sub> = r <sub>md</sub> = r <sub>ss</sub> = r <sub>dd</sub> = r <sub>sd</sub> , df = 7)	.083	.145	.319
<b>2. No sibling correlations</b>			
(r <sub>ss</sub> = r <sub>dd</sub> = r <sub>sd</sub> = 0, df = 3)	.088	.463	.206
<b>3. No parent-offspring correlations (r<sub>fs</sub> = r<sub>fd</sub> = r<sub>ms</sub> = r<sub>md</sub> = 0, df = 4)</b>			
	.257	.132	.241
<b>4. No spouse correlation (r<sub>fm</sub> = 0, df = 1)</b>			
	.087	.132	.017
<b>5. No familial resemblance (r<sub>fm</sub> = r<sub>fs</sub> = r<sub>fd</sub> = r<sub>ms</sub> = r<sub>md</sub> = r<sub>ss</sub> = r<sub>dd</sub> = r<sub>sd</sub> = 0, df = 8)</b>			
	.062	.192	.081
<b>Most parsimonious</b>			
<b>5. No familial resemblance (r<sub>fm</sub> = r<sub>fs</sub> = r<sub>fd</sub> = r<sub>ms</sub> = r<sub>md</sub> = r<sub>ss</sub> = r<sub>dd</sub> = r<sub>sd</sub> = 0, df = 8)</b>			
	.062	.192	
<b>2. and 3. No sibling or parent-offspring correlations (r<sub>ss</sub> = r<sub>dd</sub> = r<sub>sd</sub> = r<sub>fs</sub> = r<sub>fd</sub> = r<sub>ms</sub> = r<sub>md</sub> = 0, df = 7)</b>			
			.278

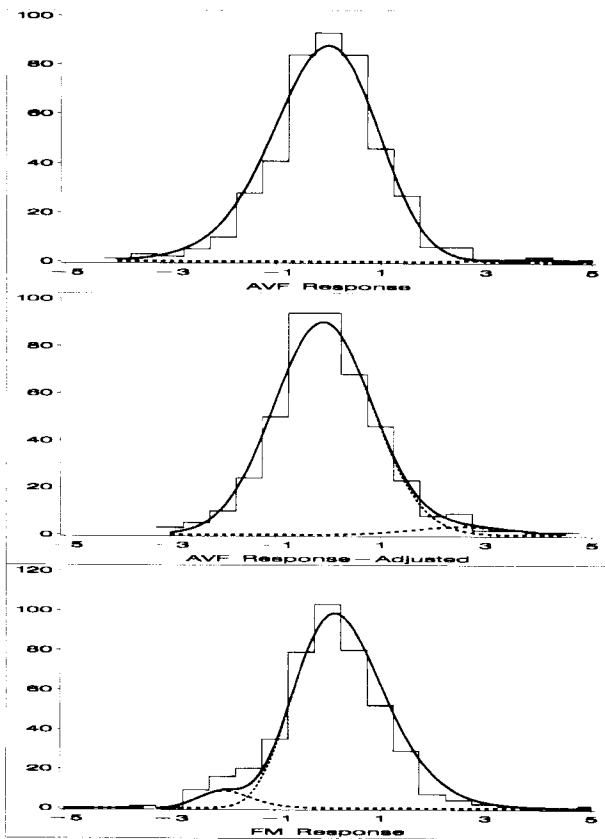
**Table 3. Maximum-Likelihood Familial Correlations ( $\pm$  SE) Under the General Models**

Parameter	Age-Adjusted AVF <sub>Δ</sub>	Age-AVF <sub>B</sub> -FM <sub>Δ</sub> -Adjusted AVF <sub>Δ</sub>	Age-Adjusted FM <sub>Δ</sub>
<i>r<sub>fm</sub></i>	.20 $\pm$ .11	.18 $\pm$ .11	.33 $\pm$ .12
<i>r<sub>fs</sub></i>	.00 $\pm$ .11	.09 $\pm$ .09	.18 $\pm$ .12
<i>r<sub>fd</sub></i>	.15 $\pm$ .08	.04 $\pm$ .08	-.06 $\pm$ .10
<i>r<sub>ms</sub></i>	-.10 $\pm$ .08	.17 $\pm$ .08	.17 $\pm$ .11
<i>r<sub>md</sub></i>	.08 $\pm$ .09	.10 $\pm$ .08	.11 $\pm$ .10
<i>r<sub>ss</sub></i>	-.25 $\pm$ .12	-.19 $\pm$ .11	.06 $\pm$ .16
<i>r<sub>sd</sub></i>	.01 $\pm$ .10	-.02 $\pm$ .13	.22 $\pm$ .13
<i>r<sub>sd</sub></i>	.15 $\pm$ .09	.05 $\pm$ .08	.09 $\pm$ .10

NOTE. Under the most parsimonious models, the only correlation significantly different from zero is the spouse correlation (*r<sub>fm</sub>* = .33  $\pm$  .12) for age-adjusted FM<sub>Δ</sub>.

*Commingling*

There are six models evaluated (mixtures of one to three distributions, each with and without skewness) for each analysis variable. For the age-adjusted AVF<sub>Δ</sub>, two skewed distributions provide the most parsimonious fit to the data. Figure 2 shows a frequency distribution for the age-adjusted AVF<sub>Δ</sub>, with the two-skewed model superimposed on the distribution. The



**Fig 2. Commingling models superimposed on frequency distributions for AVF<sub>Δ</sub> adjusted for age (top) and adjusted for age, AVF<sub>B</sub>, and FM<sub>B</sub> (middle), and for FM<sub>Δ</sub> adjusted for age (bottom). The SKUMIX parameters used to generate the curves are as follows: (top) E = 0.98, u = 0.05, d = 0, t = 4.5, q = 0.10, and p = 1.6; (middle) E = 0.88, u = 0.00, d = 0, t = 2.7, q = 0.19, p = 1; (bottom) E = 0.67, u = -0.09, d = 1, t = 2.71, q = 0.71, and p = -0.27.**

parameter estimates used to derive the curves are also shown. Two normal distributions provide the best fit for age-AVF<sub>B</sub>-FM<sub>Δ</sub>-adjusted AVF<sub>Δ</sub>, and two skewed distributions best fit the age-adjusted FM<sub>Δ</sub>. Thus, there is consistent support for commingling for all three variables.

*Segregation*

Results for the segregation analysis are presented in Table 4. For the age-adjusted AVF<sub>Δ</sub>, there is a familial effect (model 4 - model 1,  $\chi^2_5 = 25.18, P < .001$ ). The multifactorial component is not significant (model 2 - model 1,  $\chi^2_2 = 0.00, P > .999$ ), but the major effect component is significant (model 3 - model 1,  $\chi^2_3 = 24.29, P < .001$ ). The mode of transmission of the major component was tested under the major-effect-only model (ie, H = Z = 0). The recessive model (d = 0) was not rejected (model 5 - model 2,  $\chi^2_1 = 0.45, P = .502$ ), but the additive mode (d = 1/2) was rejected (model 6 - model 2,  $\chi^2_1 = 4.54, P = .033$ ), as was the dominant mode (d = 1, model 7 - model 2,  $\chi^2_1 = 4.57, P = .033$ ). Finally, the transmission probabilities were tested under the parsimonious Mendelian recessive model (model 5, H = Z = d = 0). Mendelian transmission was not rejected (model 5 - model 8,  $\chi^2_3 = 0.21, P = .976$ ), nor was the equal- $\tau$ s model (model 9 - model 8,  $\chi^2_3 = 6.64, P = .084$ ). Thus, while either the Mendelian or the equal- $\tau$ s model provided a good fit to the data by LRT, the AIC suggested that the Mendelian model (AIC = 8.66) was preferred over the equal- $\tau$ s model (AIC = 14.64).

Similar results were obtained for the age-AVF<sub>B</sub>-FM<sub>Δ</sub>-adjusted AVF<sub>Δ</sub>. The multifactorial effect was not significant ( $\chi^2_2 = 0.00, P > .999$ ), the major effect was significant ( $\chi^2_3 = 24.11, P < .001$ ), the recessive mode was not rejected ( $\chi^2_1 = 0.00, P > .999$ ), Mendelian  $\tau$ s were not rejected ( $\chi^2_3 = 0.01, P > .999$ ), and equal  $\tau$ s were not rejected ( $\chi^2_3 = 1.79, P = .617$ ). The AIC suggested that the Mendelian hypothesis (8.01) provided the best fit to the data.

Again, similar results were obtained for the age-adjusted FM<sub>Δ</sub>, except that the mode of transmission was dominant rather than recessive. The multifactorial effect was not significant ( $\chi^2_2 = 0.00, P > .999$ ) and the major effect was significant ( $\chi^2_3 = 11.75, P = .008$ ). While both the recessive mode ( $\chi^2_1 = 4.29, P = .038$ ) and the additive mode ( $\chi^2_1 = 4.35, P = .037$ ) were rejected, the dominant mode ( $\chi^2_1 = 0.00, P > .999$ ) provided a good fit to the data. The transmission probabilities were tested under a model in which there was no multifactorial effect and with a dominant mode of transmission for the major effect (ie, H = Z = 0, d = 1). The Mendelian hypothesis was not rejected ( $\chi^2_3 = 0.19, P = .979$ ), nor was the equal- $\tau$ s model rejected ( $\chi^2_3 = 3.02, P = .389$ ). The most parsimonious model by AIC (8.19) was the Mendelian hypothesis.

Table 5 provides the most-parsimonious-model parameter estimates and the percent variance accounted for by the major gene effect. There are at least three noteworthy patterns depicted. First, the only familial effect for the response to training in these HERITAGE data for both AVF and FM is due to a major locus, with no contribution from multifactorial factors such as polygenic and/or familial environment. Second, the percentage of variance in AVF<sub>Δ</sub> accounted for by the putative locus increases after adjusting for covariates, although the general pattern remains the same across adjustments. Third,

Table 4. Segregation Model-Fitting Results

Model	Age-Adjusted AVF <sub>Δ</sub>		Age-AVF <sub>B</sub> -FM <sub>Δ</sub> -Adjusted AVF <sub>Δ</sub>		Age-Adjusted FM <sub>Δ</sub>	
	-2 ln L	AIC	-2 ln L	AIC	-2 ln L	AIC
1. General Mendelian	0.21	14.21	0.01	14.01	0.19	14.19
2. No multifactorial effect (H = Z = 0)	0.21	10.21	0.01	10.01	0.19	10.19
3. No major effect (d = t = q = 0)	24.50	32.50	24.12	32.12	11.94	19.94
4. No familial effect (d = t = q - H = Z = 0)	25.39	29.39	24.12	28.12	13.76	17.76
5. Recessive (d = 0)	0.66	8.66	0.01	8.01	4.48	12.48
6. Additive (d = 1/2)	4.75	12.75	8.25	16.25	4.54	12.54
7. Dominant (d = 1)	4.78	12.75	8.25	16.25	0.19	8.19
8. Generalized τs (estimate τ <sub>1</sub> , τ <sub>2</sub> , and τ <sub>3</sub> )	0.00	14.00	0.00	14.00	0.00	14.00
9. Equal τs: (τ <sub>1</sub> = τ <sub>2</sub> = τ <sub>3</sub> = 1 - q)	6.64	14.64	1.79	9.79	3.02	11.02

NOTE. Log likelihoods (-2 ln L) were scaled by subtracting a constant from each model. For the age-adjusted AVF<sub>Δ</sub>, a value of 813.40 was subtracted; for age-AVF<sub>B</sub>-FM<sub>Δ</sub>-adjusted AVF<sub>Δ</sub>, the value was 810.45; and for age-adjusted FM<sub>Δ</sub>, the value was 769.19.

the putative loci for the response to training for FM (dominant) and AVF (recessive) are apparently different.

### DISCUSSION

In the current study, we investigated the familial etiology of total FM and AVF responses to exercise training in the HERITAGE Family Study. All subjects were uniformly sedentary at baseline measurement and were trained to the same degree (75%  $\dot{V}O_{2\max}$ ) during the 20-week training protocol. In addition, subjects were asked to keep their dietary intake constant throughout the training protocol. The training protocol produced small but statistically significant changes in adipose tissue. Both multifactorial (polygenic and familial environmental) and major gene causes for these changes were investigated. There was little evidence for any multifactorial effect on the adipose tissue responses using either familial correlation or segregation methods. The novel finding in this study is the evidence supporting putative major gene influences on each of the AVF and FM responses to training (Fig 2). The variance accounted for by the putative recessive locus for AVF<sub>Δ</sub> increased slightly (from 18% to 26%) after adjusting for baseline levels and FM<sub>Δ</sub>. The putative major gene for FM<sub>Δ</sub> accounted for 31% of the variance, and the mode of transmission was dominant. Between 60% and 80% of the variance was unaccounted for, suggesting that other factors not included in the model influence the adipose response to training.

The finding of a major locus effect in the absence of a multifactorial heritability may appear contradictory. The correlation/heritability method measures the additive variance (ie, it does not include nonadditive effects) that seems so small that we could not detect it with confidence. Finding evidence for a recessive or dominant gene (ie, nonadditive gene action at the major locus) is not a contradiction. Indeed, there seem to be

nonadditive effects detected even in this small data set. The additive variance at the recessive or dominant locus must be small, again consistent with the finding of no evidence for familial aggregation.

Previous studies found evidence for both major gene and multifactorial influences on AVF<sup>23,32,34</sup> and FM.<sup>35,36</sup> Each of the AVF studies analyzed the familial influence both before and after adjustment for the effects of FM (Table 6). In each case, a putative recessive locus accounting for over 50% of the variance and an additional multifactorial component accounting for about 20% of the variance were reported. However, after removing the effects due to FM, the evidence for a major locus was reduced. More specifically, although a major effect remained, it did not appear to be transmitted from parents to offspring according to Mendelian expectations. Finally, a recessive locus for FM that accounted for between 35% and 45% of the variance was reported. Together, these results suggest an oligogenic-pleiotropic model underlying AVF and FM. That is, a single major gene may underlie both traits (pleiotropic), and there may be additional major genes and/or familial factors specific to each trait (oligogenic).

The current study extends these results by suggesting that in addition to the putative genes influencing baseline fat accumulation, there may be other loci influencing the AVF and/or FM response to exercise training for 5 months. It is interesting that the putative genes for response AVF and FM are apparently very different, one being recessive (AVF) and the other acting in a dominant fashion (FM). Removal of the effects of baseline AVF and of the FM response strengthens the signal of the putative gene for the AVF response. This suggests that each of the putative loci for baseline AVF and response FM may have an effect on the AVF response (pleiotropy) but that there are other

Table 5. Segregation Model Parameter Estimates (± SE) Under the Most Parsimonious Models

Adjustment	V	u	t	q	% Variance
AVF <sub>Δ</sub>					
Age	1.22 ± 0.12	0.00 ± 0.05	4.18 ± 0.45	0.11 ± 0.03	18
Age-AVF <sub>B</sub> -FM <sub>Δ</sub>	1.25 ± 0.12	0.05 ± 0.05	3.18 ± 0.43	0.18 ± 0.04	26
FM <sub>Δ</sub>					
Age	1.04 ± 0.09	-0.01 ± 0.05	1.96 ± 0.18	0.70 ± 0.05	31

NOTE. V is the overall variance, u is the overall mean, t is the displacement between homozygous means, and q is the allele frequency leading to high values. For all variables, d = 0 (recessive mode of transmission), H = Z = 0 (no multifactorial effect), and the transmission probabilities are Mendelian (τ<sub>1</sub> = 1, τ<sub>2</sub> = 1/2, and τ<sub>3</sub> = 0), except that d = 1 (dominant mode) for FM<sub>Δ</sub>.

Table 6. Summary of Major Gene Studies for AVF and FM

Study	Phenotype	Major Gene	Major Gene % Variance	Multifactorial % Variance
Rice et al <sup>35</sup> (Québec Family Study)	FM	Recessive ( $q^2 = 10\%$ )	45	26
Comuzzie et al <sup>36</sup> (San Antonio Family Heart Study)	FM	Recessive ( $q^2 = 6\%$ )	37 males and 43 females	—
Rice et al <sup>34</sup> (HERITAGE Family Study)	FM	Recessive ( $q^2 = 6\%$ )	64	0
Bouchard et al <sup>32</sup> (Québec Family Study)	AVF	Recessive ( $q^2 = 10\%$ )	51	21
Rice et al <sup>34</sup> (HERITAGE Family Study)	AVF	Recessive ( $q^2 = 8\%$ )	54	17
Bouchard et al <sup>32</sup> (Québec Family Study)	AVF-FM	Non-Mendelian	—	44
Rice et al <sup>34</sup> (HERITAGE Family Study)	AVF-FM	Non-Mendelian	—	42
Current study (HERITAGE Family Study)	AVF <sub>Δ</sub>	Recessive ( $q^2 = 1\%$ )	18	0
	AVF <sub>Δ</sub> -AVF <sub>B</sub> -FM <sub>Δ</sub>	Recessive ( $q^2 = 3\%$ )	26	0
	FM <sub>Δ</sub>	Dominant ( $q^2 = 49\%$ )	31	0

NOTE. The HERITAGE study<sup>34</sup> in this table is based only on the baseline data.

loci specifically acting on the response AVF and response FM (oligogenic).

The results of this study and other studies investigating AVF and FM suggest a pleiotropic/oligogenic system (Fig 3). AVF<sub>Δ</sub> is influenced by several factors: major factors (MF in the diagram) that are also causative for baseline AVF, a major gene for FM<sub>Δ</sub> (MG<sub>3</sub>), and a major gene specific to AVF<sub>Δ</sub> (MG<sub>1</sub>). Whether these effects on AVF<sub>Δ</sub> occur directly through the inferred factors, indirectly through a phenotypic source, or both could not be distinguished. Previous studies suggest the remaining paths in Fig 3. For example, a putative locus for FM (MG<sub>2</sub>) also impacts AVF. Molecular studies are in progress to identify the genes involved in this diagram and thus to provide a resolution of the model.

These putative loci detected for the adiposity response to training may well act in regulating lipid metabolism.<sup>37</sup> During lipolysis, energy stored in fat cells (ie, triglycerides) is broken down into glycerol and free fatty acid (FFA). Glycerol is readily transported to the tissues (mainly the liver) for metabolism, while FFAs bind to carrier proteins (albumin) for transport to

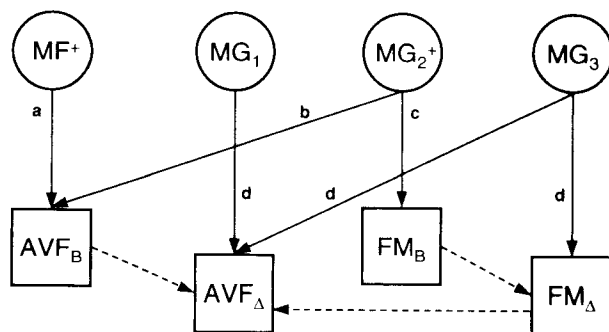


Fig 3. Schematic drawing of the etiological factors leading to variability in the AVF response to exercise training. Squares represent measured variables and circles represent inferred factors, ie, major genes (MG<sub>1</sub>-MG<sub>3</sub>) and non-Mendelian major factors (MF). Superscript + for MF and MG indicates that there is evidence for additional multifactorial effects (ie, polygenic and/or familial environmental). The paths drawn with solid lines represent the effects of the inferred factors on the variables. These paths are labeled to represent results from several studies: a, Québec Family Study (QFS)<sup>33</sup> and HERITAGE<sup>23</sup>; b, QFS<sup>32</sup> and HERITAGE<sup>34</sup>; c, QFS<sup>32,35</sup>, HERITAGE<sup>34</sup> and San Antonio Family Heart Study<sup>36</sup>; d, current HERITAGE study. Finally, the dashed lines are alternative paths among variables reflecting phenotypic causative sources.

the tissues (liver and muscle) for metabolism. Training and exercise stimulates the production of catecholamines, which in turn accelerate the  $\beta$ -adrenoceptor-mediated lipolysis rate in adipose tissues. Sex differences in the response to training also occur. The glycerol response is greater in women than in men. There is also more regional variation in women, with a greater lipolytic response in the abdominal versus peripheral tissues.

Association studies have identified at least four possible loci that may contribute to variation in the abdominal visceral area.<sup>38</sup> The ApoB locus on chromosome 2p24-23 was shown to be associated with abdominal fat.<sup>39</sup> Participants with the less frequent 13-kb allele had significantly lower AVF than subjects who were homozygous for the more frequent 11-kb allele. In other words, the 13-kb allele was associated with leanness in the abdominal visceral area. AVF, independently of total fat, also was associated with the *BclII* RFLP at the glucocorticoid receptor locus (GRL) located on 5q31-32.<sup>40</sup> Similar to the ApoB locus that was associated with leanness, the AVF association with the less frequent GRL allele (4.5 kb) was found in lean (lower tertile of percent body fat) but not in obese (upper tertile of percent body fat) participants. The fatty acid-binding protein 2 (FABP2) locus on 4q28-31 also has been associated with high levels of AVF.<sup>41</sup> Participants who were homozygous for the Thr54 allele had higher basal insulin levels, greater insulin resistance, and greater accumulation of intraabdominal fat (but not subcutaneous abdominal fat or the BMI). However, AVF intraabdominal fat in this study was measured using ultrasound techniques, and thus may be a different phenotype. Finally, the  $\beta_3$ -adrenergic receptor (ADRB3) locus on 8p12-11.2 was associated with AVF in two separate studies.<sup>42,43</sup> For example, Kim-Motoyama et al<sup>43</sup> found that the Trp64Arg mutation was associated with visceral obesity (both homozygotes and heterozygotes) and was more frequent in subjects with lower serum triglyceride levels (homozygotes only). Sakane et al<sup>42</sup> also found that in premenopausal women, visceral fat and total body fat (but not the BMI) were greater in homozygous and heterozygous carriers for the Arg64 allele. In contrast, Gagnon et al<sup>44</sup> did not find any association or linkage between the Trp64Arg mutation and AVF in the Québec Family Study, nor did the few association studies that measured AVF directly.

We are not aware of any association or linkage studies on the response in AVF due to exercise training. However, interesting candidate genes involve those showing an association or linkage with weight loss or weight gain. For example, the

uncoupling protein 1 (UCP1) locus (4q28-31, close to the FABP2 locus) was associated with a large fat gain over 12 years<sup>45</sup> and a reduction in the weight and BMI<sup>46</sup> in separate studies. The leptin gene (LEP) and several closely linked anonymous markers (D7S2519, D7S649, and D7S530) located at 7q31.3 were also associated with weight loss.<sup>47</sup> In addition, the ADRB3 locus (mentioned before as associated with AVF) was associated with weight gain over the course of 20 to 25 years in two separate studies<sup>48,49</sup> but not in others.<sup>44</sup>

In summary, the AVF and FM responses to exercise training

in these HERITAGE families may be due in part to putative major genes, with little contribution from multifactorial factors such as polygenic and/or familial environmental influences. Molecular studies are currently under way to isolate the genes suggested by these analyses. Furthermore, the fact that these putative loci account for about 20% to 30% of the variance suggests that there are other influences on these responses which are not considered in these models. Further studies investigating the source of these additional factors (eg, metabolic rates) are warranted.

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