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Heritability of respiratory sinus arrhythmia: Dependency on task and respiration rate

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Abstract

In this study, we investigated the genetic and environmental origin of individual differences in respiratory sinus arrhythmia (RSA) during rest and during four stress tasks. We used a multivariate model including age, RSA, and respiration rate. Participants were 208 male and female pairs of middle-aged twins. A model without sex differences, specifying additive genetic and unique environmental factors, showed the best fit across all conditions. Heritability of RSA ranged from 28% to 43%. Correction for respiration rate yielded RSA heritabilities of similar size. The covariance between respiration rate and RSA was best explained by a combination of correlated unique environmental and correlated additive genetic factors. Combined with data from an earlier project, RSA from 317 adolescent and 712 middle-aged individuals of both sexes was available. This large data set showed that (a) sex differences in mean RSA are absent and (b) RSA decreases considerably from adolescence (111.5 ms) to middle age (60.0 ms).

Descriptors: Heritability, Twins, Respiratory sinus arrhythmia, Respiration rate, Age, Stress tasks

Repeated increases in heart rate and blood pressure as a consequence of physical and psychological stressors are considered risk factors or indicators for cardiovascular disease (Matthews, Woodall, & Allen, 1993; Menkes et al., 1989). To gain more insight into the role of changes in blood pressure and heart rate in determining cardiovascular health, regulating mechanisms underlying these distant phenotypes must be studied. One of these mechanisms is the influence of the autonomic nervous system on the heart, which consists of activity of both sympathetic and parasympathetic (vagal) efferents that exert opposing effects on the chronotropic state of the heart by their reciprocal influence on the sinoatrial node. Respiratory sinus arrhythmia (RSA) is a specific and sensitive noninvasive index of cardiac vagal tone (Berntson et al., 1994; Cacioppo et al., 1994; Eckberg, 1983; Grossman & Wientjes, 1986; Katona & Jih, 1975; McCabe, Yongue, Ackles, & Porges, 1985). RSA is defined as the magnitude of change in heart period corresponding to the inspiratory and expiratory phases of the respiratory cycle. Heart rate typically increases during inspiration and decreases during expiration. The stronger these variations in heart rate, the larger the RSA and the stronger the vagal control of the heart.

Several studies have shown reduced heart rate variability in cardiac disease (Hayano et al., 1991; Hinkle, Carver, & Plakun, 1972; Kleiger, Miller, Bigger, & Moss, 1987; Martin et al., 1987; Singer et al., 1988) and hypertension (Julius, Pascual, & London, 1971; Malliani et al., 1991). Furthermore, RSA measures are clearly reduced with increasing age (Mølgaard, Hermansen, & Bjerregaard, 1994; Ryan, Goldberger, Pincus, Mietus, & Lipsitz, 1994) and under conditions of psychological stress (Allen & Crowell, 1990; de Geus, van Doornen, de Visser, & Orlebeke, 1990; de Geus et al., 1996; Grossman, Brinkman, & de Vries, 1992; Grossman, Stemmler, & Meinhardt, 1990a; Grossman, van Beek, & Wientjes, 1990b). Ryan et al. (1994) found significant sex differences in heart rate variability; women had slightly higher values than did men. All these facts are compatible with current views that vagal tone protects against heart disease and that high RSA may be regarded as an index of good cardiovascular health.

A striking feature of virtually all studies on RSA is that they report large individual differences in RSA, as indexed by standard deviations, both at rest and during conditions of physical and psychological challenge. Considering the potential relevance of low RSA as a risk indicator, more insight into the genetic and environmental origins of these individual differences is desirable. There has been only one study that has explored this issue (Boomsma, van Baal, & Orlebeke, 1990). In that study, RSA was measured in 160 adolescent twin pairs during a rest period and during two stressful laboratory tasks, which enabled quantification of the genetic and environmental sources of individual differences. During rest, only 25% of the variance in RSA was accounted for by genetic influences. Under task conditions however, around 50% of the variance in RSA could be explained by genetic factors, indicating a stronger genetic contribution to the variance in RSA under stress. This higher heritability could be attributed to a decrease in unique environmental variance during stress as compared with rest. Although not specifically tested, twin correlations did not suggest different heritabilities for boys and girls.

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In the present study, we investigated individual differences in RSA in middle-aged twins during rest and four different stress tasks to estimate genetic and environmental influences on RSA during these conditions. A multivariate model including age, RSA, and respiration rate was used in the quantitative genetic modeling to quantify the genetic and environmental sources of individual differences in RSA in rest and under stress while taking account of the influence of age and respiration rate. Respiration rate was incorporated into the model because various studies have raised the question of to what extent RSA is a valid index of individual differences in vagal control of the heart when the influence of respiratory parameters are not taken into account (Allen & Crowell, 1990; Grossman, Karemaker, & Wieling, 1991; Grossman & Wientjes, 1986; Hayano, Mukai, Hori, Yamada, & Fujinami, 1993; Kollai & Kollai, 1992; Kollai & Mizsei, 1990; Saul, Berger, Chen, & Cohen, 1989). Furthermore, we tested whether the origin of the covariance between RSA and respiration rate could be explained by correlated genetic factors and/or correlated environmental factors. Possible sex differences in the determinants of variance in RSA and covariance between RSA and respiration rate were investigated, and best fitting models at rest were compared with best fitting models under stress conditions.

Methods

Participants

This study is part of a project in which cardiovascular risk factors were studied in 213 middle-aged twin pairs (34–63 years of age). Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry, and solicitation through the Dutch Twin Club. In addition, a small number of pairs who heard of the study elsewhere volunteered to participate. Informed consent was obtained from all individuals. Data from five twin pairs were excluded from the sample. In one twin pair, measurements were incomplete. One pair was dropped because one member of the pair had only one lung, and three pairs were discarded because of extremely high values of RSA in one member of each pair. One monozygotic triplet was included in the sample by discarding the data from the second-born individual. In total 202 men (age, $M \pm SD$, 43.6 ± 6.4 years) and 214 women (44.7 ± 6.8 years) were included in the study. In 76 same-sex twin pairs, zygosity was determined by DNA fingerprinting (Jeffreys, Wilson, & Thein, 1985). In the remaining 94 same-sex twin pairs, zygosity was determined by questionnaire items about physical similarity and frequency of confusion by family and strangers during their childhood (Goldsmith, 1991). Classification of zygosity in these 94 same-sex twin pairs was based on a discriminant analysis, relating the questionnaire items of the 76 same-sex pairs to their zygosity based on DNA fingerprinting. In that sample, zygosity was correctly classified in 98.7% of the cases. One dizygotic pair was mistakenly classified as monozygotic. Grouped according to their zygosity and sex, the sample consisted of 43 pairs of monozygotic men (MZM), 39 pairs of dizygotic men (DZM), 48 pairs of monozygotic women (MZF), 40 pairs of dizygotic women (DZF), and 38 dizygotic pairs of opposite sex (DOS).

Procedure

Twins always came in pairs and arrived at the laboratory at about 10:00 a.m. They were asked to refrain from smoking and drinking alcohol, coffee, or tea after 11:00 p.m. the night before. Electrodes were attached for electrocardiogram (EKG) recording, and a strain-gauge was strapped around the waist to measure respiration. Participants were measured during rest, three mental stress task conditions, and a physical stressor (the cold pressor test). Mental stress tasks consisted of a choice reaction time task, a speeded mental arithmetic task, and a tone avoidance reaction time task. The reaction time and mental arithmetic task were included to ensure compatibility with a previous twin study from our laboratory in which these tasks evoked significant cardiovascular responses (Boomsma et al., 1990). The tone avoidance task was added to the design because it was expected to be an even more powerful stressor (de Geus et al., 1990). As opposed to the mental stressors, which mainly invoke cardiac responses, the cold pressor test results in a strong vascular reaction pattern (de Geus et al., 1990). Blood pressure responses to these tasks in the same participants were reported previously (Snieder, van Doornen, & Boomsma, 1995).

Each mental task condition lasted 8.5 min and was preceded by a rest period of 3 min. During testing, participants were comfortably seated in a reclined position in a dimly lit, sound-shielded testing chamber. They faced a computer screen placed 2 m in front of them and used a panel with four buttons to respond to the mental stress tasks with their preferred hand. Auditory stimuli were binaurally presented through padded earphones.

The first task was a reaction time task. Each trial started with the simultaneous onset of an auditory stimulus and the appearance of a vertical bar on the computer screen, which lowered gradually, until after 5 s it had disappeared completely and simultaneously a second auditory stimulus was heard, which was either higher or lower in pitch than the first tone. Participants had to react to high tones by pressing a panel button labeled *Yes* and to low tones by pressing a button labeled *No*. Two seconds later, participants received feedback on the computer screen indicating whether they had pushed the correct button and, for correct responses, their reaction time. The task consisted of 36 trials.

The second task was a mental arithmetic task. In this task twins were asked to add three numbers that were presented in succession on the computer screen. Five seconds after the first number appeared, the answer to the addition problem appeared on the screen. Half of the presented answers were correct and half were incorrect. Participants were required to press the *Yes* button if the presented answer was correct and the *No* button if it was incorrect. They received the same feedback as in the reaction time task, and after 2 more seconds the next trial was started. The mental arithmetic problems contained 10 levels of difficulty, ranging from three 1-digit numbers (e.g., 9 + 5 + 4) to three 2-digit numbers (e.g., 85 + 79 + 47). The mental arithmetic task also consisted of 36 trials.

The third task was a tone avoidance task. During the tone avoidance task, the twins were asked to attend the occurrence of a stimulus (an "X") that appeared briefly (500 ms) in one of the corners of the computer screen. They were to respond as quickly as possible to this stimulus by pressing the button opposite to this corner on their response panel. Incorrect or slow responses were punished with a loud noise burst that lasted 500 ms. Apart from the noise, there was an extra penalty. After every two consecutive mistakes, the original score of 500 points was reduced by 10 points. However, if they made five consecutive correct responses, the score was increased by 20 points, with a maximum score of 500 points. The foreperiod varied randomly between 500 and 1,500 ms. Reaction time had to be shorter than the response criterion, which was initially set to 650 ms. During the task, the response criterion was continuously adapted to the performance of the participant. The task was thus made more demanding if the participant started to perform better, so that the task would remain

equally stressful for all participants. The whole task consisted of about 210 trials.

Before the actual start of the experiment, participants were offered a training session during which reaction time and tone avoidance tasks were practiced. The mental arithmetic task was practiced in its entirety to determine the level of difficulty at which the participant would begin the mental arithmetic task during the actual experiment. The first problem for the mental arithmetic practice session always was at the first level of difficulty. The level of the next problems depended on the responses. The level reached after the 36 trials determined the level at which the participant started during the experimental performance of the mental arithmetic task. This procedure was developed so that the mental arithmetic task would be equally stressful for all participants.

After performance of the three mental stress tasks, basal levels of the cardiorespiratory variables were determined during an 8.5-min poststress rest period. During all rest periods, participants were asked to relax as much as possible. The poststress rest period was followed by a cold pressor test. The participants were told to immerse the preferred hand in ice water and keep it there for 2 min. Participants changed places in the testing chamber several times. While one twin was being tested, the other twin filled in questionnaires. Sequence of events was practice sessions, pause (outside the testing chamber), Rest 1, reaction time task, Rest 2, mental arithmetic task, Rest 3, tone avoidance task, another break (again outside the testing chamber), poststress rest, and cold pressor test.

Physiological Variables

Disposable pregelled Ag-AgCl EKG 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 respiration signal was recorded with a strain-gauge of hollow silastic tube strapped around the waist 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 chest circumference. This system has yielded reliable estimates of respiratory activity (Grossman et al., 1992).

The EKG and the respiration trace were displayed on a Beckman Dynograph (R611) and sampled continuously at 250 Hz via a 12-bit A-D converter. The EKG signal was recorded using a Nihon Kohden bioelectric amplifier (AB 601G) with a time constant of 0.1 s and a 30-Hz high cutoff filter. The respiration signal was collected DC and filtered by a 30-Hz cutoff filter.

EKG data were used to determine the time between successive R waves. The respiration signal was computer scored to obtain total expiration time (TTE) and total inspiration time (TTI) on a breath-to-breath basis. TTI was computed as the sum of the inspiration period and the inspiratory pause. TTE as the sum of the expiration period and the expiratory pause. Total cycle time (the sum of all four intervals) was recoded to respiration rate, expressed in cycles per minute. All breaths in a condition were averaged to yield mean values of respiratory variables for that condition. Automatic scoring of respiratory variables was checked by visual inspection of all respiratory signals in all conditions. All breathing cycles that showed irregularities such as gasps, breath holding, and coughing were rejected and removed from further processing.

Respiratory sinus arrhythmia was computed by the peak-to-trough method (Grossman & Wientjes, 1986). Although some disagreement has arisen over which method best indexes RSA (Grossman, 1992; Porges & Byrne, 1992), very high correspondence (interindividual correlations >.92) has been found between the peak-to-trough method and spectral measurements of RSA (Grossman et al., 1990b). The peak-to-trough method combines the respiratory time intervals and the interbeat intervals to obtain the shortest interbeat interval during heart rate acceleration in the inspirational phase (which included 750 ms from the following expiration to account for phase shifts) and the longest interbeat interval during deceleration in the expirational phase (including 750 ms from the following expiratory pause/inspirational 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 an RSA score of zero. Mean RSA (in milliseconds) was computed for poststress rest and task conditions by averaging the RSA values of all breaths falling within that condition.

Reactivity was calculated as the difference between RSA or respiration rate means during the tasks and during the poststress rest condition.

Statistical Analysis

To test for the effects of stress tasks, sex, and zygosity on respiration rate and RSA, a multivariate analysis of variance (MANOVA) was used. Respiration rate and RSA were analyzed as dependent variables with condition (rest, reaction time, mental arithmetic, tone avoidance, cold pressor) as within-individual variables and sex and zygosity as between-individuals variables. Because measurements in twins are not independent, a correction was made for the dependency of the observations in twins. The residual degrees of freedom for the F test were taken as half those available. This adjustment is conservative because dizygotic twins share on average only 50% of their genetic material. Tukey's HSD was used to follow up on significant main and interaction effects ($ps < .05$). To obtain a normal distribution, RSA was transformed by natural logarithm.

The (univariate) twin model Quantitative genetics is concerned with sources of individual differences in phenotypes. The phenotype is considered the sum of the effects of both the genotype and the environment. To gather insight into the genetic and environmental influences on the variance of the phenotype, data from individuals genetically informative are needed. Twins have often been used for these studies (Hewitt & Turner, 1995; Plomin, DeFries, & McClearn, 1990; Rao & Vogler, 1994). Monozygotic (MZ) twins share identical genotypes, so any differences between them are due to their environments. Dizygotic (DZ) twins, in contrast, are no more alike genetically than siblings, sharing on average 50% of their segregating genes. It is assumed that both types of twins share roughly the same environmental influences: the equal environment assumption. Although there has been some criticism of the equal environment assumption (e.g., Phillips, 1993), most studies specifically carried out to test it have supported its validity. Even if shared environment differentially affects MZ and DZ twins, it is unlikely that this difference has a substantial effect on the trait under study (Braun & Caporaso, 1993; Duffy, 1993; Kendler, Neale, Kessler, Heath, & Eaves, 1993; Leslie & Pyke, 1993; Macdonald, 1993; Plomin et al., 1990). The extent to which MZ twins are more alike than DZ twins should, therefore, reflect the importance of genetic influences. In the classic twin method, the difference between intraclass correlations for MZ twins and those for DZ twins is doubled to estimate heritability: $h^2 = 2(r_{MZ} - r_{DZ})$ (Falconer, 1989). The remaining population variance can then be attributed to environmental factors. Estimates of genetic and environmental effects based on comparisons of intraclass

correlations, however, have low power and large standard errors and do not make use of information available in variances and covariances. In recent years, genetic model fitting has become standard in twin research (Boomsma & Molenaar, 1986; Heath, Neale, Hewitt, Eaves, & Fulker, 1989; Neale & Cardon, 1992). Model fitting approaches basically involve solving a series of simultaneous structural equations to estimate genetic and environmental parameters that best fit the observed twin variances and covariances. Model fitting analyses of twin data have some major advantages over the classic twin methodology: (a) models make assumptions explicit, (b) a test of the goodness of fit of the model is provided, (c) estimates of quantitative genetic parameters and their confidence intervals are given, (d) the fit of alternative models can be compared, (e) more than two groups of twins can be analyzed simultaneously, and (f) generalization from the analysis of one variable (univariate) to multiple variables (multivariate) is relatively easy.

Genetic model fitting of twin data allows the separation of the observed phenotypic variance into its genetic and environmental components. This variance can be decomposed into several contributing factors. Additive genetic variance (V_G) is the variance that results from the additive effects of alleles at each contributing locus. Dominance genetic variance (V_D) is the variance that results from the nonadditive effects of two alleles at the same locus summed over all loci that contribute to the variance of the trait. Shared (common) environmental variance (V_C) is the variance that results from environmental events shared by both members of a twin pair (e.g., rearing, school, neighborhood, diet). Specific (unique) environmental variance (V_E) is the variance that results from environmental effects that are not shared by members of a twin pair and includes measurement error. Age can spuriously introduce a common environmental effect if the age range of the twin sample is broad and there is a significant correlation between phenotype and age. By incorporating age into the model, the influence of age on the phenotype can be quantified (Neale & Cardon, 1992; Neale & Martin, 1989). The genetic model can be represented by the following linear structural equations:

$$P_i = hG_i + dD_i + cC_i + eE_i + aA_i \quad (1)$$

$$V_P = h^2 + d^2 + c^2 + e^2 + a^2, \quad (2)$$

where P is the phenotype of the ith individual, scaled as a deviation from zero, G is additive genetic influence, D is dominance genetic influence, C is common (or shared) environmental influence, E is unique (or specific) environmental influence, and A is age. G, D, C, and E can be conceived of as uncorrelated latent factors with zero mean and unit variance; h, d, c, e, and a are regression coefficients of the observed variable on the latent factors, and they indicate the degree of relationship between latent factors and the phenotype. V_P is the phenotypic variance. Squaring the regression coefficients yields the (unstandardized) variance components ($V_G = h^2$, $V_D = d^2$, $V_C = c^2$, $V_E = e^2$, $V_A = a^2$), whose sum is equal to the total phenotypic variance. The contributions of genes, environment, and age to the total variance are often reported in their standardized form. This standardization is done by dividing the specific variance component by the total phenotypic variance (e.g., $h^2 = V_G/V_P$, where h^2 = heritability).

For MZ twins, correlations between the additive and dominance genetic factors between twin and co-twin are unity. For DZ twins, these values are 0.5 and 0.25, respectively. By definition, in both MZ and DZ same-sex pairs, correlations are unity between common environmental factors and zero between specific environmental factors. The model further assumes random mating and absence of gene–environment interaction and gene–environment correlation.

In twin studies, the effects of D and C are confounded, which means that they cannot both be included in the same univariate model. However, D and C have opposite effects on the patterns of MZ and DZ twin correlations. D tends to produce DZ twin correlations that are <50% the MZ twin correlations and C inflates the DZ correlation to be >50% the MZ correlation. Models constraining all genetic effects to be nonadditive are considered unlikely because they lack a sensible biological interpretation (Neale & Cardon, 1992).

Investigation of sex effects For the study of sex differences within a twin design, same-sex male and same-sex female MZ and DZ twin pairs and opposite-sex DZ twin pairs must be incorporated. The existence of sex differences in the influences of genetic and environmental factors on the phenotype can take several forms (Reynolds & Hewitt, 1995). First, genetic or environmental influences may differ in kind between males and females. In this case, correlations in the DZ opposite-sex twin pairs between the latent genetic or shared environmental factors will be smaller than the normal values of 0.5 and 1, respectively. Second, the same genetic and environmental effects may be present in males and females, but the magnitudes of those components may differ. Such a common-effects model can be tested by comparing a 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.

Extension to a multivariate model Although univariate genetic analysis provides estimates of the contributions of additive and nonadditive genetic effects and shared and unique environmental effects to variation in the measured phenotype, it tells nothing about factors that make sets of variables covary to a greater or lesser extent. Multivariate genetic modeling involves a decomposition of phenotypic variances and makes it possible to determine to what extent the covariation between multiple measures is due to genetic and/or environmental factors (Heath et al., 1989; Neale & Cardon, 1992). Multivariate models can thus be an important aid in unraveling the sources of interrelations between multiple variables. We therefore specified a multivariate model including RSA, respiration rate, and age that allowed us to quantify genetic and environmental influences on RSA and to take into account the influence of age and respiration rate. This model also enabled us to test whether the covariance between RSA and respiration rate was due to correlated genetic factors and/or correlated environmental factors.

Genetic Analysis

For the genetic analyses, age, respiration rate, and RSA measured in twin and co-twin were summarized into 5 × 5 variance–covariance matrices (using PRELIS; Jöreskog & Sörbom, 1986) for each of the five zygosity groups. Models were fitted to these variance–covariance matrices by the method of maximum likelihood, using Mx (Neale, 1994). Mx provides parameter estimates (h, d, c, e, a), a chi-square test of the goodness of fit of the model, and the Akaike's information criterion (AIC). The overall chi-square test measures the agreement between the observed and predicted variances and covariances in the different zygosity groups. A large chi-square value indicates a poor fit (low p value), and a small chi-square value indicates that the model is consistent with

the data (high p value). Submodels were compared by hierarchic chi-square tests, in which the chi-square value for a reduced model is subtracted from that of the full model. The degrees of freedom for this test are equal to the difference between the degrees of freedom for the full and the reduced model. Another purpose of the model-fitting procedure is to explain the pattern of covariances and variances by using as few parameters as possible. Therefore, AIC (calculated as $\chi^2 - 2df$) was used to evaluate the fit of the models. The model with the lowest AIC reflects the best balance of goodness of fit and parsimony (Neale & Cardon, 1992).

The applied multivariate model including RSA, respiration rate, and age is presented in Figure 1. The observed phenotypes for Twin 1 and Twin 2 are shown in squares, and latent factors are shown in circles. Latent factors are subdivided into genetic and environmental factors common to respiration rate and RSA (G_c, E_c) and genetic and environmental factors specific for RSA (G_s, E_s). The genetic part of the covariance between respiration rate and RSA ($h_c * h_c'$) divided by the square root of the product of the total additive genetic variance components of respiration rate ($V_G rr$) and RSA ($V_G rsa$) yields the genetic correlation between respiration rate and RSA. Accordingly, the environmental part of the covariance ($e_c * e_c'$) divided by the square root of the product of the total unique environmental variance components of respiration rate ($V_E rr$) and RSA ($V_E rsa$) yields the environmental correlation between respiration rate and RSA. Correlations between the latent genetic factors (r_{Gc} and r_{Gs}) are 1 in MZ twins and 0.5 in DZ twins. Regression coefficients of observed variables on the different latent factors are also shown. This model enabled us to differentiate between the total genetic influence on RSA, h^2_{tot}, calculated as $(h_c'^2 + h_s^2)/V_P rsa$, and the genetic influence on RSA, corrected for influence of respiration rate, h^2_{cor}, calculated as $h_s^2/(h_s^2 + e_s^2)$.

Sex differences were examined by comparing the full model, in which parameter estimates are allowed to differ in magnitude between men and women, with a reduced model, in which parameter estimates are constrained to be equal across the sexes.

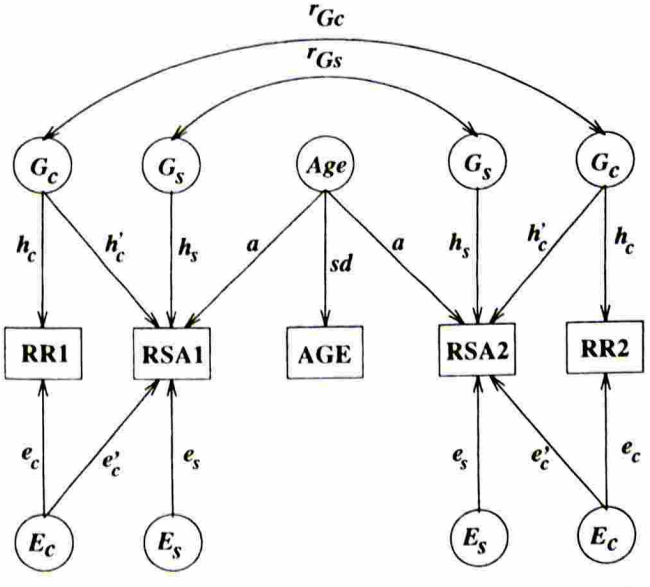

Figure 1. Multivariate model that includes RSA, respiration rate (RR), and age. The path between age and RR is not shown because it could be constrained to zero. Observed phenotypes for Twin 1 and Twin 2 are shown in squares. Latent factors are shown in circles. G_c and E_c reflect genetic and environmental influences common to RR and RSA. G_s and E_s reflect genetic and environmental influences specific for RSA. r_{Gc} and r_{Gs} are the correlations between the latent genetic factors, which are 1 in MZ twins, 0.5 in DZ same-sex twins, and can be estimated for DOS twins. Regression coefficients of observed variables on the different latent factors are also shown: h_c' = additive genetic effect common to RR and RSA, h_s = additive genetic effect specific for RSA, e_c' = unique environmental effect common to RR and RSA, e_s = unique environmental effect specific for RSA, h_c = additive genetic effect on RR, e_c = unique environmental effect on RR, a = influence of age on RSA, sd = standard deviation of age. Although models including C (ACE) and D (ADE) were also tested, C and D are not shown in the figure for reasons of clarity.

Results

Figure 2a shows means of RSA (±standard error of the mean [*SEM*]) in rest and during the four different stress tasks in men and women. A MANOVA of Condition (5) × Sex (2) × Zygosity (2) demonstrated a highly significant main effect of condition, $F(4,200) = 136.03$, $p < .001$, in the expected direction; post hoc testing of the condition means showed that RSA decreased during both the mental stressors and the cold pressor test. No main effects of sex and zygosity were found. One significant interaction was observed between sex and condition, $F(4,200) = 3.49$, $p = .009$. Post hoc testing revealed that this interaction was due to the RSA levels being more similar between the sexes during the cold pressor test than during the rest and reaction time condition.

Figure 2b shows means of respiration rate (±*SEM*) in rest and during the four different stress tasks in males and females. Also for respiration rate, a MANOVA of Condition (5) × Sex (2) × Zygosity (2) demonstrated a highly significant condition effect in the expected direction, $F(4,200) = 477.58$, $p < .001$; post hoc testing showed that respiration rate increased under all stress conditions. No other main effects were found. The only significant interaction observed was between sex and condition, $F(4,200) = 2.92$, $p = .022$. Post hoc testing revealed that this interaction was due to the respiration rate levels being more similar between the sexes during the reaction time and mental arithmetic tasks than during the cold pressor test.

In Table 1, interindividual cross correlations between age, RSA, and respiration rate are presented for men and women. Relatively high negative correlations between age and RSA and between respiration rate and RSA were found for both sexes in all conditions. No correlation was observed between age and respiration rate.

By comparing MZ and DZ twin correlations, a first impression of the magnitude of genetic influence can be obtained. A higher MZ than DZ twin correlation points to a genetic influence, which is based on the classic formula to estimate heritability: $h^2 = 2(r_{MZ} - r_{DZ})$ (Falconer, 1989). Twin correlations of RSA and respiration rate in rest and stress task conditions are shown in Table 2. In general, MZ correlations are larger than DZ correlations for both RSA and respiration rate, indicating a genetic influence. Correlations for RSA in the DOS group are relatively high, and correlations for RSA during mental stress in the DZF group were often not different from zero. Twin correlations listed in this table point to a moderate heritability for RSA and a somewhat larger heritability for respiration rate.

Model fitting showed that in all conditions the best-fitting model by AIC allowed for additive genetic and unique environmental effects, age influences on RSA only, and no sex differences in parameter estimates. Chi-square and p values of best-fitting models are shown in Table 3.

To get insight into causes of changes in standardized parameter estimates (e.g., heritabilities) due to exposure to stress conditions,

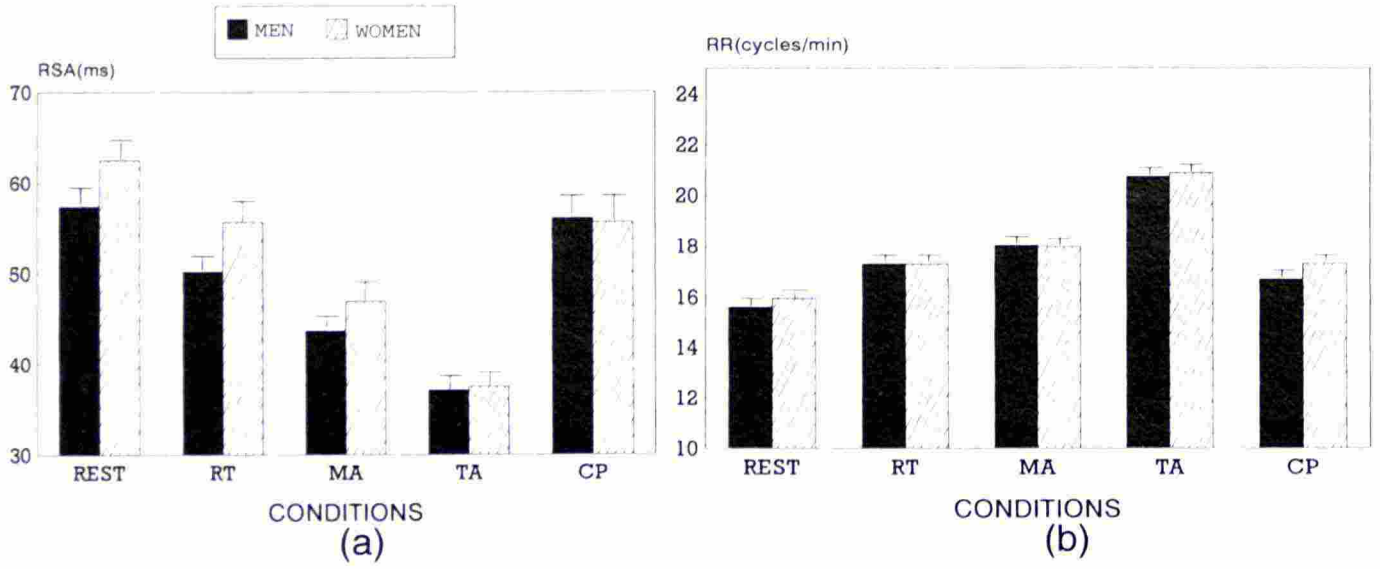

Figure 2. Means (±*SEM*) of RSA (a) and respiration rate (RR) (b) at rest and during the four different stress tasks in men and women. RT = reaction time task; MA = mental arithmetic task; TA = tone avoidance task; CP = cold pressor test.

nonstandardized variance components underlying these standardized estimates must be evaluated. Table 4 shows nonstandardized variance components of RSA and respiration rate as estimated by the best-fitting models for rest and stress conditions. Under all mental stress conditions, the total phenotypic variance of RSA decreased as compared with rest. In the reaction time and mental arithmetic task, an increase in total genetic variance of RSA accompanied a decrease in total unique environmental variance, resulting in a larger (total) heritability during these tasks as compared with rest. This pattern was reversed in the tone avoidance task, where $V_E rsa$ increased and $V_G rsa$ decreased, leading to a smaller (total) heritability for this task as compared with rest. These heritabilities of RSA and respiration rate and their 95% confidence intervals are also shown in Table 4. The 95% confidence intervals allow evaluation of the accuracy of the estimates. Heritability of respiration rate (h_c^2) did not change much over conditions; it ranged from .51 in the mental arithmetic task to .62 at rest. Total heritability of RSA, $h_{tot}^2 = (h_c'^2 + h_s^2)/V_P rsa$, varied between 28% (tone avoidance task) and 43% (mental arithmetic task). With the exception of the heritability of RSA during the tone avoidance task, the estimates suggest that heritabilities were higher during the stress tasks as compared with rest. However, confidence intervals of heritability estimates show considerable overlap during all conditions.

To investigate the origin of the covariance between respiration rate and RSA (Table 1), we tested whether one or both of the connecting paths between respiration rate and RSA (h_c', e_c') could be set to zero. Results of this test are presented in Table 3. In none of the conditions could the connecting paths be set to zero without a significant loss of fit, which suggests that the covariance between respiration rate and RSA has both a genetic and an environmental basis. To further determine the background of the correlation of respiration rate with RSA during rest and stress conditions, we need to examine standardized estimates of the different sources of variance that contribute significantly to RSA. According to the best-fitting models, five different sources of variance in RSA could be discriminated, each with a significant influence. These sources are presented in Table 5 (but see also Figure 1). From these parameters, we computed estimates of h_{cor}^2 ($=h_s^2/[h_s^2 + e_s^2]$), a measure of the relative contribution of genetic influence on RSA corrected for influence of respiration rate. As can be seen by comparison with Table 4, estimates of h_{cor}^2 were very similar to those uncorrected for the influence of respiration rate (h_{tot}^2) in all conditions. From 14% (rest) to 21% (mental arithmetic task and cold pressor test) of the variance in RSA could be explained by the influence of respiration rate ($h_c'^2 + e_c'^2$), whereas the influence of age varied between 8% (mental arithmetic and tone avoidance task) and 15% (rest). Table 5 also shows genetic (r_g) and environmental (r_e) correlations between respiration rate and RSA. The r_g and r_e values have the same sign (negative) and a comparable range

Table 1. *Interindividual Cross Correlations for Men (Below Diagonal; n = 202) and Women (Above Diagonal; n = 214)*

	Rest			RT			MA			TA			CP		
	Age	RSA	RR	Age	RSA	RR	Age	RSA	RR	Age	RSA	RR	Age	RSA	RR
Age		−.34	.01		−.34	.07		−.31	.12		−.28	.07		−.33	.09
RSA	−.43		−.35	−.36		−.42	−.34		−.49	−.32		−.45	−.39		−.50
RR	.02	−.39		−.04	−.37		.03	−.46		.02	−.39		.00	−.45	

Note. RT = reaction time task; MA = mental arithmetic task; TA = tone avoidance task; CP = cold pressor test; RR = respiration rate.

Table 2. *Twin Correlations of RSA and Respiration Rate in Rest and Stress Task Conditions*

		RSA					RR				
Group[a]	n^b	Rest	RT	MA	TA	CP	Rest	RT	MA	TA	CP
MZM	43	.45	.42	.41	.33	.45	.62	.57	.51	.49	.61
DZM	39	.20	.07	.12	−.09	.22	.12	.45	.47	.37	.20
MZF	48	.47	.62	.62	.48	.56	.72	.68	.54	.62	.55
DZF	40	.35	−.11	−.04	−.03	.19	.07	.23	.29	.24	.12
DOS	38	.26	.40	.57	.46	.47	.27	.03	.14	.10	.12

Note: RT = reaction time task; MA = mental arithmetic task; TA = tone avoidance task; CP = cold pressor test; RR = respiration rate.
[a]MZM = monozygotic men; DZM = dizygotic men; MZF = monozygotic women; DZF = dizygotic women; DOS = dizygotic opposite sex. [b]*n* = number of twin pairs. For the analyses of some tasks, *n* drops by no more than two pairs in some twin groups.

(from −.36 to −.50) and show that genetic and environmental sources influencing respiration rate and RSA overlap.

Reactivity of RSA and respiration rate to the stress tasks was calculated as the difference between mean levels of these variables during the tasks and the poststress rest condition. Table 6 shows twin correlations and Table 7 shows model fitting results for RSA and respiration rate reactivity to the tone avoidance task. No meaningful systematic pattern could be observed for the twin correlations of reactivity of RSA and respiration rate in any of the other tasks, precluding further genetic analysis. In a few instances, DZ twin correlations of respiration rate reactivity, for example, were higher than MZ twin correlations. We therefore decided to restrict our analyses to the tone avoidance task, which showed the strongest reactivity in most participants. As indicated by the low *p* values, even for this task models showed a bad fit, which suggests that results must be viewed with some caution. The relatively best fitting model allowed for additive genetic and unique environmental effects and differences in parameter estimates between men and women. The unique environmental connecting path between respiration rate and RSA reactivity (e_c') could be set to zero without a significant loss of fit, which suggests that the covariance between the change in respiration rate and the change in RSA in response to the tone avoidance task can be explained by genetic factors only. Heritability of respirator rate (h_c^2) was .22 in men and .54 in women. Heritability of RSA reactivity (h_{tot}^2) was small in both men (.24) and women (.33). This small genetic influence is almost entirely mediated by genes that are common to both RSA and respiration rate reactivity, as is indicated by the low values of heritability of RSA reactivity after correction for task-induced changes in respiration rate (h_{cor}^2: men = .02; women = .16).

Discussion

In this study, we examined the sources of individual differences in RSA at rest and under four different stress conditions. A multivariate model including age, respiration rate, and RSA was used to analyze the data. This model enabled us to estimate the size of the influence of age and respiration rate on RSA and to subdivide genetic and environmental influences into parts common and specific to respiration rate and RSA.

Models specifying only additive genetic and unique environmental factors, with parameter estimates constrained to be equal in men and women, gave the best explanation of the data, both at rest and under stress conditions. Total genetic influence on RSA (h_{tot}^2) varied between 28% (tone avoidance task) and 43% (mental arithmetic task), whereas correction of the heritability for influence of respiration rate did not make much of a difference: h_{cor}^2 varied from 32% in the tone avoidance task to 48% in the mental arithmetic task. Values at rest were intermediate for both h_{tot}^2 (31%) and h_{cor}^2 (34%).

In spite of the age differences in the twin samples (adolescents compared with middle-aged individuals in the present study), Boomsma et al. (1990) also found that the best explanation of the data was offered by a model specifying additive genetic and unique environmental factors, without sex differences. Boomsma et al.

Table 3. *Chi-square Values for Models Including Additive Genetic and Unique Enviromental Effects Without Sex Differences Testing for the Origin of Covariance Between Respiration Rate and RSA*

		Rest		RT		MA		TA		CP	
Model[a]	*df*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
h_c' and e_c' free	67	96.16[b]	.011[b]	80.14[b]	.130[b]	72.50[b]	.302[b]	63.31[b]	.605[b]	90.38[b]	.030[b]
$h_c' = 0$	68	107.05	.002	85.62	.073	82.50	.111	68.85	.449	102.49	.004
$e_c' = 0$	68	113.17	<.001	111.79	.001	106.32	.002	90.99	.033	118.96	<.001
h_c' and $e_c' = 0$	69	165.87	<.001	169.28	<.001	181.21	<.001	148.19	<.001	198.06	<.001

Note: RT = reaction time task; MA = mental arithmetic task; TA = tone avoidance task; CP = cold pressor test.
[a]h_c' = additive genetic regression coefficient common to respiratory rate (RR) and RSA; e_c' = unique environmental regression coefficient common to RR and RSA. [b]Most parsimonious solution.

Table 4. *Nonstandardized Variance Components and Total Heritabilities of RSA and Respiration Rate as Estimated by the Best-Fitting Models*

	RSA						RR				
Condition[a]	V_G	V_E	V_A	V_P	h^2_{tot}	95% CI	V_G	V_E	V_P	h^2_c	95% CI
Rest	.0844	.1471	.0406	.2721	.31	.17–.44	4.38	2.65	7.03	.62	.49–.73
RT	.0887	.1320	.0298	.2505	.35	.19–.50	4.80	3.14	7.94	.60	.46–.71
MA	.1104	.1243	.0217	.2565	.43	.27–.57	3.96	3.88	7.84	.51	.36–.62
TA	.0760	.1703	.0215	.2678	.28	.11–.44	5.57	4.66	10.23	.54	.40–.66
CP	.1224	.1464	.0392	.3080	.40	.25–.53	5.40	4.82	10.22	.53	.38–.65

Note: RR = respiration rate; V_G = total variance due to additive genes; V_E = total variance due to unique environment; V_A = total variance due to age; V_P = total variance of the phenotype; h^2_{tot} = total heritability of RSA; h^2_c = total heritability of RR; 95% CI = 95% confidence interval of heritability estimates.
[a]RT = reaction time task; MA = mental arithmetic task; TA = tone avoidance task; CP = cold pressor test; RR = respiration rate.

(1990) did not quantify the influence of age and respiration rate on RSA. Because of the restricted age range in their adolescent twin sample, it was not necessary to account for the influence of age. As respiration rate was not incorporated into the model fitting, heritabilities in the adolescent twin study can best be compared with estimates of total genetic influence (h^2_{tot}) in the present study. During rest, heritabilities were similar in magnitude in both studies (25% vs. 31%). Heritabilities for the reaction time and mental arithmetic tasks (the same tasks as in this study) were approximately 50% in adolescents, slightly higher than estimates in the middle-aged twins (35% for reaction time and 43% for mental arithmetic). Boomsma et al. (1990) thus found a higher heritability of RSA during the reaction time and mental arithmetic tasks than during rest. We had speculated that heritability would further increase as the task became more stressful. For this reason, we introduced the tone avoidance task, which was known to yield strong RSA reactivity (de Geus et al., 1990). However, the large overlap in confidence intervals of estimates during rest and all task conditions indicated that differences in RSA heritabilities among conditions were not significant in this study.

Table 5. *Standardized Estimates of Sources of RSA Variance, RSA Heritability Corrected for Respiration Rate, and Environmental and Genetic Correlations of Best-fitting Models*

	Sources of RSA variance							
Condition[a]	h'^2_c	h^2_s	e'^2_c	e^2_s	a^2	h^2_{cor}	r_e	r_g
Rest	.07	.24	.07	.47	.15	.34	−.37	−.46
RT	.04	.31	.14	.39	.12	.44	−.52	−.36
MA	.09	.34	.12	.36	.08	.48	−.50	−.46
TA	.04	.24	.14	.50	.08	.32	−.47	−.41
CP	.10	.30	.11	.37	.13	.45	−.47	−.50

Note: h'^2_c = additive genetic variance common to respiratory rate (RR) and RSA; h^2_s = additive genetic variance specific for RSA; e'^2_c = unique environmental variance common to RR and RSA; e^2_s = unique environmental variance specific for RSA; a^2 = variance of RSA attributed to age; h^2_{cor} = additive genetic variance in RSA corrected for RR; r_e = environmental correlation between RSA and RR; r_g = genetic correlation between RSA and RR.
[a]RT = reaction time task; MA = mental arithmetic task; TA = tone avoidance task; CP = cold pressor test.

In all conditions, the path from age to respiration rate could be set to zero, which corresponded to the absence of interindividual cross correlations between age and respiration rate. Age explained between 8% and 15% of the variation in RSA in different conditions within our age cohort. This result is in agreement with the known decrease in RSA with age (Mølgaard et al., 1994; Ryan et al., 1994), which is said to reflect a loss with age of flexibility of the nervous system in response to environmental demands (Porges & Byrne, 1992). In the study of Boomsma et al. (1990), both adolescent twins and their parents were evaluated, although these data were not published. The parents of the twins were similar in age to the middle-aged twins in the present study. Table 8 lists means and standard deviations of age and RSA for these three participant groups. Resting RSA values in parents were highly similar to the values in the middle-aged twins but were considerably lower than the RSA values of their twin children. The same pattern was found for RSA values during reaction time and mental arithmetic tasks (data not shown). In adolescence as well as in middle age, sex differences in mean RSA are small and nonsignificant (Table 8). This finding contrasts with results from a recent study in a much smaller sample (Ryan et al., 1994), for which a slightly higher heart rate variability was found in adult women. By combining resting RSA values of participants in both data sets (including 317 adolescent and 712 middle-aged individuals) in one figure (Figure 3), we were able to get a clearer picture of the age-induced fall in RSA. There is a steep decline in RSA from adolescence to the fourth decade, followed by a more gradual decline in RSA from middle to older age. Without longitudinal

Table 6. *Twin Correlations for Reactivity of RSA and Respiration Rate to the Tone Avoidance Task*

Group[a]	RSA	RR
MZM	.21	−.07
DZM	.19	.24
MZF	.38	.48
DZF	.24	.22
DOS	.00	.27

Note: Reactivity measured as stress level − rest level.
[a]MZM = monozygotic men; DZM = dizygotic men; MZF = monozygotic women; DZF = dizygotic women; DOS = dizygotic opposite sex.

Table 7. *Model Fitting Results for Reactivity of RSA and Respiration Rate to the Tone Avoidance Task*

Model[a]	*df*	χ^2	*p*
h_c' and e_c' free	60	113.18	<.001
$h_c' = 0$	62	121.01	<.001
$e_c' = 0$	62	118.75[b]	<.001[b]

Note: Reactivity measured as stress level − rest level.
[a] h_c' = additive genetic regression coefficient common to RR and RSA reactivity; e_c' = unique environmental regression coefficient common to RR and RSA reactivity. [b] Most parsimonious solution.

evidence, we cannot tell whether the asymptotic relation between age and RSA reflects a true asymptotic relationship or a cohort effect.

Interindividually, a negative correlation was found between respiration rate and RSA. In the five experimental conditions, 14–21% of the interindividual variance in RSA could be explained by the influence of respiration rate. This finding is in accordance with those of many other studies documenting a significant covariance (correlation coefficients between −.30 and −.70) between respiration rate and RSA at rest and during various stress tasks (de Geus, Willemsen, Klaver, & van Doornen, 1995; de Geus et al., 1996; Kollai & Kollai, 1992; Kollai & Mizsei, 1990). The empirical connection between RSA and respiration rate, for which many plausible physiological explanations have been put forward (Berntson, Cacioppo, & Quigley, 1993; Grossman, 1983; Porges, 1995), has led to the idea that RSA should be corrected for interindividual variations in respiration rate to be a valid index of cardiac vagal tone (Grossman et al., 1991). However, two recent studies have suggested that it may be more viable to use a combination of heart period and RSA to predict cardiac vagal tone (Cacioppo et al., 1994; Grossman & Kollai, 1993). Also, a theory recently proposed by Porges (1995) suggests that RSA may covary with only one of two different types or sources of vagal tone. In combination with the ongoing discussion on the method of quantification of RSA (Grossman, 1992; Porges & Byrne, 1992), the use of respiration rate to improve estimation of vagal tone remains unclear. In this study, we observed that heritabilities of RSA are similar whether they are corrected for respiration rate or not. This finding suggests that RSA correction in other between-subject designs may not be necessary to investigate individual differences.

Various studies have shown that RSA has predictive value for cardiac disease (Hayano et al., 1991; Hinkle et al., 1972; Kleiger et al., 1987; Martin et al., 1987; Singer et al., 1988). In this study, individual differences in RSA had a clear genetic background, but about 65% of the variance could be attributed to unique environmental effects. Possible environmental determinants include exercise, diet, and chronic stress. Exercisers have a larger RSA than do nonexercisers (De Meersman, 1993; Kenney, 1985), and forced bedrest reduces RSA (Hughson et al., 1994), whereas training increases RSA (Goldsmith, Bigger, Steinman, & Fleiss, 1992; Somers, Conway, Johnston, & Sleight, 1991). However, neither cross-sectional differences between exercisers and nonexercisers nor training effects are universal (de Geus et al., 1996; Sacknoff, Gleim, Stachenfeld, & Coplan, 1994), and aerobic fitness may be a more important correlate of RSA than exercise behavior. Because trainability, that is, the increase in fitness in response to exercise, is a highly hereditary trait (Bouchard, 1986), exercise can be invoked to explain environmental as well as genetic effects on RSA. Based on their genetic susceptibility, some individuals may respond to exercise with a larger increase in RSA than many others. A similar complex picture emerges for diet. Diet-induced obesity reduces RSA in dogs (van Vliet, Hall, Mizelle, Montani, & Smith, 1995), and cross-sectional associations between body weight and RSA have also been found (Freeman, Weiss, Roberts, Zbikowski, & Sparrow, 1995; Petretta et al., 1995). Body mass index (BMI) was significantly related to RSA (during rest and tasks) in our own study, although the effect was limited to men (*r*s of −.18 to −.25). By influencing BMI, diet may influence RSA, but diet determines body composition in interaction with the individual's genetic makeup (Bouchard et al., 1990).

Chronic stress may be a further factor influencing RSA; at least under short-term stressful conditions many studies have shown a decrease in RSA (Allen & Crowell, 1990; de Geus et al., 1990, 1996; Grossman et al., 1992). Although the amount of chronic stress may be largely determined by environmental factors unique to the individual (e.g., work stress), the response of the autonomic nervous system to stress may be under genetic control. To examine this, twin correlations of RSA and respiration rate reactivity were calculated. These twin correlations of reactivity yielded a pattern incompatible with any biologically plausible model for three of the tasks used. Further genetic analysis was therefore not carried out. The reason for the uninterpretable pattern of twin correlations might lie in the less reliable determination of reactivity measures of RSA and respiration rate compared with the reliability of its levels. Reactivity is calculated as the difference between two levels, which increases the error term. Another possibility is that the reaction time, mental arithmetic, and cold pressor tasks simply were not strong enough stressors to elicit a reliable RSA and respiration rate response. Even for the most stressful (tone avoidance) task, models fitted badly, thereby decreasing the weight of the reactivity results. The relatively best-fitting models showed that

Table 8. *Summary of Results from Studies of Adolescent Twins, Their Parents, and Middle-aged Twins*

	Adolescent twins[a]			Parents[a]			Middle-aged twins[b]		
Sex	*n*	Age (years)	RSA (ms)	*n*	Age (years)	RSA (ms)	*n*	Age (years)	RSA (ms)
Males	160	16.8 (1.8)	107.8 (54.0)	142	47.9 (6.2)	58.2 (35.8)	202	43.6 (6.4)	57.3 (28.9)
Females	157	16.7 (2.2)	115.4 (58.5)	154	45.4 (5.9)	65.2 (43.2)	214	44.7 (6.8)	62.5 (35.7)
Total	317	16.7 (2.0)	111.5 (56.3)	296	46.6 (6.1)	61.8 (39.9)	416	44.2 (6.6)	60.0 (32.6)

Note: Results are mean (*SD*).
[a] Unpublished data from Boomsma et al. (1990). [b] Data from this study.

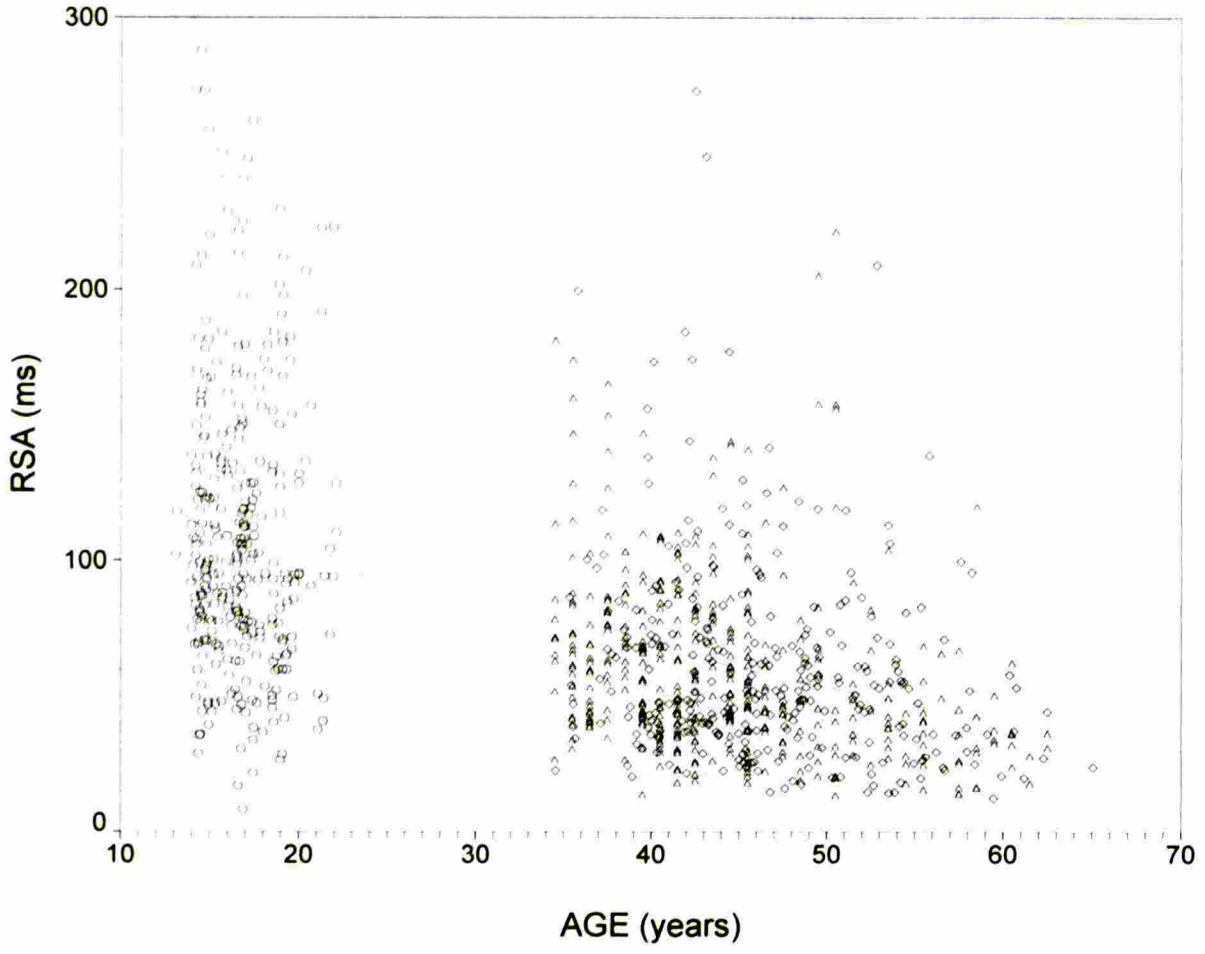

Figure 3. Relationship between age and RSA in rest in the parents (◇) and their twin children (○) from the study of Boomsma et al. (1990) and the middle-aged twins (△) from this study. The line reflects the best quadratic fit.

heritability estimates for RSA reactivity were small and differed in men and women. Substantial interindividual correlation was found between the changes in respiration rate and the changes in RSA. This correlation is well known. Within a single individual, rapid low-tidal-volume breathing will reduce the degree of RSA, and slow high-volume breathing will increase RSA (Grossman & Kollai, 1993). Our results showed that the correlation between changes in RSA and changes in respiration rate is entirely of genetic origin.

To summarize, this study is the first to quantify genetic and environmental origins of individual differences in RSA while taking into account influence of age and respiration rate on RSA. Influence of age and respiration rate on RSA was significant and should therefore be incorporated into quantitative genetic models of RSA, although correction of the heritability of RSA for influence of respiration rate did not make much difference in this study. Combining the results from this project with those of a previous one (Boomsma et al., 1990), we conclude that individual differences in RSA have a clear genetic background with heritability estimates varying between 25% and 31% at rest and 35% and 48% during stress. It is important to reflect on the role of quantitative genetic research in elucidating causes of cardiovascular disease. Future quantitative genetic studies of cardiovascular risk should shift their attention from relatively easily observable phenotypes such as blood pressure and heart rate to phenotypes representing regulatory mechanisms of the cardiovascular system. This shift is essential because an understanding of the genetic or environmental basis of the cardiovascular phenotypes could bring us closer to an understanding of the way in which disturbance of these mechanisms leads to pathology.

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