

Genetic Correlation of Exercise with Heart Rate and Respiratory Sinus Arrhythmia

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ABSTRACT

DE GEUS, E. J. C., D. I. BOOMSMA, and H. SNIEDER. Genetic Correlation of Exercise with Heart Rate and Respiratory Sinus Arrhythmia. *Med. Sci. Sports Exerc.*, Vol. 35, No. 8, pp. 1287–1295, 2003. **Purpose:** A twin design was used to test whether the association between exercise behavior and heart rate and the association between exercise behavior and respiratory sinus arrhythmia (RSA) derive from a common genetic factor. **Methods:** Data were available from 157 adolescent (aged 13–22) and 208 middle-aged twin pairs (aged 35–62), divided into five sex by zygosity groups (male and female monozygotic twin pairs, and dizygotic twin pairs of same or opposite sex). Exercise behavior was assessed as the average weekly METs spent on sports activities or other vigorous activities in leisure time (sportMETS) in the last 3 months. RSA and heart period (HP) were assessed in the time domain from the combined ECG and respiration signals. **Results:** Heritability estimates were 16% and 29% for RSA, 64% and 68% for HP, and 79% and 41% for sportMETS in young and middle-aged twins, respectively. A significant association was found between RSA and sportMETS (0.17) in the adolescent twins that derived entirely from a common genetic factor. No association was found between sportMETS and RSA in the older twins. A significant association was found between HP and sportMETS in both adolescent (0.35) and middle-aged (0.18) twins. A large contribution of common genetic factors to these associations was found amounting to 84% and 88% in the young and middle-aged twins, respectively. **Conclusions:** Although the results of this study do not preclude causal effects of exercise on RSA and heart rate, they show that the association between exercise and these cardiovascular risk factors largely derives from a common genetic factor. **Key Words:** TWINS, HERITABILITY, PHYSICAL ACTIVITY, BRADYCARDIA, PARASYMPATHETIC

Prospective studies have repeatedly suggested that regular vigorous exercise in leisure time (e.g., sports, jogging, aerobics) is associated with a reduced risk for myocardial infarction and sudden death. An exercise-induced increase in cardiac vagal nerve activity is one of the mechanisms put forward to explain this reduced risk in exercisers (2,22). Individual differences in vagal contribution to resting heart rate can be assessed as the increase in heart rate after parasympathetic blockade or the increase in heart rate from complete sympathetic blockade to complete dual sympathetic and parasympathetic blockade. Using this pharmacological blockade approach, the contribution of vagal nerve activity to resting heart rate was found to be higher in well-trained than in untrained persons in some (24,27), but not in other, studies (13,15). Most blockade studies point to a lower intrinsic heart rate as the most consistent source

of resting bradycardia in exercisers (13,15,27), and this is supported by findings in animals.

As a noninvasive alternative to pharmacological blockade, vagal contribution to resting heart rate in exercisers is increasingly quantified by measures of heart rate variability. Total heart rate variability, measured as the variance or standard deviation of the heart period (HP), provides a first crude index of vagal effects but includes the substantial contribution of sympathetic activity to variability in the low frequency ranges. In contrast, respiratory sinus arrhythmia (RSA), the heart rate variability in the respiratory frequency band, is not affected by manipulations of sympathetic activity and responds in a dose-response way to muscarinic blockers or vagal cooling (30).

Cross-sectional studies have fairly consistently suggested higher RSA in exercisers than in nonexercisers (11,12), although not all studies support this and some even report the opposite finding of lower RSA in exercisers (23). Likewise, cross-sectional studies of the association between aerobic fitness and RSA show highly significant positive correlations with the more-fit subjects having higher RSA (2,11), although exceptions have been found (6,7). All these cross-sectional studies comparing high-fit exercisers with low-fit nonexercisers suffer from the shortcoming that “correlation does not imply causation.” This can be resolved by the experimental manipulation of exercise behavior in longitudinal training studies.

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A number of such studies have supported an effect of exercise on RSA (8,17), but most failed to find a training-induced increase in RSA (4,6,7,18). We, for example, used a training-detraining paradigm in 62 young adults (6) to test the effects of aerobic fitness training and subsequent detraining on time and frequency domain measures of RSA. Although heart rate followed our (de)training manipulations closely, we found no systematic training-induced increase in RSA even after 8 months of intensive training. The clear discrepancy in the results of cross-sectional and longitudinal studies could be due to the relatively short duration of the training programs—the autonomic nervous system effects of exercising may take years to develop. Alternatively, the cross-sectional association between exercise behavior and RSA may largely derive from an unobserved underlying third factor. This may be an environmental factor like low socioeconomic status (SES). Low SES is associated with reduced exercise behavior, but it is also a source of chronic stress that, in turn, may reduce RSA. The underlying third factor may also be genetic. A favorable endowment that includes high aerobic fitness and high RSA may lead a person to more often seek out the exercise behavior he or she excels in.

This favorable genetic make-up may further include a low resting heart rate. After maximal oxygen consumption, resting heart rate is one of the parameters that fairly consistently discriminates endurance exercisers from nonexercisers. In training studies, heart rate often is seen to decrease (6,7,15,17,18,24) although strong individual differences in the size of the training effect are found (21). Importantly, heart rate rapidly increases to the initial levels during detraining manipulations (6). This strongly suggests that exercise has a causal effect on heart rate. Such a causal effect does not rule out the possibility that part of the association derives from a common underlying factor influencing both heart rate and exercise behavior.

The aim of this paper is to test the hypotheses that a) the association between exercise behavior and RSA and b) the association between exercise behavior and heart rate derive from a common genetic factor. Previous studies using a (multigenerational) family or twin design have already shown that genetic factors play a pivotal role in determining individual differences in leisure time physical activity (1,16,25). Likewise, significant genetic influences are apparent for RSA (3,5,26,28) and heart rate (20,29). No study, however, has addressed the question whether the genes influencing exercise behavior, RSA, and heart rate could be partly overlapping.

Data was available from 730 individuals in two large twin cohorts participating in the cardiovascular research program of The Netherlands Twin Registry (3,28). In these two twin cohorts, resting ECG was measured under highly comparable and standardized resting conditions, and both cohorts filled out an identical questionnaire on the average time spent per week on sports activities or other vigorous activities in leisure time. HP and RSA were assessed in the time domain from the combined ECG and respiration signals. In a twin design, structural equation modeling can be used to

estimate the genetic and environmental covariance between multiple traits measured in the same subject. This covariance, together with estimates of the genetic (heritability) and environmental contribution to the variance of the traits, can be used to estimate the contribution of genetic and environmental factors to the observed associations between multiple traits, in this case RSA, HP, and exercise behavior.

METHODS

Subjects

A total of 160 adolescent twin pairs (aged between 14 and 21) were measured between 1988 and 1990; 213 middle aged twin pairs (aged between 34 and 63) were measured between 1992 and 1994. Twins were recruited mainly through City Council population registries, but an additional portion of twins was recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of The Netherlands Twin Registry, and through the Dutch Twin Club. Informed written consent was obtained from all subjects, and approval for the protocols of both studies was obtained from the Medical Ethics Committee of the Vrije Universiteit. Four triplets were included in the sample by discarding the data from the second-born subject. Data from three adolescent pairs and five middle-aged pairs were excluded from analysis because RSA measurements were either incomplete or considered erroneous. In total, data were available for 360 male and 370 female twins. In all same-sex twin pairs, zygosity was determined from DNA sampling. Grouped according to their zygosity and sex, the sample consisted of 35 pairs of young and 45 pairs of old monozygotic males (MZM), 30 pairs of young and 37 pairs of old dizygotic males (DZM), 34 pairs of young and 48 pairs of old monozygotic females (MZF), 30 pairs of young and 40 pairs of old dizygotic females (DZF), and 28 pairs of young and 38 old dizygotic pairs of opposite sex (DOS).

Exercise Behavior

Exercise behavior was assessed by identical questionnaires in all subjects. The questionnaire was based on the standardized interview used in the Amsterdam Growth and Health Study (14). Young twins and their parents received mailed questionnaires with items on zygosity, health, alcohol and tobacco use, exercise participation, and personality. Older twins filled out the questionnaires in the laboratory when they visited for the assessment of RSA. Leisure time exercise behavior was quantified as follows:

Sport time ($\text{min}\cdot\text{wk}^{-1}$). Subjects were asked to report all weekly time spent (in a typical week) on their two favorite sports in a club or organization in the last 3 months. Sport time was computed as the average weekly minutes of sports participation done at a minimum intensity of 4.0 METs (this excludes card games, chess, bowling, fishing, etc.). Both time of training and time in competition were assessed.

Active time (min·wk⁻¹). In addition to activities in clubs, subjects reported up to three regularly performed leisure time activities (e.g., jogging, recreational cycling) and activities not unanimously qualified as “sports” but requiring physical activity at a minimum intensity of 4.0 METs (e.g., dance lessons).

sportMETs (MET·h·wk⁻¹). All sports and other physical activities ≥ 4 METs were classified in three classes of intensity. These classes were given an average metabolic rate of 5.5, 8.5, and 11 METs, respectively, where 1 MET corresponds to the average resting metabolic rate (RMR) of 3.5 mL $\dot{V}O_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The number of minutes bicycling or walking from and to the sports field/outdoor track/gymnasium/clubhouse were included (both multiplied by 5.5 METs). Time spent on the various sports and other physical activities was multiplied by the appropriate intensity in METs and summed to yield a weekly METs score.

Experimental Procedure

The twins from each twin pair were tested at the same time of day. The middle-aged twins were always tested in the morning (10:00 a.m.); the younger twins were tested in the morning (10:00 a.m.) or the afternoon (2:00 p.m.). All participants were asked to refrain from smoking, drinking alcohol, coffee, or tea after 11:00 p.m. the night before. The experimental protocol for both age cohorts was largely similar. Subjects underwent mental stress testing interspersed by periods of quiet rest (for details see 3 and 28). Briefly, electrodes were attached for ECG and impedance cardiogram recording, and a strain gauge was strapped around the waist to measure respiration. Participants were comfortably seated in reclined position in a dimly lit and sound shielded cabin. Next they practiced a number of mentally taxing tasks (a speeded two-choice reaction time task, a speeded mental arithmetic task, and a tone-avoidance four-choice reaction time task) and engaged in the execution of these tasks, interspersed with periods of resting recovery. This paper focuses on the last of the resting conditions, an 8.5-min period in which the twin was asked to sit back and relax as much as possible. All analyses will be based on the average values for HP and RSA obtained during this last resting condition.

Physiological Recording

ECG disposable pregelled Ag-AgCl electrodes (AMI type 1650–005 Medtronic) were placed on the tip of the sternum and the lateral margin of the chest, according to the standard lead II configuration. The ECG signal was recorded using a Nihon Kohden bioelectric amplifier (AB 601G) with a time constant of 0.1 s. The respiration signal was recorded with a strain gauge of hollow silastic tube strapped around the waist at a level 7 cm above the umbilicus. An acoustic tone is transmitted from one end and received at the other so that changes in the phase angle of the signal are entirely caused by changes in tube length that, in turn, reflect changes in chest circumference. The respiration signal was collected DC.

ECG data were used to determine the time between successive R-waves in ms. The ECG was converted to a HP time series by using automated software detection of the R-waves in the ECG. The R-wave detection program attends the user to possible ectopic beats or other deviations from physiological plausible criteria. For this study, heart rate was required to lie between 40 and 160 beats, and all beats deviating more than 30% from the previous beat were considered suspect. Correction of excessively short/long beats was attempted by the program by rescanning the original ECG signal using a higher/lower trigger level. If this failed, the error was brought to the attention of the user, who could either manually correct the time series, if the source of the error was obvious, or delete the fragment from further analysis.

The combined ECG and respiration signals were computer-scored to obtain RSA on a breath-to-breath basis by the peak-to-valley method (6). The shortest HP was obtained during heart rate acceleration in the inspiration phase (which was made to include 750 ms from the following expiration to account for phase shifts) and the longest interbeat interval during deceleration in the expiration phase (including 750 ms from the following expiratory pause/inspiration phase). The difference between the longest and shortest interval is used as an index of RSA. When no phase-related acceleration or deceleration was found, the breath was assigned a RSA score of zero. Mean RSA in milliseconds was computed for rest and task conditions by averaging the RSA values of all breaths falling within those conditions (including breaths with zero RSA). Automatic scoring of respiratory variables was checked by visual inspection of all respiratory signals in all conditions. Breathing cycles that showed irregularities like gasps, breath holding, coughing, etc., were not considered valid and were rejected and removed from further processing.

Analytical Approach

We used multivariate structural equation modeling to answer the main questions of our study. This technique yields separate estimates of the relative influence of genetic and environmental factors on exercise behavior, HP, and RSA, while at the same time taking account of their covariance. It further allows determination of the extent to which the correlation (or covariance) between exercise behavior and RSA and between exercise behavior and HP can be explained by common genetic or environmental factors.

Quantitative genetic model fitting. Details of model fitting to twin data have been described elsewhere (19). In short, the technique is based on the comparison of the variance-covariance matrices in monozygotic and dizygotic twin pairs and allows separation of the observed phenotypic variance into additive (A) or nonadditive (D) genetic components and shared (C) and unique (E) environmental components. The latter also contains measurement error. Dividing each of these components by the total variance yields the different standardized components of variance, for example, the heritability (h^2) that can be defined as the ratio of

additive genetic variance to total phenotypic variance. Age can spuriously introduce a common environmental effect if there is a significant correlation between the phenotype and age, because twins are always of the same age. Both exercise behavior and RSA decrease with age (11). By incorporating age into the model the influence of age on the phenotypes can be quantified and controlled for.

Input data for the model fitting analyses (age, sport-METS, HP, and RSA measured in twin and co-twin) was summarized into 7×7 variance-covariance matrices for each of the five zygosity groups in each age cohort. A triangular or Cholesky decomposition (19) was used in the multivariate model fitting to these variance-covariance matrices in both adolescent and middle-aged twins. The Cholesky decomposition represents the most general way in which the variance-covariance structure of the data can be decomposed into its genetic and environmental parts. It allows evaluation of (the significance of) the influence of genetic and environmental factors on exercise behavior, HP, and RSA and on their interrelationships.

The genetic correlation (r_g) between two traits gives an indication of the amount of overlap between (sets of) genes influencing those traits. r_g is calculated as the (additive) genetic covariance (COV_A) between two traits divided by the square root of the product of the total genetic variance components (V_A) of each of the traits. The genetic correlation between, for example, exercise behavior (METs) and RSA therefore equals: $r_g = COV_A(\text{METs}, \text{RSA}) / \sqrt{(V_A \text{METs} * V_A \text{RSA})}$. Shared and unique environmental correlations are calculated in a similar fashion.

Model fitting procedure. A series of submodels nested within the full parameter ACE or ADE Cholesky model were fitted to the multivariate variance-covariance matrices. The significance of variance components A, C, D, and AGE was assessed by testing the deterioration in model fit after each component was dropped from the full ACE or ADE model, leading to the most parsimonious (or “best fitting”) model in which the pattern of variances and covariances is explained by as few parameters as possible. Sex differences were examined by comparing the full model, in which parameter estimates are allowed to differ in magnitude between males and females, with a reduced model in which parameter estimates are constrained to be equal across the sexes. Hierarchic χ^2 tests were used to compare submodels with the full model. The difference in χ^2 values between submodel and full model is itself approximately distributed as χ^2 , with degrees of freedom (df) equal to the difference in df of submodel and full model. Model selection was also guided by Akaike’s information criterion ($AIC = \chi^2 - 2 df$). The model with the lowest AIC reflects the best balance between goodness of fit and parsimony.

Statistical Analysis and Software

Before analysis, HP and RSA (natural log) and sport-METS (square root) were transformed to obtain better approximations of normal distributions. Significances of phenotypic correlations and sex and cohort differences in mean

TABLE 1. Number of individuals (N) and mean values (SD) for age, exercise, HP, and RSA for adolescent and middle-aged twins.

	Males	Females	<i>P</i>
<i>Adolescent twins</i>			
<i>N</i>	158	156	
Age (yr)	16.7 (1.8)	16.7 (2.2)	NS
Exercise (MET · h · wk ⁻¹)	42.9 (40.5)	27.7 (29.7)	<0.05
HP (ms)	941 (160)	889 (128)	<0.025
RSA (ms)	107 (53)	116 (62)	NS
<i>Middle-aged twins</i>			
<i>N</i>	202	214	
Age (yr)	43.6 (6.4)	44.7 (6.8)	NS
Exercise (MET · h · wk ⁻¹)	14.6 (19.5)	12.5 (18.8)	NS
HP (ms)	953 (143)	903 (130)	<0.01
RSA (ms)	57 (29)	63 (36)	NS

values were tested within the structural equation modeling framework, which takes account of nonindependency of twin data and yields unbiased P values. Data handling and preliminary analyses were done with STATA. All quantitative genetic modeling was carried out with Mx software.

RESULTS

Mean values for age, exercise behavior, HP, and RSA are shown in Table 1 for both twin cohorts. In the adolescent twins, there were no effects of time of day (morning vs afternoon) on either HP or RSA. Adolescent boys showed a higher rate of sports participation and a larger HP than girls did. The longer HP for males was replicated in the middle-aged cohort. Compared with the adolescents, sports participation ($P < 0.01$) and RSA ($P < 0.01$) were clearly lower in the middle-aged cohort. Fairly large percentages of adolescent twins (23.6%) and middle-aged twins (41.8%) had a sportMETS score of zero because they did not participate at all in sports and leisure time activities more intensive than 4 METs.

Table 2 displays the phenotypic correlations between age, sportMETS, HP, and RSA. We present the overall correlations collapsed over sex, because models that best explained the covariance pattern between these variables did not show any sex differences.

Twin correlations for exercise behavior, HP, and RSA are displayed in Table 3 for each sex by zygosity group in both age cohorts. For all measures, twin correlations in monozygotic twin pairs were larger than those in dizygotic twin pairs, indicating substantial genetic influences on all traits. For sportMETS, we also checked whether the square root

TABLE 2. Correlations between age, exercise behavior, HP, and RSA in adolescent ($N = 314$) and middle-aged twins ($N = 416$).

	Age	SportMETS	ln(HP)	ln(RSA)
<i>Adolescent twins</i>				
Age	*			
SportMETS	-0.06	*		
ln(HP)	0.14	0.35	*	
ln(RSA)	-0.04	0.17	0.44	*
<i>Middle-aged twins</i>				
Age	*			
SportMETS	-0.13	*		
ln(HP)	-0.05	0.18	*	
ln(RSA)	-0.37	0.08	0.39	*

Significant ($P < 0.05$) correlations are in boldface type.

TABLE 3. Twin correlations for exercise behavior, HP, and RSA in adolescent and middle-aged twins. For SportMETS, Spearman rank correlations are given between parentheses.

	<i>N</i>	SportMETS	ln(HP)	ln(RSA)
Adolescent twins				
MZM	35	0.83 (0.71)	0.69	0.18
DZM	30	0.43 (0.38)	0.60	-0.02
MZF	34	0.78 (0.77)	0.59	0.26
DZF	30	0.51 (0.56)	0.43	-0.01
DOS	28	0.37 (0.38)	0.31	0.16
Middle-aged twins				
MZM	45	0.36 (0.39)	0.58	0.44
DZM	37	0.16 (0.20)	0.23	0.21
MZF	48	0.49 (0.56)	0.69	0.47
DZF	40	0.30 (0.23)	0.25	0.35
DOS	38	0.35 (0.35)	0.54	0.26

N, number of twin pairs; MZM, monozygotic male; DZM, dizygotic male; MZF, monozygotic female; DZF, dizygotic female; DOS, dizygotic opposite sex.

transformation gave a satisfactory approximation of normality by calculating the nonparametric Spearman rank correlations (shown between parentheses in Table 3). The pattern of regular Pearson twin correlations was very similar to Spearman twin correlations indicating a robust genetic effect.

Multivariate model fitting results for adolescent and middle-aged twins are shown in Table 4. Parameter estimates for males and females could be set equal without a significant loss in fit for both adolescent ($\Delta\chi^2 = 23.80$, $df = 21$, NS) and middle-aged twins ($\Delta\chi^2 = 20.78$, $df = 21$, NS). Dominance variation (D) did not contribute significantly and could be dropped from all ADE models (data not shown). The shared environmental component (C) could also be removed without a significant worsening of fit, implicating a model including additive genetic and unique environmental influences (AE model) without sex differences as the most parsimonious one for both age cohorts. The contribution of age to sportMETS and RSA in adolescents and to HP in middle-aged twins was not significant and could be set to zero.

Table 5 shows parameter estimates and 95% CI of the best fitting models for adolescent and middle-aged twins. The heritability estimate for RSA was small in adolescent (16%) and moderate in middle-aged twins (29%). The high heritability estimate for exercise behavior in the young twins (79%) is remarkable. Genetic factors still explained 41% of individual differences in exercise behavior of middle-aged individuals. Heritability for HP was very similar in young (64%) and middle-aged twins (68%). Age explained a substantial proportion of variance of RSA (13%) in the middle-aged cohort. No influence of age could be detected in the young cohort, probably because the age range was very small.

Figure 1A and 1B show genetic and environmental correlations and factor loadings of the best fitting models in adolescent and middle-aged twins, respectively. Squaring the factor loadings yield estimates of variance components explained by age and by genetic and environmental factors (see Table 5). The figures make clear that the association between sportMETS and RSA in adolescent twins and the association between sportMETS and HP in both adolescent

and middle-aged twins could entirely be explained by genetic factors, i.e., genetic correlations were significant but environmental correlations were not. Another way of viewing these results is displayed in Table 6. Large percentages (>80%) of the phenotypic correlation between sportMETS and RSA in adolescent twins and between sportMETS and HP in both age cohorts were explained by genetic factors. Contributions of environmental factors to these correlations were small and nonsignificant. A combination of genetic and environmental common factors is responsible for the correlation between HP and RSA in both age cohorts.

DISCUSSION

High levels of RSA are often found in regularly exercising individuals (11,12), supporting the textbook wisdom that increased cardiac vagal nerve activity accounts for part of the well-known bradycardia in exercisers. The idea that this cross-sectional association reflects a causal effect of exercise on RSA is attractive. Low RSA is an established risk factor in cardiovascular disease, and exercise-induced increases in RSA could explain part of the beneficial effects of exercising on the risk for cardiac disease (2,22). Experimental manipulation of exercise behavior in longitudinal training studies, however, has not yielded consistent evidence for an increase in RSA after training (4,6,7,15). In this study, an alternative source for the associations between exercise behavior and RSA was examined: in a young and middle-aged twin cohort, it was tested whether an underlying common genetic factor could explain part of the observed association. In agreement with this hypothesis, the association between sportMETS and RSA in young adolescents derived entirely from a common genetic factor. Furthermore, a significant contribution of a common genetic factor to the association between sportMETS and HP was found, amounting to 84% and 88% in the young and middle-aged twins, respectively. No (genetic) association was found between sportMETS and RSA in the middle-aged twins, possibly due to a restriction of the range of values (i.e., smaller variances) in both variables.

The variance-covariance pattern in the data, showing excess monozygotic similarity for sportMETS, RSA, as well as HP, was best explained by a model that specified only additive genetic and unique environmental factors without sex differences in both groups. Under this model, heritability estimates for RSA were 16% in the young and 29% in the middle-aged twins. This concurs with the findings in German middle-aged twins, where the heritability of RSA was estimated at 39% (5). Importantly, converging evidence for a genetic influence on RSA also comes from a different genetic paradigm: family studies using sibling resemblance and spouse correlations to estimate genetic and shared environmental contributions. In the Framingham Heart Study, this approach yielded heritability estimates of 16% for RSA (26).

Heritability estimates for HP were 64% (young twins) and 68% (middle-aged). These estimates add to the existing evidence (h^2 estimates ranging from 20% to 70%) for a

TABLE 4. Multivariate model fitting results for adolescent and middle-aged twins; comparisons of models are shown and *P* values, differences in chi-squares ($\Delta\chi^2$) and *df* (Δdf) for these comparisons indicated.

Model	χ^2	<i>df</i>	<i>P</i>	AIC	vs	$\Delta\chi^2$	Δdf	<i>P</i>
Adolescent twins								
Sex differences								
1) ACE	156.93	126	0.032	-95.07				
2) AE	163.03	138	0.070	-112.97	1	6.10	12	NS
3) CE	182.12	138	0.007	-93.88	1	25.19	12	0.014
No sex differences								
4) ACE	180.73	147	0.031	-113.27	1	23.80	21	NS
5) AE	183.33	153	0.048	-122.67	4	2.60	6	NS
6) CE	203.57	153	0.004	-102.44	4	22.84	6	<0.001
Middle-aged twins								
Sex differences								
1) ACE	148.94	127	0.089	-105.06				
2) AE	151.68	139	0.223	-126.60	1	4.72	12	NS
3) CE	174.90	139	0.021	-103.10	1	28.22	12	0.003
No sex differences								
4) ACE	169.72	148	0.107	-126.28	1	20.78	21	NS
5) AE	170.43	154	0.173	-137.57	4	2.83	6	NS
6) CE	191.36	154	0.022	-116.64	4	23.76	6	<0.001

χ^2 , chi-square goodness of fit statistic; *df*, degrees of freedom; *P*, *P* value; AIC, Akaike's information criterion; NS, nonsignificant; vs, versus and indicates with which model the submodel is compared. All models included age. Most parsimonious solutions are printed in boldface type.

significant genetic contribution to variation in heart rate (5,20,29). Various candidate genes for heart rate are currently being tested. For instance, a carefully designed association study in a large cohort of nuclear families from Chinese and Japanese descent (20) found a significant association between resting heart rate and a Ser49Gly polymorphism in the β -1 receptor gene. Ser49 homozygotes had heart rates that were about 5 bpm lower than the Gly49 homozygotes. Because the β -1 receptor is crucially involved in transmission of cardiac sympathetic nerve activity to the heart, an obvious chain of events would be that genetic influences on the β -1 receptor translate to genetic influences on HP.

Previous twin data have already attested to a substantial influence of genetic factors on habitual physical activity (1,25) and sports participation (16). Heritability estimates in these populations, that had an age range comparable to our middle-aged twins, have ranged from 27% to 62%, depending on the exact phenotype, i.e., moderate physical activity, vigorous exercise, or endurance sports participation. Our estimate of 41% for sportMETS in the middle-aged twins is in good agreement with these earlier studies, but the heritability of 79% in the young cohort seems very high. This may in part be attributable to the larger variance in exercise behavior in young adolescence. Also, unique environmental factors related to work and family life may become more important in adulthood reducing the impact of genetic fac-

tors, but this hypothesis needs empirical conformation from a true longitudinal twin study.

A number of different factors are likely to explain how genes influence individual differences in leisure time exercise behavior, including genetic effects on temperament, on the "activity-stat," and on the balance of rewarding versus aversive effects of acute exercise. Based on the high genetic correlation of RSA with sportMETS in the young cohort, we would like to propose an additional source of individual differences in exercise behavior. We suggest that genetic endowment for high levels of aerobic fitness may cause adolescents to take up and adhere to exercise behavior more easily. High RSA may be an indicator of individual differences in aerobic trainability, i.e., the extent of the increase in $\dot{V}O_{2max}$ in response to a standardized training program. Indirect evidence for this hypothesis comes from a study by Boutcher and Stein (4), who showed that individuals who possess high resting RSA show greater increases in $\dot{V}O_{2max}$ in response to an exercise program than subjects with low RSA. Taken the strong positive cultural attitudes toward exercise, subjects who are proficient in exercise may be more likely to take up and adhere to a lifestyle with regular exercise because it boosts their self-esteem. In addition, by enabling faster heart rate recovery, strong vagal control over heart rate may tip the balance between rewarding and aversive effects of acute exercise in favor of reward, by reducing some of the aversive effects of exercise (e.g., prolonged palpitations). In short, exercise may be more rewarding and less aversive to people with a genetic make-up that includes high RSA.

This hypothesis of "reversed causality" contrasts with the currently dominant view that the association between exercise behavior and RSA reflects a causal effect of exercise on RSA (2,11,22). This view is supported by a number of carefully controlled randomized trials in patients with impaired cardiac autonomic supply, e.g., due to neuropathy or heart disease, in whom exercise training increased RSA (see review in 22). It is not consistently supported, however, by

TABLE 5. Parameter estimates and 95% confidence intervals of the best fitting models for adolescent and middle-aged twins.

	h^2 (95% CI)	e^2 (95% CI)	age^2 (95% CI)
Adolescent twins			
SportMETS	0.79 (0.69-0.85)	0.21 (0.15-0.31)	—
ln(HP)	0.64 (0.51-0.74)	0.33 (0.24-0.46)	0.03 (0.003-0.08)
ln(RSA)	0.16 (0.02-0.37)	0.84 (0.63-0.98)	—
Middle-aged twins			
SportMETS	0.41 (0.26-0.54)	0.57 (0.44-0.73)	0.02 (0.0005-0.06)
ln(HP)	0.68 (0.56-0.77)	0.32 (0.23-0.44)	—
ln(RSA)	0.29 (0.15-0.42)	0.58 (0.45-0.72)	0.13 (0.08-0.20)

h^2 , heritability; e^2 , unique environmental variance component; age^2 , variance component due to age.

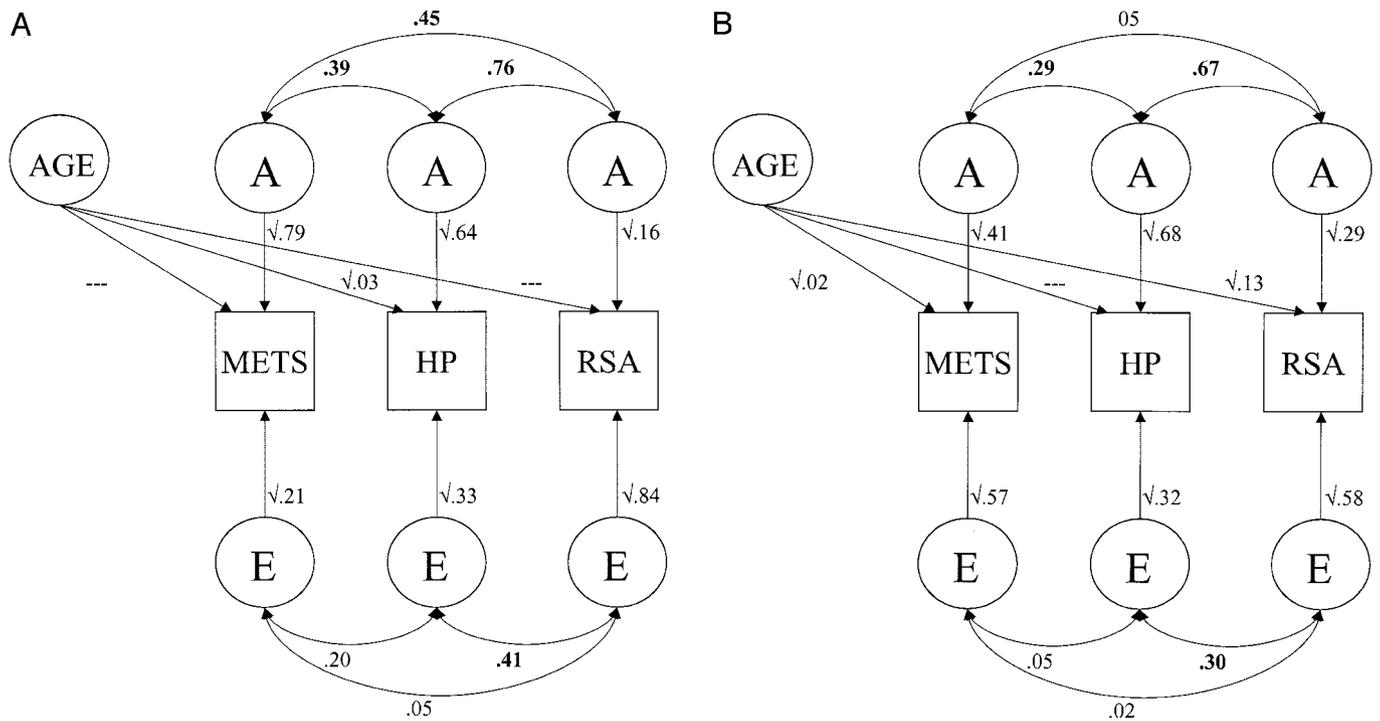


FIGURE 1—Genetic and environmental correlations and factor loadings of the best fitting models in A) adolescent and B) middle-aged twins. Factor loadings (or path coefficients) are expressed as square roots to make clear that squaring those factor loadings yield estimates of variance components explained by age, genetic, and environmental factors as shown in Table 5. Significant ($P < 0.05$) genetic and environmental correlations are in boldface type.

the findings from training studies in healthy subjects from the population at large. Most of these training studies that reported a significant pre- to posttraining increase in RSA (or in the increase in heart rate after parasympathetic blockade) failed to incorporate a nonexercising control group (17,24). All training studies that did use a (randomized) control group (4,7,15,18) or, alternatively, a training-de-training design (6) have found no significant effects of training on RSA. Also, some training studies that are cited as providing evidence for an effect of exercise on vagal contribution to resting heart rate actually only found the total heart rate variability to be increased (8), rather than variability coupled to respiration (peak-to-valley RSA, high-frequency power, or RMSSD). Total heart rate variability, although an important clinical predictor of cardiac disease, cannot be equated with RSA. Instead it reflects a complex mixture of sympathetic and parasympathetic ef-

fects on the heart rhythm, including slow sympathetic vascular effects on pre- and afterload.

We found that in healthy young twins the association between RSA and exercise behavior was largely explained by genes that influenced both variables. A genetic correlation by itself, like any phenotypic correlation, does not imply directional causality. In view of the predominantly negative findings in training studies we interpret the genetic correlation between RSA and exercise behavior to reflect the effects of 1) genes that influence RSA and, through causal effects of RSA on exercise behavior, indirectly also exercise behavior (reversed causality); or 2) pleiotropic genes that influence both exercise behavior and RSA through some common pathway (e.g., temperament).

The association between heart rate and exercise behavior was also explained by genes that influence both variables. Because there is clear evidence from training studies for a causal effect of exercise on heart rate, our interpretation of the genetic correlation between heart rate and exercise behavior must be different from that for RSA. Although strong individual differences in the size of the training effect are found (21), heart rate is seen to significantly decrease in most training studies (6,7,15,17,18,24). In further support of a causal effect, heart rate rapidly increases to pretraining level during detraining manipulations (6). Combining the high genetic correlation found in our twin study with the evidence from (de)training studies we conclude that the genes for heart rate partly overlap with the genes for exercise behavior because exercise behavior causes a decrease in heart rate.

TABLE 6. Percentage of the phenotypic correlations (see Table 2) between exercise behavior, HP, and RSA explained by genetic or environmental factors (A/E), based on the best fitting models for adolescent and middle-aged twins.

	sqrt(METS)	ln (HP)	ln (RSA)
Adolescent twins			
SportMETS	*		
ln(HP)	84/16	*	
ln(RSA)	87/13	54/46	*
Middle-aged twins			
SportMETS	*		
ln(HP)	88/12	*	
ln(RSA)	22/16	70/30	*

Significant ($P < 0.05$) contributions are in boldface type. Most of the (nonsignificant) phenotypic correlation between sportMETS and RSA in middle-aged twins could be explained by age (62%).

The above conclusion leaves open the additional possibility that genetic pleiotropy and reverse causality further contribute to the association between exercise and resting heart rate. Under favorable circumstances, a large cross-sectional twin sample can be used to resolve causality, i.e., to test whether low heart rate is truly caused by exercise behavior, whether exercise behavior is truly caused by low heart rate, or whether the correlation between exercise behavior and heart rate derives from a common (pleiotropic) genetic factor. The statistical validity of direction of causation tests in twin models depends on a complex interplay between the size of the heritability of the causative and caused traits, the difference in their heritability, the strength of the association between them, availability of estimates of the measurement error in heart rate and exercise behavior, and on the number of twins available (9). The latter seems by far the most limiting factor for extensive use of such models. Only a large sample of hundreds of mono- and dizygotic twins provides enough power to discriminate between a causal and a common genetic factor model.

Potential limitations. The very high MZ correlations (which have an upper bound in the test-retest reliability) suggest acceptable test-retest reliability of our sportMETS score. A limitation is that we focused our assessment of physical activity on moderate to intense exercise behavior in leisure time, more particularly on sports participation. Although we did include activities like recreational cycling, sensitivity of our questionnaire to various other leisure time physical activities may have been suboptimal. This leaves the possibility that RSA and heart rate are determined by nonsports-related physical activity and/or exercise with intensity below 4 METs. Another potential problem is the relatively large number of individuals with a sportMETS score of zero, which made it difficult to transform this variable to normal, necessary for maximum likelihood model fitting. However, the pattern of Spearman rank correlation coefficients, which are not sensitive to distributional assumptions, was very similar to the Pearson twin correlations of sportMETS, confirming the robustness of the genetic effect on exercise behavior.

Although heart rate variability in the respiratory frequency range is often used as an index of tonic vagal contribution to heart rate (30), it should be kept in mind that vagal nerve activity is assessed by the output variable of a complex system passed through a target organ. Individual differences in RSA may result from differences in tonic firing rate of the vagal motor neurons in the nucleus ambiguus but also from differences in which this vagal nerve activity is modulated by baroreceptor and respiratory control centers. Other confounders include cardiac compliance

(influencing the magnitude of how much cardiac filling and thereby stroke volume is altered by respiration), arterial compliance (influencing baroreceptor distension with each heart beat), baroreceptor function, central integration of baroreceptor input, and finally the sensitivity of muscarinic receptors to cholinergic stimulation.

Respiratory behavior, in particular respiration rate, has been shown to be a powerful determinant of RSA (30). Hence, the use of RSA as an index of individual differences in vagal contribution to resting heart rate may only be valid when individual differences in resting respiration rate are taken into account. However, in a previous analysis in the middle-aged twins, we clearly showed that taking respiration rate into account did not alter the estimates of heritability of RSA under either resting and stressful conditions (28). Moreover, the heritability estimates for RSA corrected for RR obtained in that analysis (31%) closely correspond to the values obtained here (29%).

The most serious challenge to using RSA as an index of vagal contribution to resting heart rate comes from the work of Goldberger and colleagues (10). They suggest that ceiling effects, possibly as a consequence of high occupancy of the available muscarinic receptors, prevent the increased acetylcholine release during expiration to linearly decrease the heart rate in highly trained athletes with very low heart rates. Our path analysis would not have properly dealt with a cubic relationship between RSA and HP. However, inspection of the scatter plots did not suggest such a cubic relationship in either age cohort, suggesting that in these populations ceiling effects did not pose a severe problem.

CONCLUSION

Population-based prospective studies showing exercise behavior to predict good health do not exclude the additional possibility that those who choose to exercise in leisure time have better health to begin with, based on their favorable genetic make-up. Although the results of this study do not preclude causal effects of exercise on RSA or heart rate, they show that the association between exercise and these cardiovascular risk factors largely derives from a common genetic factor. This provides empirical support for the long voiced caution that cross-sectional studies may overestimate the influence of exercise training by mixing the effects of genetic endowment with true causality.

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