A genetic analysis of ambulatory cardiorespiratory coupling

NINA KUPPER,[a] GONNEKE WILLEMSEN,[a] DANIELLE POSTHUMA,[a] DOLF DE BOER,[b]
DORRET I. BOOMSMA,[a] AND ECO J.C. DE GEUS[a]

[a]Department of Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands
[b]School of Sport and Exercise Sciences, The University of Birmingham, Birmingham, UK

Abstract

This study assessed the heritability of ambulatory heart period, respiratory sinus arrhythmia (RSA), and respiration rate and tested the hypothesis that the well-established correlation between these variables is determined by common genetic factors. In 780 healthy twins and siblings, 24-h ambulatory recordings of ECG and thorax impedance were made. Genetic analyses showed considerable heritability for heart period (37%–48%), RSA (40%–55%), and respiration rate (27%–81%) at all daily periods. Significant genetic correlations were found throughout. Common genes explained large portions of the covariance between heart period and RSA and between respiration rate and RSA. During the afternoon and night, the covariance between respiration rate and RSA was completely determined by common genes. This overlap in genes can be exploited to increase the power of linkage studies to detect genetic variation influencing cardiovascular disease risk.

Descriptors: Heart period, Respiratory sinus arrhythmia, Respiration, Heritability, Ambulatory, Twins

The difference in heart period during the inspiratory and expiratory phases of the respiratory cycle is known as respiratory sinus arrhythmia (RSA). RSA is affected in a dose-response way by muscarinergic blockers or vagal cooling and is regarded to be a valid noninvasive index of cardiac vagal tone (Berntson et al., 1997; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Various time and frequency domain measures of RSA have become available that produce highly comparable results in both clinical and experimental settings (Bigger et al., 1992; Grossman, van Beek, & Wientjes, 1990; Grossman, Wilhelm, & Spoerle, 2004; Penttila et al., 2001).

Studies in men and women in all age groups, using either patients or healthy controls, have collectively shown the existence of large individual differences in RSA (Ben Lamine et al., 2004; Grossman & Kollai, 1993). Studies examining these individual differences have demonstrated that lower values of RSA are associated with higher risk for cardiac disease (Bigger et al., 1995; La Rovere et al., 1998; Singer et al., 1988) and hypertension (Liao et al., 1996; Mussalo et al., 2001). Reduced RSA is also associated with anxiety (Thayer, Friedman, & Borkovec, 1996;

Watkins, Grossman, Krishnan, & Sherwood, 1998), posttraumatic stress disorder (Cohen et al., 1997), and, prospectively, with major depressive disorder (Rottenberg, Wilhelm, Gross, & Gotlib, 2002). The link between RSA amplitude and behavioral engagement can already be found in children of 3 months of age (Bazhenova, Plonskaia, & Porges, 2001) and children aged 5 to 6 years (Doussard-Roosevelt, Montgomery, & Porges, 2003).

In view of the potential relevance of individual differences in RSA to index future disease, insight is needed into its genetic and environmental origins. The twin design is a powerful method to do so (Boomsma, Busjahn, & Peltonen, 2002; Neale & Cardon, 1992). A twin study compares the intrapair resemblance for a certain trait in identical twins to the trait resemblance in fraternal twins. This allows the estimation of the relative contribution of genetic and environmental factors to variance in the trait. In addition, separate contributions can be estimated for environmental factors shared by all family members ("C") and environmental factors unique ("E") to each family member (including measurement error). Shared environment may include effects of parental socioeconomic status, diet, rearing style, and parental guidance in lifestyle choices (e.g., exercise). To obtain sufficient statistical power to detect significance of such shared environmental influences, large samples of twins are needed. Power can be further increased by adding one or more non-twin siblings of the twins (Posthuma & Boomsma, 2000). This "extended twin design" was adopted in the current study, in which RSA was obtained in 527 twins and 253 of their singleton siblings.

In previous twin studies, a significant genetic contribution to RSA has already been established. Heritability estimates ranged

from 13% to 39% (Boomsma, van Baal, & Orlebeke, 1990; Busjahn et al., 1998; Singh et al., 1999; Sinnreich, Friedlander, Luria, Sapoznikov, & Kark, 1999; Snieder, Boomsma, Van Doorren, & De Geus, 1997). The above estimates, however, were all based on the variance in RSA observed under resting conditions. Additional variance may appear under physical and psychological challenge, which are known to significantly reduce the mean value of RSA in most subjects, although large individual differences in the magnitude of RSA responsiveness are found (Cacioppo, Uchino, & Berntson, 1994; Houtveen, Rietveld, & De Geus, 2002). Using a comparable set of stressors, two large twin studies have shown a relative increase in *genetic* variance during stress (Boomsma et al., 1990; Snieder et al., 1997). Heritabilities for RSA level found at rest (24% in the adolescents, 31% in the middle-aged) increased substantially during a reaction time and mental arithmetic task (up to 51% in adolescents and 43% in the middle-aged). This suggests that genetic influences are more pronounced when the subject is challenged by mentally and emotionally taxing tasks. Accordingly, we hypothesize that heritability of RSA will be even higher when recorded over prolonged periods of time in a naturalistic setting, as such challenges are more likely to be encountered in daily life. A first aim of the present study is to assess heritability of ambulatory measured RSA levels across an entire day and night.

Many studies have documented a significant covariance between RSA and heart period at rest and during laboratory challenges (Medigue et al., 2001; Sahar, Shalev, & Porges, 2001). Short heart periods, just as low levels of RSA, have been shown to predict cardiovascular disease (Dyer et al., 1980; Jouven, Zureik, Desnos, Guerot, & Ducimetiere, 2001; Kannel, Kannel, Paffenbarger, & Cupples, 1987). In addition, heart period is significantly shorter in subjects with psychiatric disorders (Austen & Wilson, 2001; Carney et al., 1988; Lehofer et al., 1997; Rechlin, Weis, Spitzer, & Kaschka, 1994). A straightforward interpretation of RSA and heart period covariance is that low RSA and short heart period both index low cardiac vagal tone. These correlated risk factors may further reflect a common genetic susceptibility, for instance, through genes influencing parasympathetic nervous system activity. Only a single study has addressed the possibility of a genetic contribution to this association (De Geus, Boomsma, & Snieder, 2003). Common genes were indeed found to explain a large part of the association between the resting levels of heart period and RSA, although significant contribution was also found for unique environmental factors. To date, no studies have tested the contribution of genes and environment to the association of heart period and RSA under mentally challenging or naturalistic conditions.

A similar state of affairs applies to the significant covariance between RSA and respiration rate. Many studies have testified to the robustness of this association, at rest, during laboratory challenges, and during ambulatory recordings (de Geus, Willemsen, Klaver, & van Doorren, 1995; Grossman & Kollai, 1993; Grossman et al., 2004; Kollai & Kollai, 1992; Snieder et al., 1997), but only a single study has examined the source of the association in a genetically informative design (Snieder et al., 1997). Here, respiration rate–RSA covariance seemed to be entirely due to overlap in the genes contributing to these variables, both at rest and during a set of mental stress tasks. Nothing is currently known, however, about the genetics of the respiration rate–RSA covariance in naturalistic settings.

In short, the previous two bivariate twin studies on the genetics of RSA and heart period and the genetics of RSA and respiration rate were performed in a *laboratory* setting. No study has addressed the genetic architecture of the association between *ambulatory* heart period and RSA and *ambulatory* RSA and respiration rate. Furthermore, no trivariate genetic analysis of heart period, RSA, and respiration rate has been performed to date, and the possible contribution of common genes to heart period and respiration rate remains to be determined. A second aim of this study, therefore, is to estimate the relative contribution of common genetic and environmental factors to the covariance between heart period, RSA, and respiration rate measured in a naturalistic setting.

Methods

Participants

Participants were all registered in the Netherlands Twin Register (NTR). Their families were originally selected for a genetic linkage study searching for genes for depression, which is described elsewhere (Boomsma et al., 2000). Of the 1332 offspring who returned a DNA sample (buccal swabs) for the linkage study, 1008 were successfully contacted for a cardiovascular ambulatory monitoring study, of which 192 refused or were excluded. In total 816 people were willing to participate in cardiovascular ambulatory monitoring. A priori reasons for exclusion were pregnancy, heart transplantation, presence of a pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy. For 14 of the 816 participants recordings were unavailable due to equipment failures and 8 participants had either a noisy ECG or a noisy thorax impedance signal, and were therefore excluded from the analysis. Fourteen participants using rhythm-altering medication, heart rate variability-reducing antidepressants (tricyclic antidepressants and benzodiazepines), antihypertensive medication (beta-blockers), or a combination of these were excluded from the analysis. The final sample consisted of 222 identical twins (80 men), 305 fraternal twins (109 men), and 253 singleton siblings (97 men) from 339 families. Mean age was 31.0 years ($SD = 10.8$). Zygosity of the twins was determined by DNA typing. The Ethics Committee of the Vrije Universiteit approved of the study protocol and all participants gave written consent before entering the study. No payment was made for participation, but all participants received an annotated review of their ambulatory ECG recording.

Ambulatory Measurements

The Vrije Universiteit Ambulatory Monitoring System 46 (VU-AMS) continuously recorded the electrocardiogram (ECG) and changes in thoracic impedance (dZ) from a six-electrode configuration (de Geus & van Doorren, 1996; de Geus et al., 1995; Riese et al., 2003). The device automatically detects each R wave in the ECG signal, at which it reads out and resets a millisecond counter to obtain the heart period time series. The thoracic impedance (Z), assessed against a constant current of 50 KHz, 350 microamperes, was amplified and led to a precision rectifier. The rectified signal was filtered at 72 Hz (low pass) to give basal impedance Z. Filtering Z at 0.1 Hz (high pass) supplied the dZ signal, which contains three major components: high frequent impedance changes due to the ejection of blood into the aorta during systole, low frequent impedance changes due to arm and upper body movement, and, in between these frequencies, the thoracic impedance changes due to respiration. Intervals of 100 s of the dZ signal were bandpass filtered with 0.1 and 0.4 Hz cutoffs after tapering with $(\sin(x))^2$ to yield the respiration signal.

VU-AMS software (http://www.psy.vu.nl/vu-ams) was used to display the recorded heart period time series as a cardiotachogram together with the respiration signal. Suspected erroneous R wave detection (e.g., due to ectopic beats) was automatically tagged for deletion. The starting points of inspiration and expiration were automatically scored for each breath, but interactive visual inspection allowed correction of erroneous respiration scoring or the selective removal of noisy signal fragments. From the corrected signal, mean heart period, respiration rate, and RSA were computed. RSA was determined using the peak-to-trough method (Fouad, Tarazi, Ferrario, Fighaly, & Alicandri, 1984; Grossman & Kollai, 1993; Grossman et al., 1990), which combines respiratory time intervals and the heart period time series to obtain an RSA value at each breath. This method yields highly comparable results to frequency domain based methods (Grossman et al., 1990) and has the advantage of additionally providing the respiratory frequency. RSA was computed for each breath as the difference between the shortest heart period during heart rate acceleration in the inspirational phase (which was made to include 1000 ms from the following expiration to account for phase shifts) and the longest heart period during deceleration in the expirational phase (including 1000 ms from the following expiratory pause/inspirational phase). When no phase-related acceleration or deceleration was found, the breath was assigned a RSA score of zero.

Procedure

Participants were visited at home, before starting their normal daily activities. During a short interview, information on health status and current medication use was obtained. The VU-AMS was attached and its operation explained. Participants were instructed to wear the device the entire day and night up until awakening the next morning. Written instructions were supplied that explained how to respond to potential alarm beeps (e.g., on loose electrode contacts), and telephone assistance was available during waking hours. Participants were asked to keep a detailed diary. Every 30 (± 10) min the device produced an audible alarm beep to prompt them to write down a chronological account of activity, posture, location, social situation, and amount of perceived stress during each past 30 min. On the following day the researcher collected the device at home.

Statistical Analysis

Data reduction. In addition to the cardio-respiratory measures, the VU-AMS device also recorded vertical acceleration. Accelerometer output was averaged every 30 s, and used as a proxy for gross body movement. Time-stamped information from the diary about activity and posture was combined with an interactive graphical display of body movement as a function of time, which made it possible to accurately specify the exact start and end times of the changes in activity or posture that the participants had reported in the diary. We divided the entire recording into smaller intervals that were completely stationary with regard to physical activity and posture. Each interval was coded for posture (lying, sitting, standing, walking, and bicycling), activity (e.g., deskwork, housekeeping, watching TV), and location (e.g., at home, at work, at a public place). The coded intervals were never shorter than 5 min nor longer than 1 h. Based upon the reported times of dinner and lunch, awakening and bedtime, four averages were computed over all coded intervals available for each of the four periods of the day: morning, afternoon, evening, and nighttime sleep. Averages were computed once using all posture data and once using only those intervals when participants were sitting or lying. In 8% of the participants the exact time of dinner, lunch, awakening, or bedtime could not be extracted from either diary or body movement. For these participants, the missing time was imputed using the mean times of these events in the rest of the sample.

Confounding variables. Individual differences in the ambulatory physiological variables were expected to be sensitive to three main confounding variables: differences in age, sex (De Meersman, 1993; Umetani, Singer, McCraty, & Atkinson, 1998), and physical activity patterns (Osterhues, Hanzel, Kochs, & Hombach, 1997; Sacknoff, Gleim, Stachenfeld, & Coplan, 1994). To control for the first two of these variables, all correlation coefficients were age adjusted and calculated separately for the sexes. In model fitting analysis it was specifically tested whether the effects of sex and age on the means and variances could be dismissed from the model. To examine the influence of possible individual differences in physical activity patterns, analyses were performed twice, once using the averages based on all postures and once using the averages based on the data during which a participant was sitting or lying.

Twin correlations. To determine to what extent monozygotic twins are more similar than dizygotic twins or singleton siblings Pearson correlation coefficients were calculated for all groups, using SPSS software (SPSS Inc., Chicago, IL, USA). In the computation of these correlations, fraternal twin pairs and singleton siblings were regarded as a single group, as these pairs all share on average 50% of their genetic material (this assumption was tested in the model fitting). All possible fraternal twin pairs or sib pairs that could be formed within a family were used.

Structural equation modeling. To answer the question to what extent genes, common environment, and unique environment contribute to the variances and covariances between the three variables (heart period, RSA, and respiration rate), a biometrical genetic model was fitted to the observed data using the structural equation program Mx (Neale, Boker, Xie, & Maes, 2003). First, a series of unconstrained models was fitted to test the equality of means and variances for identical twins, fraternal twins, and singleton siblings. We then examined the presence of sex effects on the means and variances. Next, the significance of a linear regression of age on the three variables was tested. Lastly, we tested for heterogeneity of correlations of males versus females and of fraternal twins versus singletons. The resulting most parsimonious saturated model indicated to what extent we could limit the specification of the variance components models.

The main questions were addressed in various nested trivariate (heart period, RSA, and respiration rate) variance components models. In a twin study, the observed variance can be decomposed in four possible latent sources of variance: additive genetic effects (A), nonadditive genetic effects (D), shared environment (C), and nonshared environment (E) following Neale and Cardon (1992). However, in a design that includes identical twins, fraternal twins, and sib pairs, estimates of C and D are confounded and the observed variances and covariances only provide sufficient information to model either an ACE model or an ADE model, but not both. Based on the pattern of twin and sibling correlations we choose to model A, C, and E. For identical twins, fraternal twins, and sibling pairs alike, similarity in shared environmental influences was fixed at 100%. Similarity of additive

genetic influences was fixed at 50% for siblings and fraternal twins and at 100% for identical twins. Per definition, there was no similarity in the nonshared environmental influences.

For each of the four periods of day, a full trivariate ACE model in Cholesky decomposition (Neale & Cardon, 1992) was tested against the nested more parsimonious AE, CE, or E models. The resulting best fitting model was used to further test the source of the observed covariance between the three variables. Specifically we tested the comparative fit of models with the genetic or environmental correlations set to zero and of models with genetic or environmental correlations set to 1, that is, models in which a single genetic or environmental factor influenced heart period, RSA, and respiration rate.

Throughout, nested models were compared using the likelihood ratio test. In addition, Akaike's Information Criterion

(AIC = $\chi^2 - 2df$) (Akaike, 1987) was calculated for each model, which offers a quick approach to judging the fit of nested models. Those with lower (i.e., larger negative) values fit better than models with higher values. The final best fitting, most parsimonious model was used to estimate the genetic and environmental contribution to the covariance between heart period, respiration rate, and RSA.

Results

The average duration of valid measurement was 22:13 h (*SD* = 3:21 h). RSA was skewed at all daily periods and its natural logarithm was used in all further analyses. Figure 1 gives scatterplots of heart period and lnRSA and of lnRSA and respiration rate. Complete measurements during sitting activities
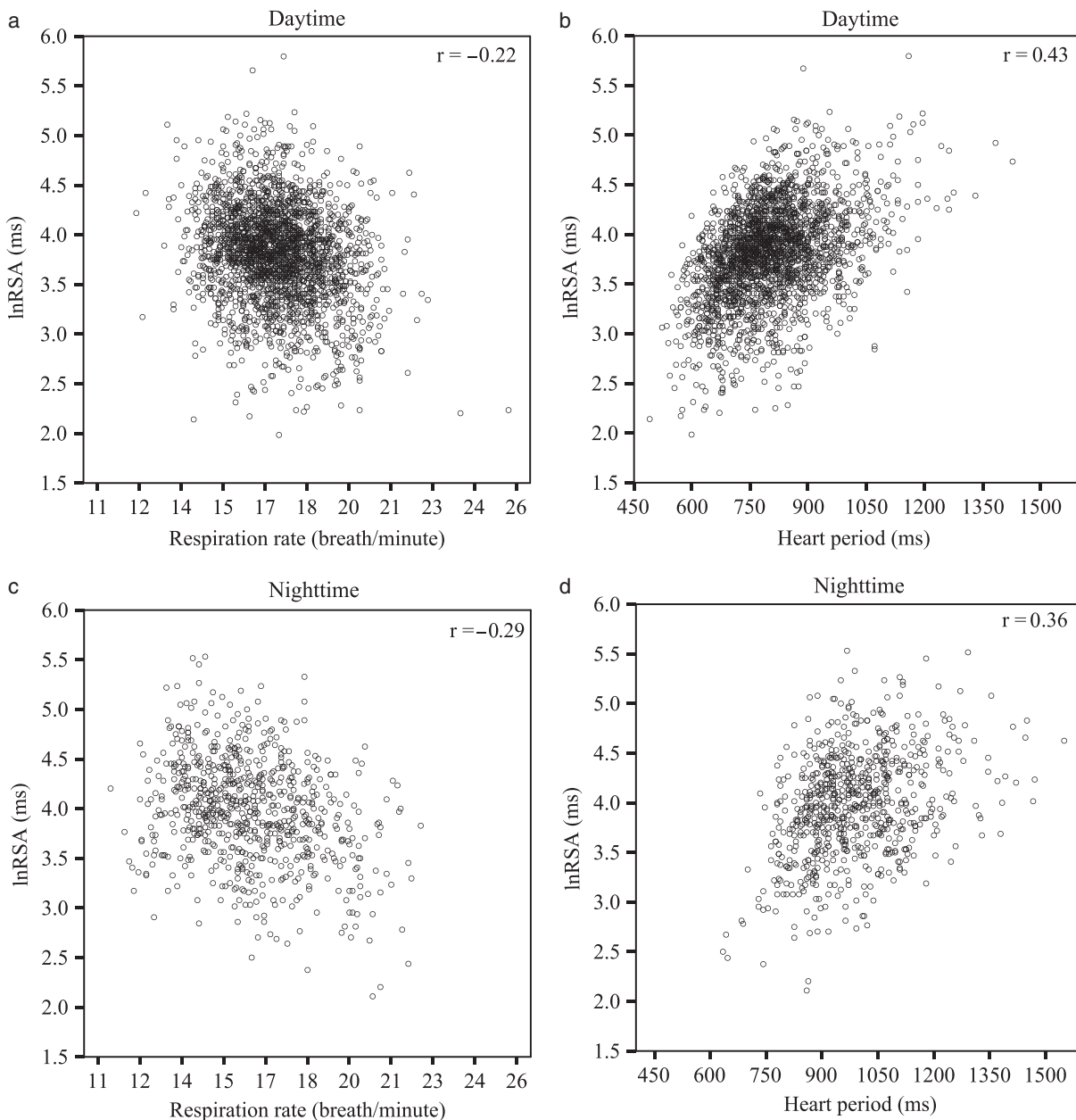
Figure 1. Scatter plots for daytime and nighttime, collapsed over sex, visualizing the relation between lnRSA and respiration rate and between lnRSA and heart period, respectively.

were obtained in 689 subjects for all four periods of the day, in 91 subjects one or more periods were missing. Because part of the sample (two in each family) was originally selected for a study in search for genes influencing anxious depression, Pearson correlation coefficients were computed between a summary score of anxious depression (see Boomsma et al., 2000, for more details) and our ambulatory variables. No meaningful association was found, suggesting that the sample selection was unbiased.

Repeated-measures ANOVA showed a significant between-subject effect of sex for heart period, $F(1,688) = 112.6$, $p < .001$, lnRSA, $F(1,688) = 7.8$, $p < .01$, and respiration rate, $F(1,688) = 4.3$, $p < .05$. Male heart period, lnRSA, and respiration rate were lower than female heart period, lnRSA, and respiration rate at all periods of day. A significant effect of the daily period was found for heart period, $F(3,686) = 1263.8$, $p < .001$, lnRSA, $F(3,686) = 55.4$, $p < .001$, and respiration rate, $F(3,686) = 203.9$, $p < .001$. This was entirely due to a decrease in respiration rate together with an increase in heart period and lnRSA during sleep in contrast to all three waking periods. Means and standard deviations of the variables are presented separately for each daily period and per sex in Table 1.

Twin and Sibling Correlations

Correlations were computed twice. For the waking periods (morning, afternoon, evening) data were used (a) from the entire recording period, that is, including different levels of physical activity and multiple postures, or (b) from periods with sitting activities only. In spite of the potential confounding effects of individual differences in the frequency of postural changes and physical activity, virtually identical patterns of twin and sibling correlations were obtained when either all data were used or sitting only data. To avoid possible interpretations based on movement artifact, however, we confined further analyses to intervals that involved a constant sitting posture (which comprised 51% of the total recording time).

Table 2 shows the resemblance between identical twins and fraternal twins/sibling pairs for heart period, lnRSA, and respiration rate. It is immediately obvious from the systematically larger identical twin than fraternal twin/sib correlations that genetic factors influence all three variables. The increase in identical

Table 1. *Means (SD) and Correlations for Heart Period, lnRSA and Respiration Rate*

	Morning	Afternoon	Evening	Night
Heart period (ms)				
Men	847 (126)	811 (121)	854 (122)	1058 (139)
Women	764 (101)	752 (94)	793 (104)	937 (109)
lnRSA (ln(ms))				
Men	3.8 (0.4)	3.7 (0.5)	3.7 (0.5)	3.9 (0.5)
Women	3.9 (0.5)	3.8 (0.5)	3.9 (0.5)	4.0 (0.5)
Respiration rate (breath/minute)				
Men	16.3 (1.4)	16.7 (1.4)	17.5 (1.6)	15.4 (2.0)
Women	16.3 (1.3)	16.8 (1.3)	17.4 (1.6)	16.1 (2.0)
Heart period and lnRSA correlation				
Men	.48**	.52**	.48**	.46**
Women	.47**	.48**	.47**	.43**
Respiration rate & lnRSA correlation				
Men	−.26**	−.23**	−.26**	−.32**
Women	−.21**	−.14*	−.23**	−.28**

Notes: Shown are means (SD) and correlations for each period of day, separately for the sexes. **$p < .01$, *$p < .05$.

Table 2. *Age-Adjusted Twin and Sibling Correlations for Heart Period, lnRSA and Respiration Rate Means*

		Respiration rate		lnRSA		Heart period	
		MZ	DZ/sib	MZ	DZ/sib	MZ	DZ/sib
Morning	Correlation	**.21**	**.15**	**.46**	**.21**	**.60**	**.31**
	N pairs	90	573	91	573	91	573
Afternoon	Correlation	**.42**	**.22**	**.51**	**.24**	**.55**	**.34**
	N pairs	90	570	91	570	92	570
Evening	Correlation	**.50**	**.21**	**.58**	**.24**	**.46**	**.28**
	N pairs	89	589	90	589	90	589
Night	Correlation	**.83**	**.50**	**.61**	**.27**	**.62**	**.38**
	N pairs	87	543	88	543	88	543

Notes: Correlations that were significant ($p < .05$) are printed boldfaced. MZ: identical twin pairs, DZ/sib: fraternal twin or sibling pairs, N pairs: number of pairs of subjects.

twin correlation for respiration rate at night further suggests higher heritability during sleep.

Structural Equation Modeling

Several assumptions of the extended twin design were tested in a series of unconstrained models. All morning, afternoon, evening, and nighttime means of heart period, lnRSA, and respiration rate could be set equal for identical twins, fraternal twins, and siblings without a significant loss of fit of the model. Dropping the linear regression effect of age or sex on all variable means, however, significantly worsened the fit. The variances were homogeneous across zygosity and across sex. Likewise, testing for heterogeneity in the correlations across sex within zygosity showed that, in all cases, the correlations were homogeneous across males and females. Importantly, equating fraternal twin correlations to correlations across any of the other sib-sib pairings (identical twin-singleton sibling, fraternal twin–singleton sibling, singleton sibling–singleton sibling) produced no significant worsening in the fit of the model. Although the main aim here was to reduce the number of parameters to be estimated, note that these results imply that the estimates obtained in twins can be generalized to singletons.

The resulting most parsimonious model (no sex or twin-singleton effects on variances or sib-pair covariances) was used to test four separate trivariate variance decomposition models, for each period of day (morning, afternoon, evening, and night). For all three variables the shared environmental component (C) could be removed without a significant loss in fit (see Table 3). This resulted in models that included only the additive genetic and unique environmental variance components (AE model). From this AE model we could not dismiss any of the genetic correlations between respiration rate, lnRSA, and heart period. The full three-factor model also had to be maintained for most of the unique environmental influence, with a few notable exceptions, visualized as the nonsignificant paths in Figure 2. A striking reduction in the importance of the environmental influences was seen at night, when both the covariance between respiration rate and heart period and between respiration rate and lnRSA became entirely genetic in origin. The final best fitting models for each of the periods of day are shown in Figure 2.

Heritability is sum of the genetic variance due to factors A1 to A3 divided by the total variance (see legend to Figure 2 for a computational example). Table 4 gives the heritability estimates for respiration rate, lnRSA, and heart period under the best

Table 3. *Summary of the Model Fitting Results*

Model	Versus	Δdf	χ^2	p	AIC
Morning					
ACE					
AE	ACE	6	3.644	0.725	-8.356
CE	ACE	6	11.128	0.085	-0.872
E	ACE	12	80.956	0.000	56.956
Reduced AE[a]	**AE**	**7**	**4.295**	**0.420**	**-9.705**
Afternoon					
ACE	—	—	—	—	—
AE	ACE	6	1.963	0.923	-10.037
CE	ACE	6	15.354	0.018	3.354
E	ACE	12	89.743	0.000	65.743
Reduced AE[b]	**AE**	**7**	**3.171**	**0.272**	**-10.829**
Evening					
ACE					
AE	**ACE**	**6**	**1.51**	**0.959**	**-10.49**
CE	ACE	6	23.93	0.001	11.93
E	ACE	12	120.773	0.000	96.773
Night					
ACE					
AE	ACE	6	4.164	0.654	-7.836
CE	ACE	6	40.584	0.000	28.584
E	ACE	12	222.107	0.000	198.107
Reduced AE[c]	**AE**	**8**	**5.606**	**0.486**	**-10.394**

Notes: Summary of the model comparison results for each daily period.
[a]No rE between respiration rate and heart period.
[b]No rE between respiration rate and lnRSA.
[c]No rE between respiration rate and lnRSA and between respiration rate and heart period. Shown are fit statistics for models with additive genetic, common environmental, and unique environmental (ACE) influences, additive genetic and unique environmental (AE) influences, common and unique environmental (CE) influences, and unique environmental (E) influences. The full models include all genetic (rA) and unique environmental (rE) correlations between respiration rate, lnRSA, and heart period. All associations between the variables have been tested for significance; only the best fitting most parsimonious model is presented. Boldfaced rows represent the model that fitted the data best. Low p values denote significant worsening of fit. AIC: Akaike's Information Criterion.

fitting models (boldfaced rows in Table 3). Heritability estimates for respiration rate were all significant, but varied greatly over the four daily periods. During waking hours, heritability ranged between 27% and 50%. At night, however, it increased up to 81%. To investigate whether it is a decrease of environmental variance or an increase of genetic variance that causes the heritability estimate to increase so dramatically at night, we examined the changes in absolute variances. We found that variance attributed to genetic effects increased much more over the four periods of day compared to the decrease found in the variance ascribed to unique environmental effects. As can be seen in Figure 2 the increase in genetic variance from evening to night for respiration rate is 196.3 ($=18.0^2-11.3^2$), whereas the decrease in environmental variance is 52.0 ($=8.7^2-11.3^2$). Thus, the increased heritability for respiration rate during the night is caused mainly by increased genetic variance in respiration rate during the night.

Heritability estimates for lnRSA ranged from 40% in the morning to 55% in the evening. As can be calculated from the path coefficients, the contribution of genes shared with respiration rate (genetic factor A1) to the heritability of lnRSA was between 8% (evening) and 16% (night). Because it has been suggested that RSA corrected for respiration rate would be a better predictor than uncorrected RSA (Grossman et al., 2004), we did an additional genetic analysis for respiratory-corrected

RSA. These heritability estimates were 38%, 47%, 46%, and 46% for the daily periods, respectively, which is lower than the estimates for uncorrected RSA in the multivariate analyses.

Heritability estimates for heart period ranged from 37% in the morning to 48% at night. The contribution to this heritability of genes that are shared between respiration rate and heart period was between 1% (morning) and 4% (afternoon). The contribution of genes shared only with lnRSA (genetic factor A2), which may reflect genes affecting cardiac vagal control, was between 6% (night) and 17% (afternoon). This leaves a substantial unique genetic influence (genetic factor A3) on heart period, which may reflect genetic effects on the intrinsic pacemaker frequency and the sympathetic nervous system.

Table 5 shows the standardized covariances (correlations) between respiration rate, lnRSA, and heart period (upper off-diagonal elements), as well as the standardized genetic contributions to the covariance between these three variables (lower off-diagonal elements). The genetic contribution to the covariance between two variables is calculated as the ratio of the genetic covariance (which is the genetic correlation between two variables and the product of the squared roots of the two heritabilities) to the total observed covariance. The remaining part of all covariances is explained by unique environmental factors. The correlation between morning lnRSA and respiration rate, for instance, is -0.23, with genes contributing 62% to this covariance and unique environment 38%.

lnRSA and heart period covaried significantly at all four periods, with correlation coefficients ranging between .35 and .45. The contribution of common genetic factors to the heart period–lnRSA covariance between the measures varied between 30% and 52%. The correlations between respiration rate and lnRSA were all significant and ranged between -0.17 and -0.24. The contribution of common genetic factors to the respiration rate–lnRSA covariance varied between 62% and 100%. The correlation between respiration rate and heart period was only significant at night (-0.19). All of this respiration rate–heart period covariance was explained by genetic factors.

Discussion

This study assessed the heritability of ambulatory heart period, RSA, and respiration rate in 339 families and tested the hypothesis that the covariance between these variables is caused by common genetic factors. Our study had a number of strengths in design that provide confidence in its outcome. The extended twin design increases statistical power to distinguish between genetic and common and unique environmental influences compared to a design that includes only identical and fraternal twins (Posthuma & Boomsma, 2000). Furthermore, it allowed us to show that results obtained in singleton siblings do not differ from those obtained in twins. The absence of any twin-singleton difference replicates previous findings in other cardiovascular risk factors (de Geus, Posthuma, Ijzerman, & Boomsma, 2001) and indicates that our heritability estimates can safely be generalized to the population at large. The current study estimated the heritability of RSA to lie between 40% and 55%. This agrees with a previous twin study, in which RSA heritability (based on spectral power in the heart period time series) was estimated to be 39% across a short 30-min recording interval in 141 twin pairs (Busjahn et al., 1998). On the other hand, using a sib–parent design, Singh et al. (1999) found that genetic factors accounted for only 16% of the
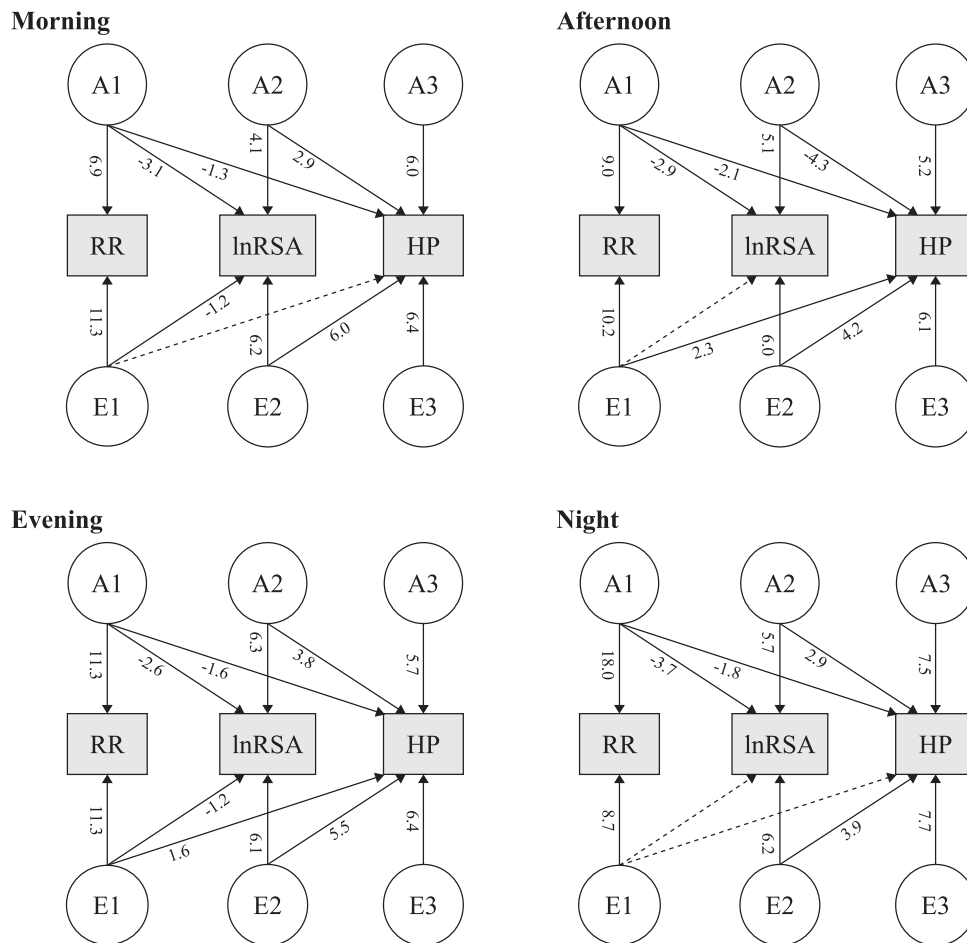
Figure 2. The most parsimonious decomposition of the variance and covariance for respiration rate (RR), lnRSA, and heart period (HP). For each of the daily periods, factor A1 represents the genetic influences on respiration rate, which are partly shared with lnRSA and heart period. Factor A2 represents the genetic influences on lnRSA, which are partly shared with heart period, but unshared with respiration rate. Factor A3 represents the remaining genetic influences on heart period that are unshared with respiration rate and lnRSA. Notation for the unique environmental factors (E1 through E3) follows analoguous reasoning. The numbers next to the paths represent the unstandardized path coefficients. Nonsignificant paths are indicated by dotted lines. Heritability can be computed by standardizing these coefficients following path tracing rules. For example, heritability of morning lnRSA is

$$\frac{\text{Summed genetic variance}}{\text{Total variance}} = \frac{((-3.1)^2 + 4.1^2)}{((-3.1)^2 + 4.1^2 + (-1.2)^2 + 6.2^2)}.$$

interindividual variation in high frequency power. In that study, however, high frequency power was obtained from a 2-h interval recorded during a routine examination at the Framingham Heart Study clinic, which is more similar to a laboratory, rather than ambulatory, recording. Other laboratory twin studies have also reported more modest heritability estimates for resting RSA, varying from 19% to 23% (Boomsma et al., 1990; Snieder et al., 1997).

When RSA is measured during stressful tasks, however, heritability increases to reach levels comparable to those found in the current ambulatory study (Snieder et al., 1997). This pattern of results is in keeping with the idea that under conditions of challenge or in naturalistic settings other (or more) aspects of cardiovascular regulation come into play, allowing genetic differences between individuals to become more pronounced (see also Boomsma, Snieder, de Geus, & van Doornen, 1998). However, it is unclear whether the effects of the same genetic

factors that operate at rest are amplified by stress, or whether new genetic factors emerge during stress. Heritability estimates do not tell us *which* genes are contributing in *what way*. It may well be that different genetic pathways operate to affect resting RSA measured in a standardized setting compared to ambulatory RSA recorded over prolonged periods of time in a naturalistic setting.

Indeed, it is possible that genes that cause *low* RSA at rest act to cause *high* RSA under ambulatory conditions. This could explain the apparent contradiction in two studies that have examined the genetic basis of RSA by a candidate gene approach. An ambulatory study by Busjahn et al. (1998) in Caucasian twins, found that subjects homozygous (DD) for an insertion/deletion polymorphism in intron 16 of the angiotensin-converting enzyme gene had significantly higher levels of ambulatory RSA. The opposite finding was found, however, using laboratory recordings in African American subjects (Thayer et al., 2003). Although

Table 4. *Heritability Estimates for Respiration Rate, lnRSA, and Heart Period*

	Respiration rate	lnRSA	Heart period
h^2 morning	27% (12–41)	40% (27–54)	37% (24–50)
h^2 afternoon	44% (28–57)	49% (34–59)	45% (28–54)
h^2 evening	50% (37–61)	55% (43–65)	40% (24–53)
h^2 night	81% (74–86)	54% (41–64)	48% (26–68)

Notes: Shown are the heritability estimates (h^2) for respiration rate, RSA, and heart period, under the best fitting models. The 95% confidence intervals are presented in parentheses. All values are significant at a .05 level.

ethnicity was invoked as a plausible explanation for the discrepancy in results, contribution of the difference in ambulatory versus laboratory design cannot be excluded.

In the present study, heritability of ambulatory heart period is estimated to be between 37% and 48%. Previous twin studies that assessed the heritability of ambulatory heart period (Fagard, Loos, Beunen, Derom, & Vlietinck, 2003; Jedrusik et al., 2003) estimated heritability of the 24-h mean of heart period to be slightly higher, between 51% and 70%. When we compare all estimates from ambulatory studies (including our own) to the published twin data on resting laboratory values, we find no evidence that the heritability of heart period increases in ambulatory recordings over laboratory recordings as it did for RSA. Laboratory studies in adult twins have reported heritabilities of resting heart period to lie between 50% and 77% (Ditto, 1993; Fagard et al., 2003; Li et al., 2001; Russell, Law, Sholinsky, & Fabsitz, 1998). It should again be noted that the same heritability may arise from different genetic pathways influencing heart period in different measurement settings.

Table 5. *Standardized Covariances between Respiration Rate, lnRSA and Heart Period and the Genetic Contributions to These Covariances*

	Respiration rate	lnRSA	Heart period
Morning			
Respiration rate	—	− **.23**	− .07
lnRSA	**62% (34–85)**	—	**.42**
Heart period	**100%**	**30% (10–48)**	—
Afternoon			
Respiration rate	—	− .17	.02
lnRSA	**100%**	—	**.45**
Heart period	**45% (13–72)**	**52% (32–66)**	—
Evening			
Respiration rate	—	− **.24**	.01
lnRSA	**69% (37–96)**	—	**.42**
Heart period	**49% (3–95)**	**47% (28–63)**	—
Night			
Respiration rate	—	**.27**	**.19**
lnRSA	**100%**	—	**.35**
Heart period	**100%**	**49% (27–68)**	—

Notes: Significance at a .05 level is indicated in bold. The upper-diagonal elements report the standardized phenotypic covariances between the variables (= correlations), and the lower-diagonal elements show the genetic contributions (%) to these covariance. The 95% confidence intervals are presented in parentheses. Note that a nonsignificant correlation between the variables can still have significant genetic contributions, if the genetic and unique environmental correlations are of opposite sign, as was the case for daytime correlations between heart period and respiration rate.

In the present study the genetic contribution to the variance in daytime respiration rate was between 27% and 50%. Only one previous study reported on the heritability of respiration rate. In that study respiration rate was measured under rest and stress conditions in a laboratory setting (Snieder et al., 1997). The genetic component of variance was estimated to be around 62% for the resting condition and between 51 and 60% for stress conditions. The lower estimates in our study compared to Snieder et al. (1997) may reflect the fact that during the laboratory experiment there was no talking. Talking influences respiration and increases the environmental component of the variance decomposition to the detriment of the genetic component. Interestingly, in the current study a strong increase in the heritability of respiration rate was seen at night, suggesting that respiration rate is more under genetic control during sleep. Neurobiologically this makes good sense. During sleep respiratory frequency will most purely reflect intrinsic rhythmogenesis by the brain stem and some aspects of respiratory rhythmogenesis may even be entirely specific to sleep (Mackiewicz & Pack, 2003). Indeed, heritability increased mainly because of an increase in total genetic variance, suggesting that new genetic variation is being expressed during sleep. This clearly demonstrates the advantage of ambulatory recording over resting recordings, in particular the added value of nighttime recordings.

Genetic Contributions to the Covariances

RSA was significantly correlated to respiration rate and heart period. About 6% of the between-subject variance in RSA was explained by respiration rate, which is comparable to the association found in many laboratory studies (Grossman & Kollai, 1993; Houtveen et al., 2002; Ritz, Thons, & Dahme, 2001). The association between ambulatory RSA and heart period was stronger, with RSA explaining about 25% of the between-subject variance in heart period. This is again comparable to what is found in laboratory settings (de Geus et al., 2003; Medigue et al., 2001; Sahar et al., 2001). A significant association between heart period and respiration rate was found at night only, and this association was completely due to genetic effects. It should be noted that our focus was on between-subject covariance only. Much higher correlations between respiration rate and RSA and RSA and heart period are found in a pure within-subject analysis of ambulatory recordings (Grossman et al., 2004).

In agreement with our hypothesis, significant genetic correlations between heart period, RSA, and respiration rate were found at all periods of the day. This suggests that common genes influence individual variation in respiration rate, RSA, and heart period. The genetic correlations are not unity, though, suggesting that additional unique genes contribute to each of the variables. It is important to note here that our results do not reveal the exact causal chain of events that give rise to the common genetic factor. The common genetic factor may act entirely through one of the traits, that is, they may influence respiration rate, which influences RSA, which in turn influences heart period. It may also primarily affect heart period, which in turn affects respiration rate and RSA, possibly through baroreflex action on respiratory generator neurons. Finally, the common genetic factor may reflect pleiotropic genes that influence each of the traits, but without any causal effects of the traits on each other. Pleiotropic genes simultaneously affecting between-subject variance in respiration rate, RSA, and heart period may be found at different levels. They may reflect individual differences in the general state of arousal, for instance, as part of neuroticism or depression

(Carney et al., 2001) that would affect both respiratory drive and tonic vagal drive. A pleiotropic genetic basis of respiration rate, RSA, and heart period may also derive from genes affecting general aspects of neurotransmission (e.g., serotonergic receptors or secondary signaling proteins) in either limbic or brain stem areas involved in cardiovascular and respiratory control (Richerson, 2004; Severson, Wang, Pieribone, Dohle, & Richerson, 2003).

It has been suggested that RSA is a more valid index of between-subject differences in cardiac vagal control when individual differences in respiratory behavior are taken into account (Grossman & Kollai, 1993; Grossman et al., 2004) and that the validity of heart rate variability measures as predictors for cardiovascular disease could potentially improve from a correction for respiration rate. If, however, the genetic correlation between respiration rate and RSA shown in our analyses derives from pleiotropic genes, a correction for respiration rate could actually remove individual differences in cardiac vagal control. Our genetic analysis of the respiratory-corrected RSA showed heritability to decrease substantially compared to the estimates based on uncorrected RSA. Resolving the nature of the common genetic factor causing respiration rate and RSA to covary is an important future mission, but for now, removing genetic variance shared by RSA and respiration rate by using residualized scores seems unfounded. We suggest that future studies employ a multivariate design retaining both respiration rate and RSA as predictor variables.

Our results showed that RSA and heart period share another set of genes that are independent of respiration rate (factor A2 in Figure 2). A putative explanation for the additional genetic factor A2 in Figure 2 is that it represents respiration-independent gene effects on central vagal tone or the efficacy of muscarinergic effects on the sinoatrial node. Decreases in cardiac vagal control are well known to increase heart period as well as decrease RSA (Adinoff, Mefford, Waxman, & Linnoila, 1992; Berntson et al., 1994). The significant genetic contribution to the covariance between heart period and RSA is in accord with a previous investigation in a young and middle-aged twin cohort (virtually nonoverlapping with the present cohort) in which a common genetic factor contributed 54% and 70% respectively to the association between heart period and RSA (De Geus et al., 2003). In view of the clinical importance of cardiac vagal control in cardiology (Bigger et al., 1995; Tsuji et al., 1996), finding genes influencing both heart period and RSA is an important objective.

The power to detect such genes, particularly in linkage studies, is known to increase when a multivariate approach is used (Amos, de Andrade, & Zhu, 2001; Evans, 2002). This could be done using repeated measures of the same variable as well as using different, but genetically correlated, variables. The latter multivariate strategy has already successfully been applied in whole genome scans for blood pressure that added body mass index as a second phenotype (Turner, Kardia, Boerwinkle, & Andrade, 2004). Prolonged ambulatory recording of respiration rate, RSA, and heart period has an inherent repeated-measure structure, and the three variables are highly genetically correlated. Measurement in a naturalistic setting has the additional advantage of providing a window on the function of the autonomic nervous system in the context of repeated and ecologically valid stressors. We conclude therefore, that ambulatory measurement of cardiovascular and respiratory signals can help shorten the search for the actual genetic polymorphisms that influence cardiovascular disease risk.

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