Genetic Epidemiology of Risk Factors for Coronary Heart Disease

Harold Snieder

GENETIC EPIDEMIOLOGY OF RISK FACTORS FOR CORONARY HEART DISEASE

A study of middle-aged twins

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Harmen Snieder

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Promotor:	prof.dr J.F. Orlebeke
Copromotoren:	dr D.I. Boomsma
	dr L.J.P. van Doornen
Referent:	dr M.C. Neale

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To my parents
whom I will always be grateful
for sharing their genes
and environment with me

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dr E.J.C. de Geus
dr P. de Knijff

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Introduction

Genetic epidemiology of risk factors for coronary heart disease

Coronary heart disease (CHD) results from a complex interplay of multiple etiological factors. Due to this complexity, much remains unknown about the processes that determine the build-up of the atherosclerotic plaque on the arterial wall, which eventually lead to the occlusion of (coronary) arteries. To gain a better insight into the complex etiology of atherosclerosis most research has confined itself to (putative) risk factors of CHD (Rao & Vogler, 1994). Each of these risk factors may exhibit familial aggregation or variability. Sources of familial aggregation of (risk factors of) disease are studied within genetic epidemiology. According to the often cited definition of Morton and Chung (see Khoury, Beaty & Cohen, 1993), genetic epidemiology consists of two main components: 1) the study of the etiology of disease among groups of relatives to unravel the causes of family resemblance and 2) the study of inherited causes of disease in populations. In the study reported in this thesis, cardiovascular risk factors were measured in middle-aged twins. In connection with the appropriate model fitting techniques, derived from quantitative genetics, a twin design enables quantification of genetic and environmental sources of familial resemblance of risk factors for CHD.

Familial aggregation of CHD and its risk factors

Since long it has been recognized that the occurence of premature coronary heart disease runs in families. Fifty to 80% of early CHD in the general population is found in only 5 to 15% of the families (Hopkins & Williams, 1989). A positive family history of CHD is thus an important risk factor, especially for the occurrence of *early* CHD death. This familial risk of premature CHD can be due to the action of either genetic influences or environmental influences that are shared by family members, or both. Twin studies are able to discriminate between both sources of familial resemblance. For discretely defined characteristics like the presence or absence of disease, the degree of resemblance can be expressed in terms of concordance rates. Since monozygotic (MZ) twins share all their genes and dizygotic (DZ) twins on average only 50%, a significantly higher concordance for CHD among MZ compared with DZ pairs suggests a genetic component. Early twin studies from the Norwegian, Danish and Swedish twin registries found an increased concordance for the risk of death from CHD among MZ compared to DZ twins (see for a review: De Faire & Pedersen, 1994). In a Norwegian study of 2000 MZ and 3000 DZ twin pairs, for example, concordance rates for CHD death before age 60 were 0.83 in MZ twins against 0.22 in DZ twins (Hopkins & Williams, 1989). The

importance of genetic factors in CHD was confirmed by a recent study, using data from 26 years of follow-up of 21,004 Swedish twins. The relative hazard of dying from CHD when one's twin died of CHD was significantly higher in MZ than in DZ twins of both sexes (Marenberg, Risch, Berkman, Floderus, & de Faire, 1994). These results suggest that sharing genes is more important than sharing family environment in determining mortality from cardiovascular disease in both men and women.

The genetic influence on morbidity and mortality of CHD is probably mediated by the genetic influence on its risk factors. Genes have, for example, been shown to influence traditional risk factors like blood pressure (Snieder, van Doornen & Boomsma, 1995), total serum cholesterol (Heller, de Faire, Pedersen, Dahlén & McClearn, 1993; Boomsma et al., 1996), obesity and distribution of body fat (Bouchard, 1988,1991; Fabsitz, Carmelli & Hewitt, 1992; Meyer, 1995; Herskind, McGue, Sφrensen & Harvald, 1996) and diabetes mellitus (Groop & Eriksson, 1992; Beck-Nielsen & Groop, 1994; Pierce, Keen & Bradley, 1995). Also more behaviorally related risk factors like smoking (Boomsma, Koopmans, van Doornen & Orlebeke, 1994; Koopmans, van Doornen & Boomsma, 1994; Heath & Madden, 1995), physical exercise (Boomsma, van den Bree, Orlebeke and Molenaar,1989; Koopmans, van Doornen & Boomsma, 1994) and diet (Fabsitz, Garrison, Feinleib & Hjortland, 1978; Boomsma, 1990; De Castro, 1993) are known to be influenced by genetic factors. Even after correction for abovementioned risk factors, however, family history of CHD remained an independent risk factor (Hopkins & Williams, 1989). This finding was replicated by twin studies of CHD mortality, which found that genetic factors remained to have an independent influence on mortality from CHD (De Faire & Pedersen, 1994). These results suggest that the genetic risk for CHD is only partly mediated by abovementioned risk factors. Furthermore, the prediction of new cases of CHD on the basis of knowledge of traditional risk factors is limited. Together they explain only about half of the variance in CHD incidence (van Doornen, 1991). The inability of known risk factors to predict new cases of CHD satisfactorily, implicates that the causal path from genes to the actual occurence of a heart attack via the whole range of possible intermediate steps is still imperfectly understood. Other, as of yet still unknown, risk factors therefore have to be considered. Van Doornen (1991) states that attention to the dynamics of risk factors may increase our understanding of very early disregulations in the body that eventually lead to higher static levels of traditional risk factors. Physiological mechanisms that are activated as part of the response to psychological stress have, for example, been hypothesized to precede hypertension and higher levels of serum cholesterol in later life. Therefore, the measurement of reactivity and variability of risk factors like blood pressure and elements of the lipid system may add to the predictive power of risk for CHD factors, over and above the prediction on the basis of their static measurement. Intermediate phenotypes and mechanisms, like measures of stress-reactivity for example, thus may influence CHD risk through their effects on levels of traditional risk factors. More knowledge of these intermediate phenotypes and their genetic and environmental causes, will probably improve our

understanding of the etiology of traditional risk factors and eventual CHD.

Sing and Moll (1989) proposed a model for linking genes to endpoints of CHD, dividing intermediate risk factors into gene products and measures of metabolism. Pearson (1991) expressed similar ideas by proposing a subdivision of risk factors for atherosclerosis in proximal and distal ones. Distal risk factors would be those with the biological capability of being direct causes for the growth of atherosclerotic plaques. Proximal risk factors would also be associated with atherosclerosis but rather by causing or working through the distal factors. Another scheme which discriminates four steps in the development of atherosclerosis was devised by Hopkins and Williams (1989). Risk factors could be subdivided into initiators, promoters, potentiators and precipitators. With this scheme the amazing number of more than 250 putative risk factors was classified. However, not only the search for new (intermediate) risk factors is likely to lead us to a better understanding and prediction of new cases of CHD. More recent models increasingly recognize the complex multifactorial origin of CHD. Therefore, a stronger emphasis is put on the complex interactions of the multiple risk factors for CHD, which have, for example, been described as a network of intermediary traits (Sing et al., 1992) or a tangled web (Krauss, 1991).

Influence of sex and age

There is a marked difference in CHD incidence between the sexes (Goldbourt, 1994). Before the age of menopause, the male:female incidence ratio is reported to be 9:3 (Lobo, 1990). Being male is thus an important risk factor for CHD. Data from the Framingham study, for example, showed that being male was even a more important risk factor than well established factors like smoking and cholesterol level (Breslow, 1991). It is therefore of great importance to consider sex differences in studying the architecture of genetic and environmental sources of individual differences in risk factors for CHD. Another important aspect of cardiovascular risk is that it is not static, but changes over time. The CHD risk profile at one age may well be different from the profile at a later age. Both the mean and variance of risk factor traits may change with age (Sing & Moll, 1989; Reilly, Kottke & Sing, 1990), which implicates that the expression of genetic and environmental influences on CHD risk factors may also be age-dependent.

Outline of the project

The studies reported in this thesis are part of a project in which cardiovascular risk factors were studied in a group of 213 middle-aged twins, aged between 34 and 63 years. Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry and sollicitation through the Dutch Twin Club. In addition, a small number of twins who heard from the study in another way volunteered to participate. Twins always came in pairs and arrived at the laboratory at about 10.00 am. For the rest of the day (till about 3.00 pm) the pair was subjected to a large range of tests and measurements, which were selected to yield an as complete as possible assessment of risk factors for CHD (see Table 1.1 for a summary). This thesis reports on part of these measurements and variables. Others will be the subject of future publications.

Main questions of the thesis

It has now become possible to define a simple model, which offers a framework for the main questions of this thesis (Figure 1.1).

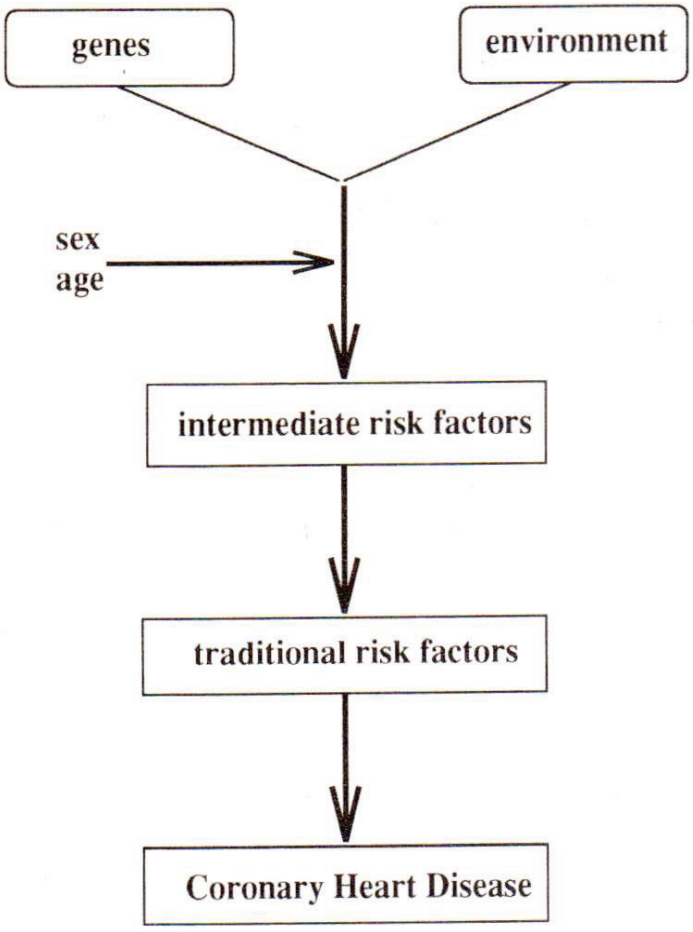

Figure 1.1: General model which offers a framework for the main questions of this thesis.

Table 1.1: Overview of variables measured in 213 middle-aged twins

Biochemical Determinations			
Lipid System	Carbohydrate Metabolism	Haemostasis	Stress Hormones
Total cholesterol	Insulin	Fibrinogen	Noradrenalin[a]
LDL, HDL	Glucose	t-PA	Adrenalin[a]
ApoA1, ApoB		vWf	Cortisol[b]
Lipoprotein(a)			
Free Fatty Acids			

Cardiorespiratory System during rest and 4 stress tasks	
Measurements	Derived Variables
Systolic Blood Pressure	Respiratory Sinus Arrhythmia
Diastolic Blood Pressure	Pre Ejection Period
Mean Arterial Pressure	Stroke Volume
Respiration Rate	Cardiac Output
Heart Rate	Total Peripheral Resistance
Continuous Blood Pressure	Baroreflex Sensitivity
	Approximate Entropy

Anthropometric Measurements	
Measurements	Derived Variables
Length	Body Mass Index
Weight	Waist Hip Ratio
Waist Circumference	Percentage Body Fat
Hip Circumference	
4 Skinfolds	

Questionnaires		
Personality	Health Behavior	Miscellaneous
Type A	Smoking	Socio-Economic Status
Trait Anger	Alcohol Use	Education
Hostility	Physical Exercise	Social Contact between Twins
Depression	2-day Dietary Record	Family History of CVD & DM
Vital Exhaustion		Personal Disease History
Psychosomatic Complaints		Medication Use

Abbreviations: LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, ApoA1 = Apolipoprotein A1, ApoB = Apolipoprotein B, t-PA = tissue Plasminogen Activator, vWf = von Willebrand factor, CVD = Cardiovascular Disease (Myocardial Infarction, Stroke and Hypertension), DM = Diabetes Mellitus.
[a]in urine, [b]in saliva

Genes and environment exert their influence on the eventual probability of CHD via intermediate phenotypes and traditional risk factors. Intermediate phenotypes in this case are thought to reflect all possible mechanisms, phenotypes or processes which might influence traditional risk factors. All factors of which the predictive value for CHD has been proved more or less unequivocally, are regarded as traditional risk factors. Furthermore, Figure 1.1 shows that the influence of genes and environment may be dependent on age and sex. The main questions that are addressed in this thesis are accordingly: 1) What are the relative contributions of genetic and environmental factors on intermediate and traditional risk factors? and 2) Are these relative contributions a function of age or sex?
The specified model is deliberately kept simple as its main goal is to exemplify the research questions. We recognize, however, that it does not fully reflect the complex etiology of CHD. More complex models offer a better approximation of reality. It can be argued, however, that appreciating complexity means asking complex questions, which can only be answered with more basic knowledge.

Knowledge of the relative contribution of genetic and environmental influences (and their dependency on age and sex) on risk factors for CHD may have important implications regarding the choice of effective strategies for treatment and prevention of CHD disease (Rao & Vogler, 1994; Hewitt & Turner, 1995). First, the existence of genetic predispositions allows the possibility of identification of subgroups of the population that may be most at risk. Second, the influence of environmental factors implies that succesful intervention is certainly possible. On the other hand this does not imply that a trait which expresses a high heritability is untreatable. A highly heritable trait like familial hypercholesterolemia (caused by a mutation in the low density lipoprotein (LDL) receptor), can for example be treated well with the new generation of cholesterol-lowering drugs (HMG-CoA reductase inhibitors), that inhibit cholesterol production by the liver (Leitersdorf, 1994).

Before proceeding with an outline of the thesis, a few words on the twin design, and the way in which sex and age effects can be studied within this design, seem appropriate.

Twin methodology and genetic analysis

The twin model

Quantitative genetics is concerned with sources of individual differences in phenotypes. It therefore considers a phenotype as the sum of the effects of both a genotype and an environment. To gather insight into the genetic and environmental influences on the variance of the phenotype, data from genetically informative subjects are needed. For this goal the study of twins is a suitable method with a long history (Plomin, DeFries & McClearn, 1990; Rao & Vogler, 1994; Hewitt & Turner, 1995). Monozygotic twins share identical genotypes, so any differences between them are theoretically due to their environments. Dizygotic 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 on the equal environment assumption (e.g. Phillips, 1993), most studies specifically executed to test it, have proved it to be valid. Even if shared environment differentially affects MZ and DZ twins, it is unlikely that this has a substantial effect on the trait under study (Plomin, DeFries & McClearn, 1990; Kendler, Neale, Kessler, Heath & Eaves, 1993; Leslie & Pyke, 1993; Braun & Caporaso, 1993; Duffy, 1993; Macdonald, 1993). The extent to which monozygotic twins are more alike than dizygotic twins should, therefore, reflect genetic influences. In the classic twin method, the difference between intraclass correlations for monozygotic twins and those for dizygotic twins is doubled to estimate heritability [$h5=2(r_{MZ} - r_{DZ})$; 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 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 in order to estimate genetic and environmental parameters that best fit observed familial (twin) correlations. Model fitting analyses of twin data have some major advantages above the classic twin methodology: 1) models make assumptions explicit. 2) a test of the goodness-of-fit of the model is provided. 3) estimates of quantitative genetic parameters and their standard errors are given. 4) the fit of alternative models can be compared. 5) more than 2 groups of twins can be analyzed simultaneously. 6) more complex models can be tested by extending the twin design with measures of other family members. 7) generalization from the analysis of one variable (univariate) to multiple variables (multivariate) is relatively easy. 8) time series analysis of longitudinal data becomes possible.

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, neighbourhood, 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 also 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 the phenotype and age, which is the case for many CHD risk factors. By incorporating age into the model, the influence of age on the phenotype can be quantified (Neale & Martin, 1989; Neale & Cardon, 1992). The genetic model can be represented by the follo-

wing linear structural equations:

1) $P_i = hG_i + dD_i + cC_i + dE_i + aA_i$
2) $V_P = h^2 + d^2 + c^2 + e^2 + a^2$

where P is the phenotype of the *i*th individual, scaled as a deviation from zero. G is additive genetic influence, D, dominance genetic influence, C, common (or shared) environmental influence and E, unique (or specific) environmental influence, A is age. G, D, C and E can be conceived of as latent factors with zero mean and unit variance. h, d, c, e and a are factor loadings of the observed variable on the latent factors, they indicate the degree of relationship between latent factors and the phenotype. V_P is the phenotypic variance. Because the latent factors have unit variance, squaring the factor loadings yields the variance explained by the various components ($V_G = h^2$, $V_D = d^2$, $V_C = c^2$, $V_E = e^2$ and $V_A = a^2$). 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, on the basis of the opposite effects of D and C on the MZ and DZ twin correlations it can be decided which effect is more likely. D tends to produce DZ twin correlations less than one half the MZ twin correlations and C inflates the DZ correlation to be greater than one half the MZ correlation. Models constraining all genetic effects to be non-additive are considered unlikely, because they lack a sensible biological interpretation (Neale & Cardon, 1992).

Although univariate genetic analysis provides estimates of the contributions of additive and non-additive 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 not only involves a decomposition of phenotypic variances, it also 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 risk factors of CHD.

Investigation of sex and age effects

For the study of sex differences within a twin design both same-sex male and

female MZ and DZ twin pairs and opposite-sex DZ twin pairs have to 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. Alternatively, there may be differences in phenotypic variance, even though the same relative magnitudes of genetic and environmental effects are found in males and females. This model is called the scalar-effects model, as the difference in phenotypic variance is accounted for by a scalar multiplier.

As mentioned earlier, it may well be possible that different genes (or environments) are influential on CHD risk factors, in different periods of life. Longitudinal studies, in which genetically informative subjects are measured repeatedly, are normally needed to test such an hypothesis. In this study an alternative approach was taken. Data from an earlier project (Boomsma, 1992) in which parents with their twin offspring were measured were combined with data from middle-aged twins, collected in this project. Combining the data of both projects enabled the specification of an extended quantitative genetic model with which the genetic transmission from parent to offspring could be estimated. Thereby offering a test for the age-dependency of genetic or environmental influences (Stallings, Baker & Boomsma, 1989; Snieder, Boomsma & van Doornen, 1993; Snieder, van Doornen & Boomsma, 1995).

Outline of the thesis

Chapters 2 and 3 of this dissertation focus on variables that can be regarded as intermediate risk factors. The second chapter is concerned with Respiratory Sinus Arrhythmia (RSA), an index of the vagal control of the heart. As such, RSA is thought to play an important role in blood pressure regulation. The major aim of this study, was to estimate the influence of genetic and environmental factors on RSA during rest and mental and physical stress, while taking account of the influence of age and respiration rate. Further, it was investigated whether the origin of the covariance between RSA and RR is genetic, environmental or both. To be able to answer those questions, a multivariate model including RSA, respiration rate and age was applied.

The third chapter focuses on fasting insulin, which is considered the best marker of insulin resistance in population based studies. Insulin resistance is thought to be a predictor of cardiovascular disease via a cluster of interrelated cardiovascular risk factors, which has been called "Syndrome X" or "insulin resistance syndrome". The main aim of the study reported in this chapter, was to estimate the relative

influence of genes and environment on fasting insulin. Furthermore, the origin of the covariance between fasting glucose and fasting insulin was investigated with a reciprocal causation model.

Chapters 4 and 5 report on studies that investigated the age-dependency of genetic and environmental influences on blood pressure and the lipid system. In addition to blood pressure and total plasma cholesterol, usually regarded as traditional risk factors, intermediate traits like blood pressure reactivity (Chapter 4) and apolipoproteins (Chapter 5) were incorporated in the studies. In both chapters a quantitative genetic model was used, in which in addition to the data of middle-aged twins collected in this project, data from parents and their twin offspring, collected in an earlier project, could be analysed. With this extended model the age-depency of the expression of genes and environment on the CHD risk traits were estimated.

Finally, Chapter 6 provides a discussion of the results, the practical implications of these results for treatment and prevention of CHD, and possible directions of future research.

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2

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

Harold Snieder, Dorret I. Boomsma, Lorenz J.P. van Doornen
and Eco J.C. de Geus

INTRODUCTION

Repeated increases in heart rate and blood pressure as a consequence of physical and psychological challenge, are supposed to have a role as a risk factor or indicator for cardiovascular disease (Menkes et al., 1989; Matthews, Woodall & Allen, 1993). 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 have to be studied. One of these mechanisms is the influence of the autonomic nervous system on the heart. Its influence 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) has shown to be a specific and sensitive non-invasive index of cardiac vagal tone (Cacioppo et al., 1994; Berntson et al., 1994). 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.

Recently, however, various studies have raised the question to what extent RSA is a valid index of tonic vagal control of the heart when the influence of respiratory parameters are not taken into account (Grossman & Wientjes, 1986; Saul, Berger, Chen & Cohen, 1989; Allen & Crowell, 1990; Kollai & Mizsei, 1990; Grossman, Karemaker & Wieling, 1991; Kollai & Kollai, 1992; Hayano, Mukai, Hori, Yamada & Fujinama, 1993; Grossman & Kollai, 1993). Many studies have documented a significant covariance (correlation coefficients between -.30 and -.70) between respiration rate and RSA at rest and during various stress tasks (Kollai & Mizsei, 1990; Kollai & Kollai, 1992; de Geus et al., 1993; de Geus, Willemsen, Klaver & van Doornen, 1995). Rapid low-tidal volume breathing will reduce the degree of RSA, and slow high-volume breathing will increase RSA. These variations in RSA produced by variations in respiration rate and volume need not reflect changes in cardiac vagal tone (Grossman & Kollai, 1993). This implicates that only when

respiratory variables are held constant, or are statistically controlled for, there is a reasonable correspondence between variations in vagal tone and RSA amplitude.

A strong RSA is regarded as an index of good cardiovascular health. Several studies have shown reduced heart rate variability in cardiac disease (Hinkle, Carver & Plakun, 1972; Kleiger, Miller, Bigger & Moss, 1987; Martin et al., 1987; Singer et al., 1988; Hayano et al., 1991) and hypertension (Julius, Pascual & London, 1971; Malliani et al., 1991). Furthermore, RSA measures are clearly reduced with increasing age (Mφlgaard, Hermansen & Bjerregaard, 1994; Ryan, Goldberger, Pincus, Mietus & Lipsitz, 1994) and under conditions of psychological stress (Kamphuis & Frowein, 1985; Grossman & Svebak, 1987; Aasman, Mulder & Mulder, 1987; Mulder, 1988; Langewitz & Rüddel, 1989; Allen & Crowell, 1989; de Geus, van Doornen, de Visser & Orlebeke, 1990; de Geus, van Doornen & Orlebeke, 1993) even when changes in respiration rate are accounted for (Grossman, Stemmler & Meinhardt, 1990; Grossman, van Beek & Wientjes, 1990; Grossman, Brinkman & de Vries, 1992). A recent study (Ryan et al., 1994) found significant sex differences in heart rate variability, women having slightly higher values than men.

A striking feature of virtually all studies on RSA, is that they report large individual differences as indexed by standard deviations. Standard deviations are large at rest as well as during conditions of physical and psychological challenge. This means that there are large individual differences in baseline RSA as well as in RSA responses to various stimuli. 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). This study used twins to quantify the genetic and environmental sources of individual differences. In 160 adolescent twin pairs, RSA was measured during a rest period and 2 stressful laboratory tasks. During rest, only 25% of the variance in RSA was accounted for by genetic influences. Under task conditions, 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 was attributed to a decrease in unique environmental variance during stress compared to rest. Although not specifically tested, twin correlations did not suggest different heritabilities for boys and girls. Also, no effort was made to correct RSA for differences in age and respiration rate.

In the present study, we investigated RSA in middle-aged twins during rest and four different stress tasks, with the aim to estimate genetic and environmental influences on RSA during these conditions. A multivariate model including age, RSA and respiration rate (RR) was used in the quantitative genetic modeling. This enabled us to quantify the genetic and environmental sources of individual differences in RSA in rest and under stress, while simultaneously taking account of the influence of age and respiration rate. Furthermore, it was tested whether the origin of the covariance between RSA and RR could be explained by correlated genetic factors, correlated environmental factors or both. Possible sex differences in the determinants of variance in RSA and covariance between RSA and RR were

investigated and best fitting models in rest were compared with best fitting models under stress conditions.

METHODS

Subjects

This study is part of a project in which cardiovascular risk factors were studied in 213 middle aged twin pairs (aged between 34 and 63). Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry and sollicitation through the Dutch Twin Club. In addition, a small number of twins who heard from the study in another way volunteered to participate. Data from 5 twin pairs were excluded from the sample. In one twin pair measurements were incomplete. One pair was dropped because one member of the twin 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 subject. In total 202 males (age: 43.6 ± 6.4) and 214 females (age: 44.7 ± 6.8) 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 males (MZM), 39 pairs of dizygotic males (DZM), 48 pairs of monozygotic females (MZF), 40 pairs of dizygotic females (DZF) and 38 dizygotic pairs of opposite sex (DOS).

Procedure

Twins arrived at the laboratory at about 10.00 a.m. They were asked to refrain from smoking, drinking alcohol, coffee or tea after 11.00 p.m. the night before. Electrodes were attached for Electrocardiogram (ECG) recording and a strain-gauge was strapped around the waist to measure respiration. Subjects were measured during rest, three mental stress task conditions and a physical stressor: the Cold Pressor test (CP). Mental stress tasks consisted of a Choice Reaction Time (RT) task, a speeded Mental Arithmetic (MA) task and a Tone Avoidance (TA) reaction time task. The RT and MA task were used to ensure compatibility with a previous twin study from our laboratory, in which these tasks evoked significant cardiovascular responses (Boomsma, van Baal & Orlebeke, 1990). The TA 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 CP test results in a strong vascular reaction pattern (de Geus et al., 1990).

Each mental task condition lasted 8.5 minutes and was preceded by a rest period of three minutes. During testing, subjects were comfortably seated in reclined position in a dimly lit and sound shielded cabin. They faced a computer screen placed at two meters 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 the RT task. In the RT 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 seconds it had disappeared completely and simultaneously a reaction stimulus was heard. Subjects had to react to high tones by pressing a button on a panel labeled 'Yes' and to low tones by pressing a button labeled 'No'. Two seconds later subjects received feedback on the computer screen, indicating whether they had pushed the correct button and, in case the response was correct, also their reaction time. The task consisted of 36 trials.

The second task was the MA task. In the MA task twins had to add 3 numbers that were presented in succession on the computer screen. Five seconds after the first number the answer to the addition problem appeared on the screen. Half of the presented answers were correct, half incorrect. Subjects 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 RT task and after 2 more seconds the next trial was started. The MA problems contained 10 levels of difficulty: ranging from 3 1-digit numbers (e.g. 9+5+4) to 3 2-digit numbers (e.g. 85+79+47). The MA task also consisted of 36 trials.

The third task was the TA task. During the TA task the twins had to attend the occurence of a stimulus (an 'X') that flared up shortly (500 msec.) in one of the corners of the computer screen. They had to respond as fast as possible to this stimulus by pressing the button opposite to this corner on their response panel. Incorrect or too slow responses were punished with a loud noise burst that lasted 500 msec. 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 1500 msec. Reaction time had to be shorter than the response criterium, which was initially set to 650 ms. During the task the response criterium was continuously adapted to the performance of the twin. The task was thus made more demanding if the subject started to perform better, with the intention to make the task equally stressful for all subjects. The whole task consisted of about 210 trials.

Before the actual start of the experiment, subjects were offered a training session during which RT and TA task were practised for a short time. The MA task was practised entirely to determine the level of difficulty at which the subject had to start the MA task during the actual experiment. The first problem for the MA practice

session always was at the first level of difficulty. The level of the next problems depended on the twins responses. The level reached by the twins after the 36 trials determined the level at which the twin started during the experimental performance of the MA task. This procedure was developed so that the MA task would be equally stressful for all twins.

After performance of the three mental stress tasks, basal levels of the cardiorespiratory variables were determined during a 8.5 minute post-stress rest period. During all rest periods the twin was asked to relax as much as possible. The post-stress rest period was followed by a Cold Pressor test (CP). The subject was told to immerse his or her preferred hand in ice water and keep it there for a period of 2 minutes. Subjects changed places in the cabin several times. When one subject was tested, the other subject filled in questionnaires. Sequence of events was: practice sessions, pause (outside of the cabin), rest1, RT, rest2, MA, rest3, TA, another break (again outside of the cabin), post-stress rest, CP.

Physiological variables

ECG disposable pregelled Ag-AgCl electrodes (AMI type 1650-005 Medtronic) were placed on the tip of the sternum and the lateral margin of the chest, according to the standard lead II configuration. The respiration signal was recorded with a strain-gauge of hollow silastic tube strapped around the waist at a level 7 cm above the umbilicus. An acoustic tone is transmitted from one end and received at the other so that changes in the phase angle of the signal are entirely caused by changes in chest circumference. Previously this system has shown to yield reliable estimates of respiratory activity (Grossman, Brinkman & de Vries, 1992).

The ECG 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 ECG signal was recorded using a Nihon Kohden bioelectric amplifier (AB 601G) with a time constant of 0.1 sec and a 30 Hz high cut-off filter. The respiration signal was filtered by a 30 Hz cut-off filter as well.

ECG data were used to determine the time between successive R-waves in msec. The respiration signal was computer-scored to obtain Total Expiration Time (TTE), and the Total Inspiration Time (TTI) on a breath to breath basis (in msec.). TTI was computed as the sum of inspiration period and inspiratory pause. TTE as the sum of the expiration period and expiratory pause. Total Cycle Time (the sum of all four intervals) was recoded to RR, expressed in cycles per min. 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. Breathing cycles that were not considered valid were rejected and removed from further processing.

Respiratory Sinus Arrhythmia was computed by the peak-to-trough method (Grossman & Wientjes, 1986). Very high correspondence (interindividual correlations above .92) has been found between the peak-to-trough method and spectral measurements of RSA (Grossman, van Beek & Wientjes, 1990). The peak-to-trough method combines the respiratory time intervals and the inter beat

intervals to obtain the shortest inter beat interval during heart rate acceleration in the inspirational phase (which was made to include 750 milliseconds from the folowing expiration to account for phase shifts) and the longest inter beat interval during deceleration in the expirational phase (including 750 milliseconds 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 a RSA score of zero. Mean RSA in milliseconds was computed for post-stress 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 RR means during the tasks and the post-stress rest condition.

Statistical analysis

To test for the effects of stress tasks, sex and zygosity on RR and RSA, MANOVA was used. RR and RSA were analyzed as dependent variables with condition (Rest, RT, MA, TA, CP) as within factors, and sex and zygosity as between factors. As 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 HSD was used to follow up on significant main and interaction effects (p's < 0.05). To obtain a normal distribution, RSA was transformed by natural logarithm.

Genetic analysis

For the genetic analyses, age, RR and RSA, measured in twin and co-twin, were summarized into 5 x 5 variance-covariance matrices using PRELIS (Jöreskog & Sörbom, 1986), for each of the 5 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 Akaike's information criterion (AIC). The overall χ^2 tests the agreement between the observed and predicted variances and covariances in the different zygosity groups. A large chi-square indicates a poor fit (low probability), while a small chi-square indicates that the model is consistent with the data (high probability). Submodels were compared by hierarchic χ^2 tests, in which the χ^2 for a reduced model is subtracted from that of the full model. The degrees of freedom (df) for this test are equal to the difference between the df 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 χ^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).

A multivariate model with RR entered as the first and RSA as the second variable was used to answer the research questions. Age was incorporated into the model to account for the well know negative correlation between RSA and age. The model is presented in Figure 2.1.

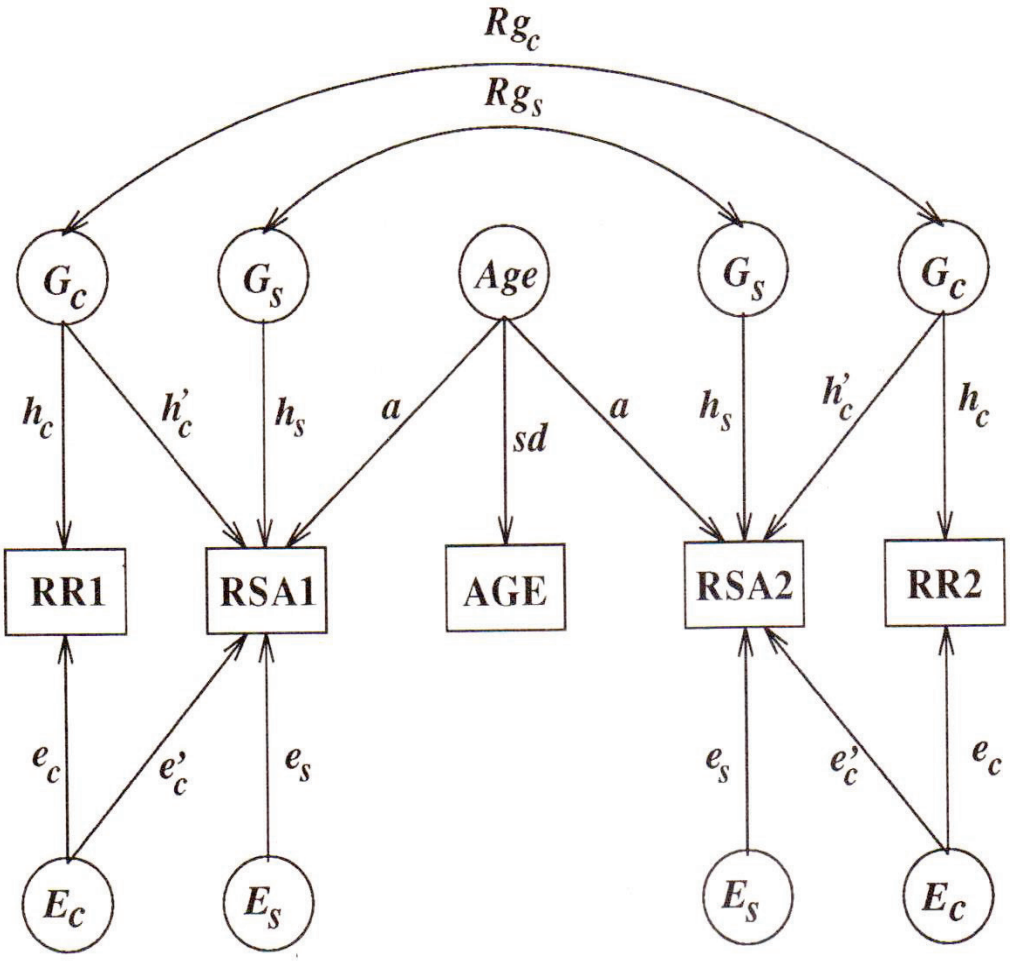

Figure 2.1: Multivariate model with RR entered as the first and RSA as the second variable. Age is also included in the model. The path between age and RR is not shown because it could be constrained to zero. Observed phenotypes for twin 1 and twin 2 are shown in squares. Latent factors are shown in 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 1 in MZ twins, 0.5 in DZ same-sex twins and can be estimated for DOS twins. Factor loadings of observed variables on the different latent factors are also shown: h'_c = additive genetic variance common to RR and RSA, h_s = additive genetic variance specific for RSA, e'_c = unique environmental variance common to RR and RSA, e_s = unique environmental variance specific for RSA, h_c = additive genetic variance in RR, e_c = unique environmental variance in RR, a = variance in RSA attributed to age, 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.

The observed phenotypes for twin 1 and twin 2 are shown in squares, whereas latent factors are shown in circles. Latent factors are subdivided into genetic and environmental factors common to RR and RSA (G_c and E_c), and genetic and

environmental factors specific for RSA (G_s and E_s). The covariance between RR and RSA consists of a genetic part (h_c * h'_c) and an environmental part (e_c * e'_c). These covariances divided by the square root of V_{G}*rr**V_{G}*rsa* and V_{E}*rr**V_{E}*rsa* respectively, yield the genetic and environmental correlation between RR and RSA. Correlations between the latent genetic factors (r_{Gc} and r_{Gs}) are 1 in MZ twins and 0.5 in DZ twins. Factor loadings 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_{tot}^2, calculated as: $(h'^2_c + h_s^2)/V_P$*rsa*] and the genetic influence on RSA, corrected for influence of RR [h_{cor}^2, 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 males and females, with a reduced model in which parameter estimates are constrained to be equal across the sexes. In addition to sex differences in magnitude, it was tested whether gene effects are sex-specific: r_{Gc} and r_{Gs} in the DOS group were estimated as free parameters (i.e., allowed to be smaller than the normal value of 0.5) or fixed to zero.

RESULTS

Figure 2.2 shows RSA in rest and during the 4 different stress tasks in males and females. MANOVA 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 was due to the RSA levels being more similar between the sexes during the cold pressor test than during the rest and RT condition.

Figure 2.3 shows RR in rest and during the 4 different stress tasks in males and females. Also for RR, MANOVA demonstrated a highly significant condition effect in the expected direction ($F(4,200)=477.58$, $p<.001$): post-hoc testing showed that RR 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 was due to the RR levels being more similar between the sexes during the RT and MA tasks than during the CP test.

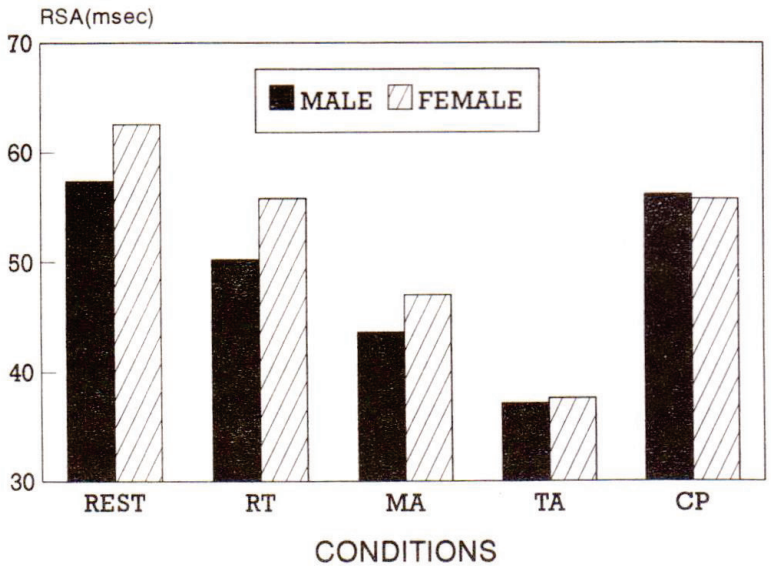

Figure 2.2: RSA in rest and during the 4 different stress tasks in males and females. RT = Reaction Time task, MA = Mental Arithmetic task, TA = Tone Avoidance task, CP = Cold Pressor task.

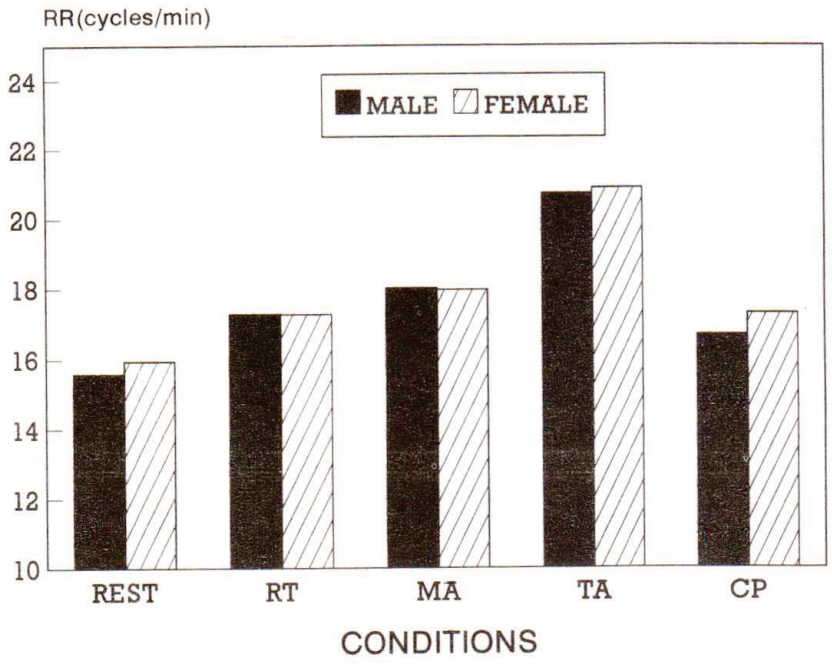

Figure 2.3: RR in rest and during the 4 different stress tasks in males and females. For abbreviations see Figure 2.2.

In Table 2.1 intraindividual cross correlations between age, RSA and RR are presented for males and females. Relatively high negative correlations between age and RSA and between RR and RSA were found for both sexes in all conditions. No correlation was observed between age and RR.

Table 2.1: Intraindividual cross correlations for males (below diagonal; n=202) and females (above diagonal; n=214) between age, respiratory sinus arrhythmia and respiration rate

	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	

Abbreviations: RSA = Respiratory Sinus Arrhythmia, RR = Respiration Rate, RT = Reaction Time task, MA = Mental Arithmetic task, TA = Tone Avoidance task, CP = Cold Pressor task.

Twin correlations of RSA and RR in rest and stress task conditions are shown in Table 2.2. In general MZ correlations are larger than DZ correlations for both RSA and RR, 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.

Table 2.2: Twin correlations of respiratory sinus arrhythmia and respiration rate in rest and stress task conditions for each zygosity group

	Rest			RT			MA			TA			CP		
	N	RSA	RR	N	RSA	RR	N	RSA	RR	N	RSA	RR	N	RSA	RR
MZM	43.45	.62		43	.42	.57	43	.41	.51	42	.33	.49	43.45	.61	
DZM	39.20	.12		39	.07	.45	39	.12	.47	38	-.09	.37	39.22	.20	
MZF	48.47	.72		47	.62	.68	48	.62	.54	48	.48	.62	47.56	.55	
DZF	40.35	.07		40	-.11	.23	40	-.04	.29	40	-.03	.24	40.19	.12	
DOS	38.26	.27		36	.40	.03	37	.57	.14	38	.46	.10	38.47	.12	

Abbreviations: N = Number of twin pairs, MZF = Monozygotic Females, MZM = Monozygotic Males, DZF = Dizygotic Females, DZM = Dizygotic Males, DOS = Dizygotic Opposite Sex.
For further abbreviations see Table 2.1.

Table 2.3 shows χ^2-values and probabilities of multivariate models for rest and stress conditions. In the upper part of the table models are shown, in which parameter estimates are allowed to be different between the sexes. In the lower part of the model these estimates are set equal. The upper part of the table also shows GE

models testing whether gene effects are sex-specific: r_{Gc} and r_{Gs} in the DOS group were estimated as free parameters (i.e., allowed to be smaller than the normal value of 0.5) or fixed to zero. Only in the RT and TA task a model in which r_{Gc} was set to zero instead of 0.5, fitted slightly better. This was most probably due to the low RR correlation in the DOS group in these conditions. In all conditions, models estimating additive genetic (G) and unique environmental (E) factors fitted best. The GE model in which parameter estimates for males and females were constrained to be equal, did not fit significantly worse than the GE model in which parameter estimates were allowed to be different in magnitude for males and females. This means that the models without sex differences offer the most parsimonious solution. In the mental stress conditions (RT, MA and TA), the fit of the models is better (lower χ^2-values and higher probabilities) than during rest and the CP test.

Table 2.3: χ^2 values with p between parentheses of multivariate models for rest and stress conditions, with (sex differences) and without sex differences (no sex differences). Variations of the GE model with sex differences, testing wether gene effects are sex-specific, are also shown

Model	df	Rest	RT	MA	TA	CP
Sex differences						
GCE	54	88.42 (.002)	70.03 (.070)	63.46 (.177)	57.68 (.341)	86.16 (.004)
GDE	54	84.58 (.005)	68.42 (.090)	64.35 (.158)	55.57 (.415)	82.42 (.008)
GE						
r_{Gc} & r_{Gs}free	58	90.18 (.004)	76.34 (.054)	68.70 (.159)	58.47 (.458)	86.04 (.010)
r_{Gc} & r_{Gs}=0.5	60	**90.18 (.007)**	78.29 (.057)	**68.95 (.201)**	59.19 (.506)	**86.44 (.014)**
r_{Gc}=0,r_{Gs}=0.5	60	93.28 (.004)	**76.41 (.075)**	69.27 (.193)	**58.78 (.520)**	86.58 (.014)
CE	60	109.08 (.000)	88.68 (.009)	76.09 (.079)	70.08 (.175)	100.49 (.001)
No sex differences						
GCE	64	95.90 (.006)	78.19 (.109)	70.87 (.260)	63.31 (.501)	90.38 (.017)
GDE	64	90.93 (.012)	74.40 (.176)	69.70 (.292)	61.41 (.569)	87.20 (.028)
GE	67	**96.16 (.011)**	**80.14 (.130)**	**72.50 (.302)**	**63.31 (.605)**	**90.38 (.030)**
CE	67	115.30 (.000)	92.41 (.022)	81.19 (.114)	74.87 (.238)	107.87 (.001)

Abbreviations: df = degrees of freedom, G = Additive genetic variance, D = Dominance genetic variance, E = Unique environmental variance, C = Shared environmental variance, r_{Gc}= correlation in the DOS group between latent genetic factors common to RR and RSA, r_{Gs}= correlation in the DOS group between latent genetic factors specific for RSA. Most parsimonious solutions for models with and without sex differences are printed in **boldface** type. For further abbreviations see Table 2.1.

In Table 2.4, variations of the GE models without sex differences are shown. In this case the influence of age on RR and RSA in all 5 conditions is estimated. Influence of age on RR could be set to zero without a significant loss in fit and is therefore not shown in Figure 2.1. If, on the other hand, the influence of age on RSA was also set to zero, the fit of the models became significantly worse.

Table 2.4: χ^2 values with p between parentheses of GE models without sex differences, estimating the influence of age on respiration rate and respiratory sinus arrhythmia for rest and stress conditions

Model	df	Rest	RT	MA	TA	CP
a_{RSA} & a_{RR} free	66	96.10 (.009)	80.12 (.114)	70.92 (.201)	62.76 (.590)	89.90 (.027)
a_{RR}=0	67	**96.16 (.011)**	**80.14 (.130)**	**72.50 (.302)**	**63.31 (.605)**	**90.38 (.030)**
a_{RSA} & a_{RR}=0	68	156.93 (.000)	125.81 (.000)	106.02 (.002)	95.79 (.015)	143.24 (.000)

Abbreviations: RSA = Respiratory Sinus Arrhythmia, RR = Respiration Rate, a_{RSA} = influence of age on RSA, a_{RR} = influence of age on RR.
Most parsimonious solution is printed in **boldface** type. For further abbreviations see Table 2.1.

To test for the origin of the covariance between RR and RSA, it was investigated whether one or both of the connecting paths between RR and RSA (h'_c and e'_c) could be set to zero. Results are presented in Table 2.5. In none of the conditions, the connecting paths could be set to zero without a significant loss in fit. This suggests that the covariance between RR and RSA has both a genetic and an environmental basis.

Table 2.5: χ^2 values with p between parentheses of GE models without sex differences, testing for the origin of covariance between respiration rate and respiratory sinus arrhythmia for rest and stress conditions

Model	df	Rest	RT	MA	TA	CP
h'_c & e'_c free	67	**96.16 (.011)**	**80.14 (.130)**	**72.50 (.302)**	**63.31 (.605)**	**90.38 (.030)**
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 (.000)	111.79 (.001)	106.32 (.002)	90.99 (.033)	118.96 (.000)
h'_c & e'_c=0	69	165.87 (.000)	169.28 (.000)	181.21 (.000)	148.19 (.000)	198.06 (.000)

Abbreviations: h'_c = additive genetic factor loading common to RR and RSA, e'_c = unique environmental factor loading common to RR and RSA.
Most parsimonious solution is printed in **boldface** type. For further abbreviations see Table 2.1.

Table 2.6 shows non-standardized variance components of RSA and RR 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 to rest. In the RT and MA task an increase in total genetic variance of RSA went together with a decrease in total unique environmental variance. This pattern was reversed in the TA task where V_E*rsa* increased and V_G*rsa* decreased.

Table 2.6: Variance components of RSA and RR as estimated by the best fitting models for rest and stress conditions

	RSA				RR		
	V_G	V_E	V_A	V_P	V_G	V_E	V_P
Rest	.0844	.1471	.0406	.2721	4.38	2.65	7.03
RT	.0887	.1320	.0298	.2505	4.80	3.14	7.94
MA	.1104	.1243	.0217	.2565	3.96	3.88	7.84
TA	.0760	.1703	.0215	.2678	5.57	4.66	10.23
CP	.1224	.1464	.0392	.3080	5.40	4.82	10.22

Abbreviations: 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.
For further abbreviations see Table 2.1.

Table 2.7 presents standardized parameter estimates of best fitting models for rest and stress conditions. Variance in RSA can be attributed to 5 different sources (see also Figure 2.1) that all have significant influence. The relative contribution of total genetic influence on RSA ($h_{tot}^2 = (h'_c{}^2 + h_s^2)/V_P rsa$) varied between 28% (TA) and 43% (MA). Furthermore, 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 RR, were very similar (from 32% in TA to 48% in MA). From 14% (Rest) to 21% (MA, CP) of the variance in RSA could be explained by the influence of RR ($h'_c{}^2 + e'_c{}^2$), whereas the influence of age varied between 8% (MA, TA) and 15% (Rest). Heritability of RR (h_c^2) did not change much over conditions. It ranged from .51 in the MA task to .62 in Rest. Table 2.7 also shows genetic (r_g) and environmental (r_e) correlations between RR and RSA. r_g and r_e have the same sign (negative) and a comparable range (from -.36 to -.50).

Table 2.7: Standardized parameter estimates of best fitting models for rest and stress conditions

	RSA							RR		$r_{(RSA,RR)}$	
	h'^2_c	h^2_s	h^2_{tot}	h^2_{cor}	e'^2_c	e^2_s	a^2	h^2_c	e^2_c	r_g	r_e
Rest	.07	.24	.31	.34	.07	.47	.15	.62	.38	-.46	-.37
RT	.04	.31	.35	.44	.14	.39	.12	.60	.40	-.36	-.52
MA	.09	.34	.43	.48	.12	.36	.08	.51	.49	-.46	-.50
TA	.04	.24	.28	.32	.14	.50	.08	.54	.46	-.41	-.47
CP	.10	.30	.40	.45	.11	.37	.13	.53	.47	-.50	-.47

Abbreviations: h'^2_c= additive genetic variance common to RR and RSA, h^2_s= additive genetic variance specific for RSA, h^2_{tot}= total additive genetic influence on RSA, h^2_{cor}= additive genetic variance in RSA corrected for RR, e'^2_c= unique environmental variance common to RR and RSA, e^2_s= unique environmental variance specific for RSA, a^2= variance in RSA attributed to age, h^2_c= additive genetic variance to RR, e^2_c= unique environmental variance to RR, r_g= genetic correlation between RSA and RR, r_e= environmental correlation between RSA and RR. For further abbreviations see Table 2.1.

Table 2.8 shows twin correlations for RR and RSA reactivity to the different stress tasks. Reactivity is expressed as the difference between rest and stress level. No meaningful systematic pattern could be observed for the twin correlations of reactivity of RSA and RR. Therefore no further genetic analysis was conducted.

Table 2.8: Twin correlations of reactivity of RSA and RR to different stress task conditions for each zygosity group

	RT		MA		TA		CP	
	RSA	RR	RSA	RR	RSA	RR	RSA	RR
MZM	.28	-.07	.26	.05	.20	-.08	.07	.11
DZM	.27	.55	.27	.19	.19	.24	.05	.13
MZF	.25	.44	.23	.12	.38	.48	.28	.26
DZF	.00	.12	.19	.22	.24	.22	.04	-.11
DOS	.04	.14	.06	.10	.00	.27	.22	.02

Reactivity measured as: stress level - rest level. For further abbreviations see Table 2.1.

DISCUSSION

This study investigated the sources of individual differences in RSA, an index of cardiac vagal tone, in rest and under four different stress conditions. A multivariate model including age, RR and RSA was used to analyze the data. This enabled us to estimate the size of the influence of age and RR on RSA. At the same time, genetic and environmental influences, could be subdivided into parts common and specific to RR and RSA.

Models specifying only additive genetic (G) and unique environmental (E) factors, with parameter estimates constrained to be equal in males and females, gave the best explanation of the data, both in 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 RR did not make much difference: h_{cor}^2 varied from 32% in the Tone Avoidance task to 48% in the Mental Arithmetic task. Values in rest were intermediate for both h_{tot}^2 (31%) and h_{cor}^2 (34%).

In spite of the age difference of the twin samples (adolescent, compared to middle-aged in this study), the study of Boomsma, van Baal and Orlebeke (1990) also found that the best explanation of the data was offered by a model specifying G and E, without sex differences. Boomsma, van Baal and Orlebeke (1990) did not quantify the influence of age and RR on RSA. Because of the restricted age range in the adolescent twin sample, it was not necessary to account for the influence of age. As RR was not incorporated into the model fitting, heritabilities in the adolescent twin study can best be compared with estimates of total genetic influence (h_{tot}^2) in this study. During rest heritabilities were similar in magnitude (25% vs 31%). Heritabilities for both tasks (RT and MA; the same tasks as in this study) were around 50%; slightly higher than estimates in our study (35% for RT and 43% for MA). Boomsma, van Baal and Orlebeke (1990) thus found a higher heritability under stress, which was due to a smaller contribution of unique environmental variance as compared to rest. Our study showed that this stress-induced rise in heritability is not a universal phenomenon. Results in the RT and MA task were similar: total unique environmental variance decreased and total genetic variance increased, which led to a higher h_{tot}^2 than in rest. During the TA task, however, the pattern was reversed: total unique environmental variance increased and total genetic variance decreased, which led to a heritability of the TA task (28%), which was even lower than in rest (31%).

In all conditions, the path from age to RR could be set to zero, which corresponded to the absent intraindividual cross correlations between age and RR. 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 (Mφlgaard, Hermansen & Bjerregaard, 1994; Ryan et al., 1994), which is said to reflect a loss with age in flexibility of the nervous system in response to environmental demands (Porges & Byrne, 1992). In the study of Boomsma, van Baal

and Orlebeke (1990) not only adolescent twins but, although these data were not published, also their parents were measured. These parents were of similar age as the middle aged twins in this study. Their RSA values, both in rest and during RT and MA tasks, were highly similar to the values in the middle aged twins, but considerably lower than the RSA values of their children. (see Figure 2.4).

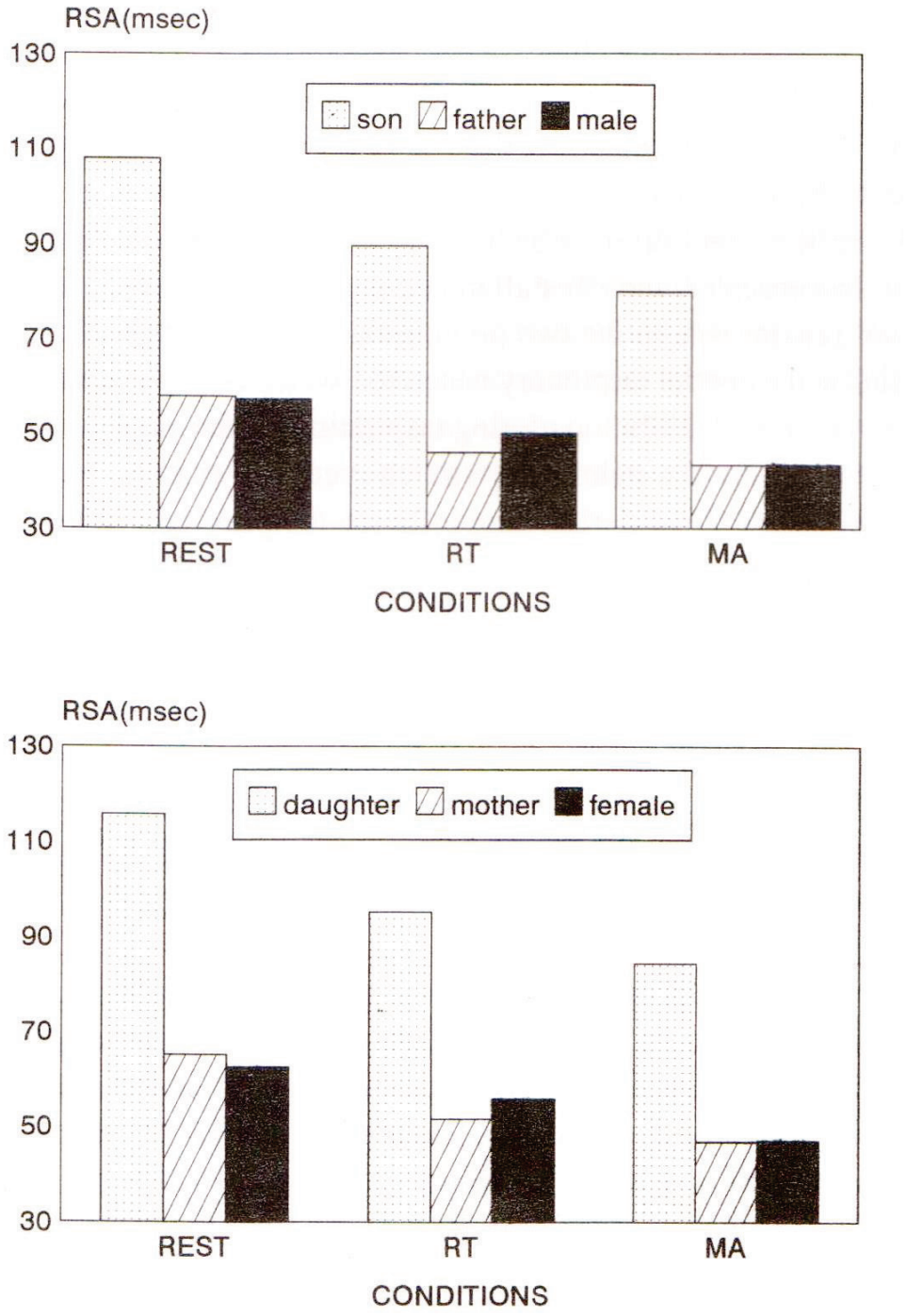

Figure 2.4: RSA in rest and during RT and MA tasks in parents and their children (data from the study of Boomsma, van Baal and Orlebeke, 1990) compared to males and females from this study.

The parents were of similar age as the middle aged twins in this study. A) Sons, their fathers and middle aged males. B) Daughters, their mothers and middle aged females.

Figure 2.4 also shows that the decrease in RSA between adolescence and middle age is larger than the age related decrease in our own age cohort (34 to 63) which accounted for only 8% to 15% of the variance.

In the 5 experimental conditions, 14% to 21% of the interindividual variance in RSA could be explained by the influence of RR. The neurophysiological basis of RSA and the closely related role of respiration therein, has recently been described by Berntson, Cacioppo & Quigley (1993). RSA has both tonic and phasic determinants, which in turn have their primary origin in central nerve networks or in peripheral afferent inputs. The tonic influence of the vagus consists of a central vagal drive, which represents the aggregate of tonic central excitatory influences on vagal motor neurons, and a reflex vagal drive, which arises from the vagoexitatory actions of chemoreceptor, baroreceptor, and other afferent inputs to the nucleus tractus solitarius. Respiration has a major role in the two predominant sources of phasic modulation of RSA. The first is the central respiratory generator, which is responsible for excitation (during expiration) and inhibition (during inspiration) of the vagal motor neurons. The second is formed by the pulmonary stretch receptors that have an inhibitory influence on tonic vagal drive during lung inflation (inspiration). In this study, the covariance between RR and RSA turned out to have both a genetic and a unique environmental origin. Due to the highly complex basis of the relationship between RR and RSA it is virtually impossible to infer (on the basis of this study) which of these neurophysiological mechanisms, responsible for the RR-RSA covariance, are mainly determined by genetic and which by unique environmental influences. Only around 20% of the total genetic influence on RSA in the different experimental conditions, is mediated by RR (h'^2_c/h^2_{tot}). This means that RR-independent genetic effects, for instance on central vagal drive or baroreceptor action must also play an important role.

Grossman and coworkers (1991) reasoned that RSA should be corrected for between and within subjects variations in RR in order to stay a valid index of cardiac vagal tone. In this study we observed that heritabilities of RSA are similar whether they are corrected for RR or not. Possibly, this implies that RSA correction in other between subjects designs may not be necessary to investigate individual differences.

To investigate whether changes in RSA in response to the stress tasks were influenced by genetic factors, twin correlations of RSA and RR reactivity were calculated. These twin correlations of reactivity yielded a pattern incompatible with any biologically plausible model. 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 RR compared to the reliability of its levels. Reactivity is calculated as the difference between two levels, which increases the error term. Another possibility is that someone's reactivity simply is a less reliable person characteristic. These effects might also explain the less consistent heritabilities of blood pressure reactivity compared to those of blood pressure levels, we reported earlier (Snieder, van Doornen & Boomsma, 1995).

To summarize, this study was the first to quantify genetic and environmental origins of individual differences in RSA, while simultaneously taking account of influence of age and respiration rate on RSA. Influence of age and RR 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 RR did not make much difference in this study. The RR-RSA covariance turned out to have both genetic and environmental determinants. The design of this study did not allow, however, to decide which neurophysiological causes of the RR-RSA covariance were mainly genetic and which mainly environmental. 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 like blood pressure and heart rate to phenotypes representing regulating mechanisms of the cardiovascular system. This shift is considered essential as understanding the genetic or environmental basis of the latter phenotypes could bring us closer to an understanding of the way in which disturbance of these mechanisms leads to pathology. This study is a first step towards this goal.

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3

A bivariate genetic analysis of fasting insulin and glucose

Harold Snieder, Dorret I. Boomsma, Lorenz J.P. van Doornen
and Michael C. Neale

INTRODUCTION

Resistance to insulin-stimulated glucose uptake, also called insulin resistance or impaired insulin sensitivity, is considered a major risk factor for progression to both coronary heart disease (CHD) and non-insulin-dependent diabetes mellitus (NIDDM) (Reaven, 1988; Groop & Eriksson, 1992; Beck-Nielsen & Groop, 1994; Pierce, Keen & Bradley, 1995). At first, insulin-resistant individuals are able to keep a normal glucose homeostasis by developing hyperinsulinaemia (Reaven, 1988). This compensatory response of the endocrine pancreas is not without its price, however. Insulin resistance and its resulting hyperinsulinaemia may lead to a cluster of metabolic abnormalities which comprise a syndrome of interrelated risk factors for cardiovascular disease. This syndrome is known as "syndrome X" (Reaven, 1988, 1993, 1994) or "insulin resistance syndrome" (Haffner et al., 1992; Wajchenberg, Malerbi, Rocha, Lerario & Santomauro, 1994; Grootenhuis, 1994). Insulins resistance is most accurately measured by the euglycemic hyperinsulinaemic clamp technique. This method is, however, laborious and therefore not easily applicable in epidemiological studies. The best estimate of insulin resistance in population studies is fasting insulin (Laakso, 1993). Elevated fasting insulin precedes other features of the insulin resistance syndrome like hypertension (Reaven, 1990, 1991), a low HDL cholesterol concentration and an increase in triglycerides (Haffner et al., 1992). Moreover, fasting insulin turned out to be an independent predictor of CHD mortality in a large prospective study of over 7000 men (Fontbonne & Eschwège, 1991; Fontbonne, 1993).

A number of studies have shown that insulin resistance and hyperinsulinaemia are familial characteristics, which may point to a genetic basis of insulin resistance. Haffner, Stern, Hazuda, Mitchell and Patterson (1988), for example, observed a "genetic dosage" effect in Mexican-Americans: fasting insulin levels increased as a function of whether neither, one or both of the subject's parents had NIDDM. Insulin sensitivity has shown a high degree of familial aggregation in both Caucasians (Martin, Warram, Rosner, Rich & Krolewski, 1992) and Pima indians (Lillioja et al., 1987). Further work suggested that insulin resistance in Pima

indians may be determined by a single gene with a codominant mode of inheritance (Bogardus et al., 1989). Also in Caucasians evidence for a major locus was found for both fasting insulin and 1-hour-stimulated insulin levels (Schumacher, Hasstedt, Hunt, Williams & Elbein, 1992). Recently, a number of studies have measured insulin resistance with the hyperinsulinaemic clamp in healthy offspring of parents with NIDDM (Erikkson et al., 1989; Vaag, Henriksen & Beck-Nielsen, 1992; Gulli, Ferrannini, Stern, Haffner & DeFronzo, 1992). In spite of having a normal oral glucose tolerance, these first degree relatives showed a significant reduction in their insulin sensitivity, even several decades before they are expected to develop NIDDM (Beck-Nielsen & Groop, 1994). Results of these studies further indicate that the inherited insulin resistance seems to be located mainly in the non-oxidative pathway of glucose metabolism in skeletal muscles, i.e. glycogen synthesis. All proteins participating in insulin signaling and glucose processing in skeletal muscle may therefore, theoretically be involved in the pathophysiology of insulin resistance (Groop & Eriksson, 1992; Beck-Nielsen & Groop, 1994).

The search for the genetic basis of insulin action via candidate genes and linkage analyses has, to date, been only minimally positive, leaving most insulin resistance unexplained (Flier, 1992; Hansen, 1993; Raffel, Shohat & Rotter, 1994). A number of factors are held responsible for this result. First, insulin resistance may have a polygenic basis: multiple loci with small effect may be involved (Rich, 1990; Groop & Eriksson, 1992; Beck-Nielsen & Groop, 1994). Furthermore the penetrance is dependent on age and on environmental factors like smoking (Facchini, Hollenbeck, Jeppesen, Chen & Reaven, 1992; Attvall, Fowelin, Lager, von Schenck & Smith, 1993), diet (Lovejoy & DiGirolamo, 1992; Sharma, 1992; Mayer, Newman, Quesenberry & Selby, 1993) and physical exercise (Laws & Reaven, 1991; Lampman & Schteingart, 1991; Mikines, 1992).

Quantitative genetic approaches enable estimation of the relative importance of polygenic and environmental influences on a phenotype, provided the data are gathered in genetically informative subjects like, for example, nuclear families or twins. The relative influence of genes can be expressed as heritability, which is defined as the proportion of population variance attributable to genetic factors. Substantial genetic variation was detected for glucose intolerance, a correlate of insulin resistance, in the NHLBI study of male veteran twins: a heritability of .88 was reported for one hour glucose tolerance (Feinleib et al., 1977). Only two studies reported the heritability of fasting insulin. Iselius et al. (1982) applied a path analytic model to data from 155 nuclear families and reported a heritability of fasting insulin of .40 in both children and adults. In the second study (Schumacher et al., 1992), a segregation analysis of fasting insulin was performed on data from 16 pedigrees ascertained through siblings with NIDDM. Evidence was found for the effect of a single major locus. 33.1% of the variance in fasting insulin was attributed to this major locus and 11.4% to polygenic inheritance. Thus, in total 44.5% of the variance was due to genetic factors.

In normal circumstances the concentration of blood glucose is regulated within close boundaries (Brück, 1983). Insulin plays an important role in

maintaining blood glucose homeostasis by stimulating the uptake of glucose in the cell. This homeostatic regulation is similar to a feedback loop between glucose and insulin. Furthermore, fasting glucose and fasting insulin tend to show a positive correlation in population samples of older age, which is probably a reflection of a weakening homeostatic control of the blood glucose concentration with aging (Grootenhuis, 1994; Beck-Nielsen & Groop, 1994).

The principal aim of this study was to estimate the relative influence of genes and environment on fasting insulin, considered as a proxy for insulin resistance, and to investigate sex-differences in genetic and environmental estimates. Furthermore, it was tested whether the origin of the covariance between fasting glucose and fasting insulin could best be explained by a unidirectional causation from glucose to insulin (or vice versa), or a reciprocal causation of insulin and glucose (i.e. a feedback loop). To resolve these questions we measured fasting insulin and fasting glucose in middle-aged twins and used a reciprocal causation model to quantify the contributions of genes and environment to the variance and model the covariance of these variables.

METHODS

Subjects

This study is part of a project in which cardiovascular risk factors were studied in 213 middle aged twin pairs (aged between 34 and 63). Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry and sollicitation through the Dutch Twin Club. In addition, a small number of twins who heard from the study in another way volunteered to participate. Informed consent was obtained from all subjects. Data from 4 subjects were excluded from the sample. In one subject no blood could be obtained. Two subjects were dropped because they were insulin dependent diabetics, and one subject was discarded because of a glucose value higher than 7.8 mmol/l, which is an indication of diabetes mellitus according to WHO criteria (see e.g. Mooy, de Vries, Heine & Bouter, 1995). One (monozygotic) triplet was included in the sample by discarding the data from the second born subject. In total 204 males (age: 43.7 ± 6.5) and 218 females (age: 44.7 ± 6.8) were included in the study. In 76 same-sex twin pairs zygosity was determined by DNA fingerprinting (Jeffreys, Wilson & Thein, 1985; Meulenbelt, Droog, Trommelen, Boomsma & Slagboom, 1995). 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

the following number of twin pairs: 43 pairs of monozygotic males (MZM), 39 pairs of dizygotic males (DZM), 49 pairs of monozygotic females (MZF), 39 pairs of dizygotic females (DZF) and 39 dizygotic pairs of opposite sex (DOS).

Blood sampling and biochemical assays

Twins arrived at the laboratory at about 10.00 a.m. They were requested to fast, refrain from smoking and the use of alcohol, coffee and tea after 11.00 p.m. the preceding night. Blood was collected by venipuncture and sampled in EDTA tubes. The tubes were placed on ice and centrifuged promptly (30 minutes, 2000g) at 4 ^{o}C to separate plasma from cells. Aliquots of plasma were snap-frozen using liquid nitrogen and stored at -20 ^{o}C until processing. Fasting insulin was measured with a commercial radioimunoassay kit (INS-RIA-100, Medgenix diagnostics, Brussels, Belgium). The lower limit of sensitivity of this assay for insulin was 3.0 mU/l. Fasting glucose was determined with a Dimension clinical chemistry system (DuPont, Wilmington, U.S.A.). Each time a substantial number of samples had been collected, plasma samples were sent to the laboratory and analysed subsequently. Analyses took place on 8 different occasions.

Genetic analysis of fasting insulin and fasting glucose

Thirty of the 204 males (14.7%) and 53 of the 218 females (24.3%) had fasting insulin values below the detection limit of the assay (3 mU/l). Though it was certain that these subjects had values below 3 mU/l, which is informative from a clinical-diagnostic point of view, the exact value of their fasting insulin thus remained unknown. A simple solution to this problem would be to assign a fixed value (e.g. 2 mU/l) to those subjects. This, however, leads to a truncated, and thus non-normal, distribution of fasting insulin for both males and females, which may be of influence on the quantitative genetic modeling. We therefore decided to account for the truncation problem more accurately, within the genetic model fitting (see Appendix).

Inspection of the data of one random chosen member of each twin with ANOVA, showed that the occasion of measurement had a significant influence on the means of both fasting insulin and glucose. As two members of a twin pair were always measured on the same occasion, this effect could spuriously induce an increase in twin correlation. This occasion effect was accounted for in the model fitting by estimating separate means for each occasion.

Previous to all data analysis and model fitting fasting insulin and glucose were transformed by natural logarithm to obtain a normal distribution.

Model fitting was done with Mx (Neale, 1994), a computer program specifically designed for the analysis of genetically informative subjects. With Mx, a full model was defined which thus accounted for both truncation and occasion of measurement and in which for each of the 5 sex-by-zygosity groups variances and covariances of the initial 4x4 matrices of glucose and insulin were estimated freely. Furthermore, the initial means of glucose and insulin were estimated for each sex separately. These means and variance-covariance matrices formed the basis for

further quantitative genetic analysis of glucose and insulin. The 4x4 variance-covariance matrices in each of the 5 zygosity groups, were expressed in terms of a reciprocal causation model. Model fitting provided parameter estimates (h, d, c, e) and was done by a user defined fit function. Submodels were compared by hierarchic χ^2 tests, as the difference between the function value for a reduced model and that of the full model (Δ 2lnL) is χ^2 distributed. The degrees of freedom (df) for this test are equal to the difference between the df for the full and the reduced model.

A reciprocal causation model of glucose and insulin was fitted to the data. This model was chosen because of the similarity to the physiological feedback loop between glucose and insulin, which plays an important role in the maintainance of a constant glucose concentration in the blood plasma (Brück, 1983). The model is presented in Figure 3.1. The observed phenotypes for twin 1 and twin 2 are shown in squares, and latent factors are shown in circles. Factor loadings of observed variables on the different latent factors are depicted beside the arrows. Correlations between the latent genetic factors in both models are 1 in MZ twins and 0.5 in DZ twins.

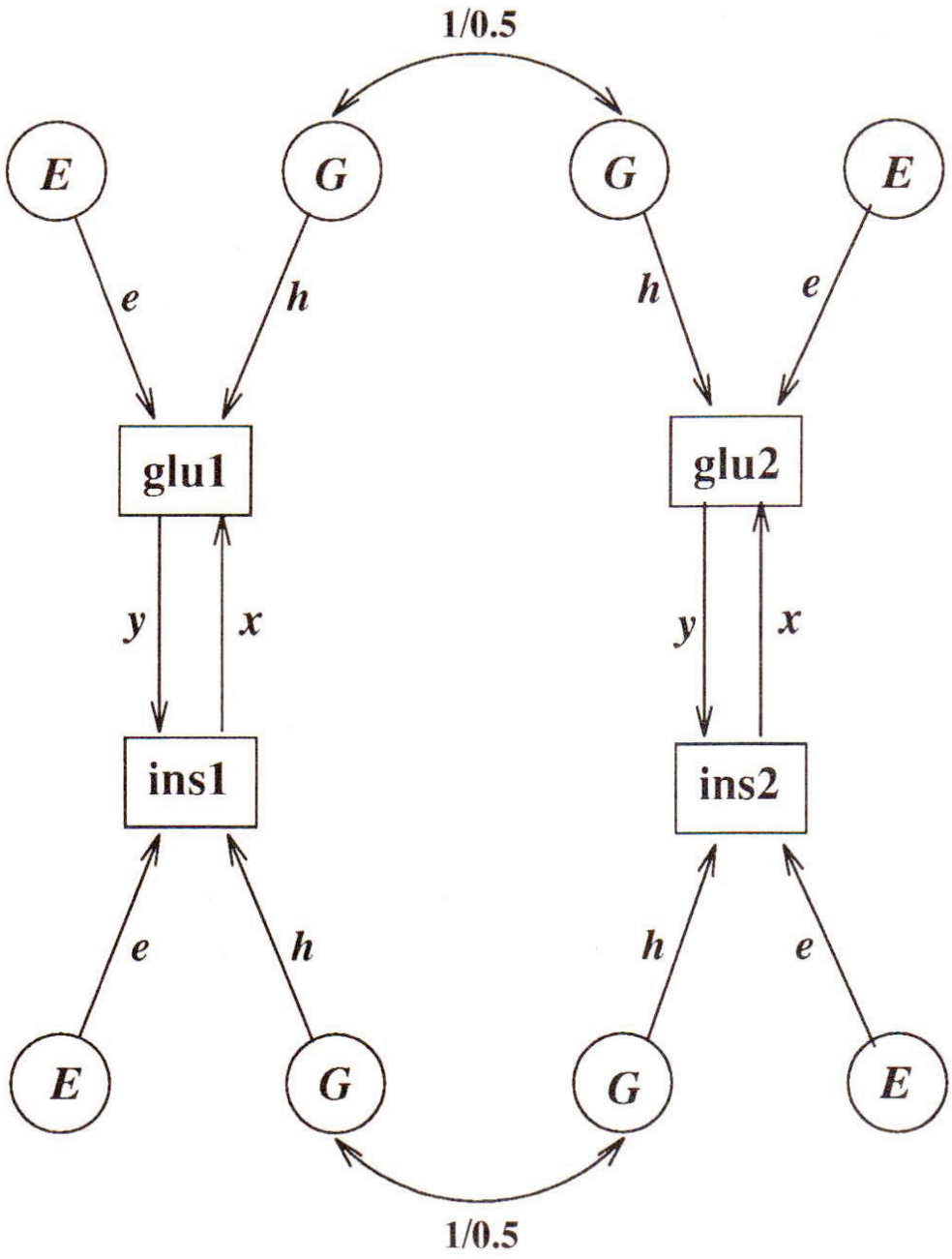

Figure 3.1: Reciprocal causation model for glucose and insulin. Observed phenotypes for twin 1 and twin 2 are shown in squares. Latent factors are shown in circles. G and E reflect genetic and environmental influences on glucose and insulin. Factor loadings of observed variables on the different latent factors are depicted beside the arrows: h = additive genetic influence, e = unique environmental influence, x = causal path from insulin to glucose, y = causal path from glucose to insulin. Correlations between the latent genetic factors are 1 in MZ twins and 0.5 in DZ twins. Although models including C (ACE) and D (ADE) were also tested, C and D are not shown in the figure for reasons of clarity.

For the reciprocal causation model it is assumed that the correlations between the genetic and environmental determinants of glucose with those of insulin are all zero, which implies that the association between glucose and insulin arises solely because of the reciprocal causal influences of insulin on glucose (x) and glucose on insulin (y) (a "feedback loop" between glucose and insulin). Under the model presented in Figure 3.1, total phenotypic variances (V_P) of insulin and glucose are equal to:

$$V_P ins = ((h^2 ins + e^2 ins) + y^2(h^2 glu + e^2 glu)) / (1-xy)^2$$
$$V_P glu = ((h^2 glu + e^2 glu) + x^2(h^2 ins + e^2 ins)) / (1-xy)^2$$

These formulae show that $V_P ins$ consists of a part due to insulin and a part due to glucose mediated through y, the path from glucose to insulin. The same kind of reasoning applies to $V_P glu$. From these formulae it follows that the additive genetic component of variance (V_G) for insulin and glucose is equal to:

$$V_G ins = (h^2 ins + y^2 h^2 glu) / (1-xy)^2$$
$$V_G glu = (h^2 glu + x^2 h^2 ins) / (1-xy)^2$$

Standardized heritabilities (h^2) for insulin and glucose can be calculated from V_G and V_P: $h^2 = V_G/V_P$.

Sex differences in components of variance were examined by comparing a reciprocal causation 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. In a similar way, heterogeneity of the means of glucose and insulin was tested across males and females. Only this was done within the full model in which variances and covariances were estimated freely for each sex-by-zygosity group.

RESULTS

In Table 3.1, means before truncation correction[1] and after truncation correction of fasting insulin and glucose are shown for males and females. Effects of sex on the means of insulin and glucose were tested by hierarchic χ^2 tests. In submodels of the full model, parameter estimates for the means were set equal across males and females. Results are shown in Table 3.2. A significant sex difference was found for insulin but not for glucose. For fasting insulin, values were higher in males than in females (see Table 3.1).

[1] Means of fasting insulin before truncation correction, were calculated after all values below the detection limit were assigned a value of 2 mU/l.

Table 3.1: Means before truncation correction[a] and after truncation correction of fasting insulin and fasting glucose for males and females

		Insulin(mU/l)[b]		Glucose(mmol/l)[b]	
	n	before corr.	after corr.	before corr.	after corr.
Males	204	5.45	5.65	6.12	6.11
Females	218	4.49	4.46	5.98	6.03

Abbreviations: n = number of subjects.
[a] Means of fasting insulin after truncation correction, were calculated after all values below the detection limit were assigned a value of 2 mU/l.
[b] For insulin and glucose, antilogs are reported.

Table 3.2: Effects of sex on the means of fasting insulin and glucose tested by hierarchic χ^2 tests.

Model	-2lnL	∆df	∆(2lnL)	p
Full model	2598.74			
Insulin				
no sex differences	2620.15	8	21.41	<.01
Glucose				
no sex differences	2607.81	8	9.07	ns

Abbreviations: -2lnL = minus twice the log-likelihood, df = degrees of freedom, ∆df = (df *full model)* - (df *submodel*), ∆(2lnL) = (2lnL*full model)* - (2lnL*submodel*), p = probability

The effect of the correction for truncation only, and the correction for both truncation and occasion of measurement on the twin correlations of fasting insulin and fasting glucose in the 5 different zygosity groups can be seen in Table 3.3. The corrections hardly affected the twin correlations for glucose. Only after correction for both effects, correlations seemed to go down slightly. For fasting insulin, corrections had a substantial influence on the twin correlations. Especially the correction for occasion of measurement induced a decline in twin correlations. In general, after correction for truncation and occasion of measurement, MZ correlations were larger than DZ correlations for both insulin and glucose, indicating a genetic influence. For insulin however, MZ correlations were only slightly higher than DZ correlations, which implies that the proportion of variance due to genetic influences on fasting insulin is small.

Table 3.3: Twin correlations of fasting insulin and fasting glucose before correction and after correction for truncation only (trunc corr.), and after correction for both truncation and occasion effect (both corr.)

		Insulin			Glucose		
	N	before corr.	trunc. corr.	both corr.	before corr.	trunc. corr.	both corr.
MZM	43	.41	.28	.15	.56	.54	.48
DZM	39	.10	.19	.04	.02	.02	-.03
MZF	49	.38	.44	.45	.72	.73	.61
DZF	39	.42	.45	.29	.27	.27	.19
DOS	39	.25	.21	-.06	.19	.18	.17

Abbreviations: N = Number of twin pairs, MZF = Monozygotic Females, MZM = Monozygotic Males, DZF = Dizygotic Females, DZM = Dizygotic Males, DOS = Dizygotic Opposite Sex.

Table 3.4 shows function values (-2lnL), and hierarchic χ^2 tests of submodels of the reciprocal causation model. In the upper part of the table models are shown, in which parameter estimates are allowed to be different between the sexes. In the lower part of the model these estimates are set equal. Models estimating only additive genetic (G) and unique environmental (E) factors fitted best. Parameter estimates for males and females within the GE model could be set equal without a significant loss in fit. This means that the GE model without sex differences offers the most parsimonious solution. Variations of the GE model without sex differences, testing whether the reciprocal paths (x or y) could be set to zero are also shown. None of these paths could be set to zero without a loss in fit, implicating that both causal paths are needed to explain the phenotypic correlation between insulin and glucose. The predicted phenotypic correlation between glucose and insulin within the best fitting model was .23 (not shown). Computed for uncorrected values, this correlation was .15 for males and .10 for females.

Table 3.5 presents standardized parameter estimates of the best fitting reciprocal model (GE without sex differences). As expected from the twin correlations a small heritability for fasting insulin was found (.21). For fasting glucose heritability was .47. Reciprocal paths were of opposite sign, which points to a negative feedback loop.

Table 3.4: Reciprocal causation models, with (sex diff.) and without sex differences (no sex diff.). Variations of the GE model without sex differences, testing whether x or y can be set to zero, are also shown

Model	-2lnL	df	∆df	∆(2lnL)	p
Sex differences					
GCE	2661.391	963			
GDE	2661.436	963			
GE	2664.896	967	4	3.505	ns[a]
CE	2678.493	967	4	17.102	<.0025[a]
No sex differences					
GCE	2675.596	971			
GDE	2674.666	971			
GE	**2675.621**	**973**	**2**	**0.025**	**ns[b]**
CE	2688.867	973	2	13.271	<.0025[b]
GE					
full model	**2675.621**	**973**			
x = 0	2688.606	974	1	12.985	<.001[c]
y = 0	2680.802	974	1	5.181	<.025[c]
x & y = 0	2712.317	975	2	36.696	<.001[c]

Abbreviations: x = causal path from insulin to glucose, y = causal path from glucose to insulin.
Most parsimonious solution is printed in **boldface** type.
[a] Compared to GCE (sex diff.); [b] Compared to GCE (no sex diff.); [c] Compared to GE (no sex diff.).
For further abbreviations see Table 3.2.

Table 3.5: Parameter estimates from best fitting reciprocal causation model

Insulin		Glucose		Reciprocal paths	
h^2	e^2	h^2	e^2	x	y
0.21	0.79	0.47	0.53	0.57	-0.35

Abbreviations: h^2= additive genetic variance, e^2= unique environmental variance, x = causal path from insulin to glucose, y = causal path from glucose to insulin.

DISCUSSION

This study investigated the relative contribution of genes and environment to individual differences in fasting insulin, and sex-differences in estimates of these contributions. A reciprocal causation model including fasting insulin and fasting

glucose was used to analyze the data. This also enabled us to test whether the origin of the covariance between fasting glucose and fasting insulin could best be explained by a unidirectional causation from glucose to insulin (or vice versa), or a reciprocal causation of insulin and glucose (i.e. a feedback loop). Before we were able to explore these issues, we had to account for two effects within the model fitting: a truncation effect on fasting insulin and an effect of the occasion of measurement on both fasting glucose and insulin.

Plasma samples were analysed on 8 different occasions, which turned out to have a significant influence on the means of both fasting insulin and glucose. As two members of a twin pair were always measured on the same occasion, the occasion of measurement could thus induce a spurious increase in twin correlations. Accounting for the occasion effect in the model fitting indeed showed a decline in twin correlations of especially fasting insulin. For the determination of fasting insulin an assay was used with a lower detection limit of 3.0 mU/l. 14.7% of all males and 24.3% of all females had values below this detection limit. Within the quantitative genetic model fitting, the bivariate distribution of glucose and insulin was corrected for this truncation effect. The combination of both the correction for truncation and for occasion of measurement turned out to have a large impact on the twin correlations of fasting insulin. This suggests that, in case variables are truncated and/or show an occasion effect, these corrections are highly important in order to obtain unbiased quantitative genetic parameters estimates.

A model without sex differences specifying both reciprocal paths and only additive genetic and unique environmental factors, gave the best explanation of the data. None of the reciprocal paths could be set to zero without a loss in fit. This implicates that a reciprocal causation gives a better explanation of the covariance between insulin and glucose than a unidirectional causation from glucose to insulin or vice versa. Estimates of reciprocal paths within the best fitting model were of opposite sign, which indicates that the covariation between insulin and glucose can be described by a negative feedback loop. Within the most parsimonious model, only 21% of the variance in fasting insulin in both males and females was explained by additive genetic factors. The rest of the variance could be attributed to unique environmental factors. Our study was the first to look into possible sex differences. However, differences in parameter estimates between males and females were absent.

In a study of middle-aged male twins, Feinleib et al. (1977) observed a heritability of .88 for the glucose value, measured one hour after an oral glucose load of 50 gram. This large genetic influence on glucose intolerance, a correlate of insulin resistance just like hyperinsulinaemia, clearly contrasts to our finding of a low heritability (.21) of fasting insulin. The two other studies that reported heritabilities of fasting insulin (Iselius et al., 1982 and Schumacher et al., 1992) also found higher values: .40 and .44 respectively. An explanation for these differences may be offered by different subject characteristics and/or differences in methodology in the latter two studies as compared to our study.

The first issue concerns the ascertainment of subjects. Iselius et al. (1982) studied 155 nuclear families. 96 of these families were ascertained through a diabetic proband parent. Schumacher et al. (1992) studied 16 large pedigrees ascertained through two or more siblings with NIDDM, although the segregation analysis they performed was restricted to 206 family members and 65 spouses with normal glucose tolerance. This segregation analysis allowed the attribution of part of the genetic variance to a major gene effect. The authors admit, however, that the ascertainment of the pedigrees through NIDDM cases might have resulted in the high frequency (25%) of the hyperinsulinaemic allele among measured individuals. Conversely, our study comprised non-diabetic twins, who were *not* selected on the basis of family members with NIDDM, which may partly explain the lower heritability estimate in our study. Also in our data, selecting a subgroup of twins of which one or both members had a glucose value higher than 6.0 mmol/l, increased the difference between the MZ and DZ twin correlation for fasting insulin, pointing to a higher heritability. This increase in heritability of fasting insulin in subgroups with higher glucose values, might indicate that subjects characterized by higher glucose values may be genetically disposed to develop insulin resistance and hyperinsulinaemia (Beck-Nielsen, 1995; Snieder, Boomsma & van Doornen, 1995a).

With respect to differences in methodology, the use of an environmental index in the path model applied by Iselius et al. (1982), may have led to biases in their heritability estimate. Their environmental index was created by multiple regression on body mass index, use of tobacco, alcohol and other drugs, type of work, physical exercise and amount of stress in work. An environmental index may have two opposing effects on heritability estimates (Vogler, Rao, Laskarzewski, Glueck & Russel, 1987; Vogler et al., 1989; Rao & Vogler, 1994). First, the index may contain variables that are at least partly under genetic control which would result in an underestimation of heritability. This effect is not unthinkable, as recent findings indicate that even "purely environmental" variables like life events and social support, for example, have a genetic component (Plomin, 1995). Further, the index may fail to capture most of the familial environment which would lead to an overestimation of heritability. Another methodological point concerns the study of Schumacher et al. (1992). In their data analysis, evidence for a major gene affecting insulin levels was detected only when the variance in insulin values attributable to body mass index (BMI) was removed. Actually, when segregation analysis was done on fasting insulin unadjusted for BMI, the data were best explained by an environmental model.

Although fasting insulin is the best indicator of insulin resistance in population studies, correlations observed by Laakso (1993) between fasting insulin and insulin sensitivity (measured as whole-body glucose uptake during a euglycemic hyperinsulinaemic clamp) were far from perfect, especially in subjects with impaired glucose tolerance (-.47) and NIDDM (-.48). Therefore, fasting insulin is an adequate surrogate for direct measurement of insulin resistance in persons with normal glucose tolerance only (Laakso, 1993; Anderson et al., 1995). As our sample

consisted of middle-aged twins, it is certainly possible that a considerable number of subjects may have had impaired glucose tolerance (Mooy, 1995). Future studies on the genetics of insulin resistance, therefore have to apply measures that give a good and consistent approximation of this phenotype, even in people with impaired glucose tolerance [see Anderson et al. (1995) for measures of insulin sensitivity based on a frequently sampled intravenous glucose tolerance test].

To summarize above considerations, a low heritability of fasting insulin as found in our study of non-diabetic middle aged twins, not neccesarily indicates that heritable influences on insulin resistance are unimportant. Although genes may not be able to explain a large part of the variance of fasting insulin in the general population, evidence seem to point to the existence of a subgroup of people that express a genetically determined susceptibility to develop insulin resistance and subsequent hyperinsulinaemia. On the other hand, we found that 80% of the variance in fasting insulin could be explained by unique environmental factors, which emphasizes the importance of environmental influences. Most probably, a genetic susceptibility for insulin resistance comes to expression as a result of environmental triggers, like a high-fat, low-fiber diet (Sharma, 1992; Lovejoy & DiGirolamo, 1992; Mayer et al., 1993), a lack of physical activity (Laws & Reaven, 1991; Lampman & Schteingart, 1991; Mikines, 1992) and smoking (Facchini et al., 1992; Attvall et al., 1993), which are known to impair insulin sensitivity. Whatever the true identity of environmental influences on insulin resistance may be, this study shows that these influences are not shared by family members but are specific to an individual.

Future data on the genetics of insulin resistance, may provide more insight into the causes of two major diseases of the western world: non-insulin-dependent diabetes and cardiovascular disease. Evidence for a genetic basis of NIDDM is manifold. Twin studies show very high concordance rates in identical twins (Barnett, Eff, Leslie & Pyke, 1981; Newman et al., 1987), NIDDM clusters in families and its prevalence varies among various ethnic groups (Bennet & Stern, 1991; Pierce, Keen & Bradley, 1995). According to the "insulin resistance/pancreatic exhaustion" hypothesis, compensatory hyperinsulineamia to maintain glucose homeostasis, develops as a result of chronic insulin resistance, which eventually leads to an exhaustion of the pancreatic ß-cells and an impairment of insulin secretion. Most likely, several genes are responsible for both the impairment in insulin sensitivity and the deterioration of insulin secretion (Beck-Nielsen & Groop, 1994; McCarthy, Froguel & Hitman, 1994).

Mortality from cardiovascular disease is also highly genetically determined (Marenberg, Risch, Berkman, Floderus & de Faire, 1994), which is probably mediated by the genetic influence on its risk factors. Genes have, for example, been shown to influence blood pressure (Snieder, van Doornen & Boomsma, 1995b), HDL cholesterol and triglycerides (Heller, de Faire, Pedersen, Dahlén & McClearn, 1993; Boomsma et al., 1996) obesity (Bouchard, 1991; Fabsitz, Carmelli & Hewitt, 1992), distribution of body fat (Bouchard, 1988) and haemostatic factors (Fay & Ginsburg, 1994). All these cardiovascular risk factors are interrelated, and form what is called the "insulin resistance syndrome" or "Syndrome X". Carmelli, Cardon and

Fabsitz (1994) found evidence for a common latent factor mediating the clustering of hypertension, NIDDM and obesity. This common factor was influenced by both genetic (59%) and environmental (41%) effects. Although the identity of the latent common factor could not be determined from the available data, insulin resistance was proposed by Carmelli et al. (1994) as a possible candidate.

Since insulin resistance is a common predecessor of both NIDDM and cardiovascular disease, it is tempting to hypothesize that the genetic basis of both NIDDM and cardiovascular disease is partly explained by the putative genetic influence on insulin resistance. The exact molecular mechanism that explains insulin resistance, however, remains unknown. A number of candidate genes and mechanisms have been examined, like the insulin and insulin receptor gene (Elbein, Ward, Beard & Permutt, 1988; McClain, Henry, Ullrich, & Olefsky, 1988; Elbein, Hoffman, Bragg & Mayorga, 1994), glucose transporter genes (Mueckler, 1990) and substances inhibiting insulin receptor function (Flier, 1992; Kahn, 1995), but only a very small proportion of the genetic defects in insulin action are traceable to these causes. Postreceptor defects in insulin action provide the best candidate to explain the majority of cases of insulin resistance (Flier, 1992; Hansen, 1993; Raffel, Shohat & Rotter, 1994). More specifically, there is evidence that the main defect is located in the process of glycogen synthesis in skeletal muscle (Eriksson et al., 1989; Gulli et al., 1992; Vaag, Henriksen & Beck-Nielsen, 1992). In accordance to that, Vaag and coworkers (1992) observed that the activation of the glycogen synthase enzyme was reduced in parallel to the reduction in glycogen synthesis in first degree relatives of patients with NIDDM. This opens the prospect of a genetic defect in glycogen synthase as a cause of insulin resistance. Recently a Xba1 polymorphism of the glycogen synthase gene was found, which is enriched in Finnish patients with NIDDM (Groop et al., 1993). This Xba1 polymorphism is due to a single basepair change in an intron, and can therefore only be considered as a marker of NIDDM (Beck-Nielsen & Groop, 1994). Moreover, this association has not been duplicated in other populations (Elbein et al., 1994). Furthermore, stimulation of glycogen synthase activity in skeletal muscle is the result of a cascade of phosphorylation and dephosphorylation reactions. It is quite possible that defects anywhere in this cascade are involved in the development of insulin resistance (Groop & Eriksson, 1992; Beck-Nielsen & Groop, 1994; Kahn, 1995). Recent evidence, for example, indicates that obesity-induced insulin resistance is caused by the (tumour necrosis factor-α mediated) inhibition of insulin receptor substrate-1, which is one of the first steps in the insulin receptor signalling pathway (Hotamisligil et al., 1996).

In conclusion, the low heritability of fasting insulin we found need not imply that genetic influences on insulin resistance are unimportant. The importance of genetic influences may be restricted to a subgroup of people with a genetically determined susceptibility to develop insulin resistance and subsequent hyperinsulinaemia. Future studies on the genetics of insulin resistance also have to apply measures that show a greater correspondence to this phenotype. The substantial influence of unique environmental factors on fasting insulin, may offer a hopeful

perspective for the treatment and prevention of insulin resistance. This is all the more important as insulin resistance is regarded a primary factor in the development of both NIDDM and CHD.

APPENDIX

The analysis of truncated data requires careful specification of the likelihood. If we assume that both the insulin and glucose measures are normally distributed [see, e.g., Ferrannini, Haffner & Stern (1990)], then the likelihood of a pair of twins measured on these two variables may be written as:

$$L(I_1, I_2, G_1, G_2) = \phi(I_1, I_2, G_1, G_2) \quad (1)$$

where φ1 is the multivariate normal probability density function (pdf), given by

$$\varphi(\boldsymbol{x}) = \det(2\pi\boldsymbol{\Sigma})^{-m/2} \exp -.5(\boldsymbol{x} - \mu)'\boldsymbol{\Sigma}^{-1}(\boldsymbol{x} - \mu)2$$

in which: **Σ**3 is the covariance matrix of the vector of *m* observed variables **x**, whose mean vector is μ.

The software package Mx (Neale, 1994) allows computation of truncated multivariate normal integrals, via the \mnor and \momnor functions. It also computes the likelihood under the multivariate normal pdf of a vector of observed scores (the maximum likelihood method for rectangular or variable length raw data files). Third, it will compute the moments of the truncated normal distribution. It does not have any direct computation of the above multivariate normal functions truncated and integrated over a subset of the dimensions. In a large sample, such computations would be cpu-intensive, requiring separate integrations for each pair in the sample. Instead, we can rearrange the above expressions to reduce them to forms that can be computed within Mx.

Pairs concordant for being above the detection limit present no special problem - we can use equation 1 above. In the remaining cases, we would like to find the likelihood of the Glucose measures and any Insulin measures above the detection limit. We can find the conditional distribution of the Glucose observations from the moments of the truncated normal distribution (see, e.g., Neale, Eaves, Kendler & Hewitt, 1989). A computationally efficient way to obtain this is to compute the moments of the truncated (below limit) Insulin measures, and then use the Pearson-Aitken-Lawley selection formula (Aitken, 1934) to obtain the moments of the not-selected variables. The justification for this approach lies in a rearrangement of Bayes' theorem:

$$p(A \mid B) = \frac{p(A \,\&\, B)}{p(B)}$$

so

$$P(A\&B) = p(A \mid B)p(B)4 \quad (2)$$

in our case, we seek $p(A\&B)$ where A is the Glucose measures and above detection limit Insulin measures and B is the below detection limit Insulin measures. The moments of B may be obtained from Mx's momnor function, and the Pearson-Aitken formula gives us the the moments of the conditional distribution $A|B$. We then compute the likelihood in the usual way (a reduced form of equation 1) for the observations under this conditional

distribution to obtain $p(A|B)$. The probability of observing B [$p(B)$ in equation 2] is simply the multivariate normal integral from minus infinity to the detection limit over all variables below the limit. Thus in the case where only Insulin on twin 1 is below the limit,

$$p(B) = \int_{-\infty}^{t} \phi(I_1)\, dI_1 \qquad 5$$

and where both twins are below the limit we have:

$$p(B) = \int_{-\infty}^{t}\int_{-\infty}^{t} \phi(I_1, I_2)\, dI_2\, dI_1 \qquad 6$$

These terms differ according to whether the subjects are MZ or DZ, but otherwise would be expected to be the same for all subjects, in the absence of occasion effects (see below).

Writing the m dimensional integral from $-\infty$ to t as $\int_B$, the log likelihood for all pairs $j = 1, \ldots n$ is

$$\log(L) = \sum_{j}^{n} \left(\log(\phi(A \mid B) + \log(\int_B) \right)$$

where the second term is constant for all pairs of a given zygosity. Thus we have

$$\log(L) = n \log(\int_B) + \sum_{j=1}^{j=n} \log(\phi(A \mid B))$$

so that only one integration step is required per zygosity group, instead of for every subject.

Occasion effects on the sample resulted in different means for each occasion of measurement. These in turn affect the amount of truncation represented by an absolute detection limit of 3 mU/l. Therefore separate terms of the form $\int_{-\infty}^{t} \phi(B_k)\, d_{Bk}$ are needed for each occasion of measurement.

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4

Developmental genetic trends in blood pressure levels and blood pressure reactivity to stress[1]

Harold Snieder, Lorenz J.P. van Doornen & Dorret I. Boomsma

INTRODUCTION

This chapter has two main aims. The first is to describe the changes in heritability of blood pressure levels and blood pressure reactivity that occur during the lifespan. The second is to disentangle the genetic and non-genetic causes of stability and change in these parameters. In order to achieve these goals, both empirical studies and developmental models will be discussed.

After an initial examination of blood pressure levels and reactivity, the results of twin studies on subjects of different ages are compared to address the question of whether heritabilities are age-specific. The next section focuses on parent-offspring studies and studies with a special interest in age-dependent genetic and environmental effects. Following this presentation, the very limited number of longitudinal genetic studies of blood pressure are discussed, and an extended parent-offspring design is described and illustrated with relevant data from our own laboratory. This design, coupled with the appropriate modeling, shows how one can estimate genetic stability in the absence of longitudinal data. Finally, we discuss the implications of such studies and speculate about what directions such research might take in the future.

Blood pressure levels

Blood pressure levels have been shown to be related to the risk of coronary heart disease and stroke. Risk increases linearly from a systolic blood pressure (SBP) of 110 mmHg on, and even increases progressively in the higher pressure range (Pooling Project, 1978; Kannel, 1976). In Western societies blood pressure rises with age. This trend, however, is not a simple linear one, and it differs between males and females. In boys, a strong rise in SBP of about 15 mmHg occurs in the first 8 weeks

[1] This chapter is a slightly revised version of chapter 6 in J.R.Turner, L.R. Cardon & J.K. Hewitt (Eds.). *Behavior genetic approaches in behavioral medicine*. New York: Plenum, 105-130.

of life; in girls this rise occurs earlier and is even steeper. In both sexes SBP remains stable until the age of 12 months. Diastolic blood pressure (DBP) declines after birth in both boys and girls, reaching its minimum in 2-3 months, but is back at birth level by the end of the first year. From then on there is a gradual increase in SBP (+20 mm Hg) and DBP (+15 mm Hg) in both sexes until puberty.

During adolescence, SBP again rises, this time more steeply in boys than in girls. At the age of 20, SBP in boys is nearly 10 mm Hg higher than in girls, whereas DBP is only slightly higher (Lauer, Burns, Clarke & Mahoney, 1991; Labarthe, Mueller & Eissa, 1991; van Lenthe & Kemper, 1993). These sex differences remain until the age of 50.

During the middle-age period (40-60 years) SBP rises about 10 mm Hg in males and the variance in values also increases. DBP levels and their variance remain fairly constant in this period (Pooling Project, 1978). Recently, these age trends in levels and variances during middle age were confirmed in a large Norwegian sample and shown to apply to females as well (Tambs et al., 1992). The SBP rise from 40 years of age on is steeper in females. After the age of 50, women have a higher SBP than men. Menopause as such does not seem to be responsible for this difference (Lerner & Kannel, 1986; Matthews et al., 1989). SBP, but not DBP, continues to rise until the age of 70. After this age both SBP and DBP decline. In later life, females have higher SBP and DBP than males (Valkenburg, Hofman, Klein & Groustra, 1980).

The age-specific increase in SBP and DBP, and the sex differences in this increase, suggest that different mechanisms have their influence on blood pressure in different periods of life and that they may not be the same, or have the same timing, in males and females. The balance between genetic and environmental influences on blood pressure thus may also vary as a function of age and sex. There may even be "critical periods" in which exposure to certain environmental factors, or the expression of particular genes, will have a decisive impact on future blood pressure, whereas exposure to the same factors in other stages of life may be relatively inconsequential (Unger & Rettig, 1990).

Blood pressure reactivity

Exaggerated cardiovascular reactivity to stress is hypothesized to play some role in the development of hypertension (Manuck, Kasprowicz & Muldoon, 1990). Support for this idea is furnished by studies showing that increased cardiovascular reactivity to stress is associated with stronger rises of blood pressure with age. This was shown by Menkes et al. (1989) for the response to the cold pressor test and by Matthews, Woodall, and Allen (1993) for the response to mental stressors. Promising as these results may be, these observations do not necessarily imply a causal role of exaggerated stress reactivity for the development of high blood

pressure. Such stress reactivity could be just a risk marker reflecting underlying structural or functional vascular changes (Folkow, Hallback, Lundgren & Weiss, 1970; Adams, Bobik & Korner, 1989).

When investigating the contribution of genetic factors to blood pressure reactivity, it is again of interest to know whether this influence varies with age. A change in reactivity with age would perhaps point to this possibility. A decline in heart rate responsiveness to mental stress with increasing age has been observed in several studies (Faucheux et al., 1989; Garwood, Engel & Capriotti, 1982; Barnes, Raskind, Gumbrecht & Halter, 1982; Furchtgott & Busemeyer, 1979; Gintner, Hollandsworth & Intrieri, 1986). This observation fits the general finding of attenuated cardiac responsiveness to ß-receptor agonists. For blood pressure reactivity one would predict an increased stress reactivity with age on physiological grounds. α1-Receptor-mediated functions remain preserved with aging, whereas ß-receptor-mediated vasodilation and arterial compliance decrease with age (Folkow & Svanborg, 1993). The empirical support for an age trend in blood pressure reactivity is, however, not convincing. Matthews and Stoney (1988) found larger SBP responses in adults as compared to children, but no association within the adult age range (31-62). Garwood et al. (1982) observed a small positive correlation of SBP reactivity with age (r=0.25) in the range between 30 and 79 years. Other studies, however, found no age effect (Faucheux et al., 1989; Gintner et al., 1986; Steptoe & Ross, 1981). In none of these studies was DBP reactivity age-dependent.

The absence of a convincing age trend in blood pressure reactivity does not refute, however, the possibility that genetic or environmental influences on reactivity, or both, differ by age. A role for genetic factors in stress reactivity finds support in studies showing that a family history of hypertension in subjects who are still normotensive themselves is associated with higher blood pressure reactivity to stressors (De Visser, 1994).

Currently we know very little about developmental trends in the heritability of important cardiovascular and other (psycho)physiological parameters, and we know even less about causes of stability and change in these variables. As stated, the age trends in blood pressure level may indicate the involvement of different genetic or environmental factors, or both, at different ages. The same applies to the increases in phenotypic variance, especially for SBP, across the life span. This increase may be due to an increase in the amount of genetic variance, nongenetic variance, or both. Such changes in variance components can imply changes in heritabilities with age ($h^2 = Vg/Vp$, where h^2 = heritability, Vg = genetic variance and Vp = total phenotypic variance) as well as differences in correlations among relatives of different ages. For example, if the amount of genetic variance is the same at different ages while the amount of environmental variance increases as people grow older, the covariance between parents and offspring will be the same as the covariance among siblings (assuming that the same genes are expressed at different ages and that shared environmental and dominance influences are negligible). However, the

parent-offspring *correlation* will be lower than the sibling correlation, due to the increase in variance in the parental generation.

Changes in phenotypic variance and in the part of the variance that may be attributed to genetic and environmental factors, i.e., heritability and environmentality (Plomin, DeFries & McClearn, 1990), may be detected in cross-sectional or longitudinal studies. However, only longitudinal studies, in which the same subjects are measured repeatedly, are informative about the stability of genetic and environmental factors. Contrary to popular points of view, genetically determined characteristics need not be stable, nor are longitudinally stable characteristics always influenced by heredity (Molenaar, Boomsma & Dolan, 1991). During development, stability of a quantitative trait such as blood pressure may be due to stable environmental influences, while instability may be due to distinct subsets of genes turning on and off. This chapter considers the kinds of empirical evidence that will help us to understand the genetic and environmental influences on the stability and change of blood pressure as a dynamic characteristic.

1. TWIN STUDIES

Blood pressure levels

If twins within a specific age range are measured in studies estimating the genetic influence on blood pressure level, heritability values for this specific age range are obtained. The available twin studies are listed in Table 4.1. These studies did not take an explicit interest in age, but studies are listed in ascending order according to age of the twin sample.

Such a systematic overview of all studies may reveal any age-dependent trends in heritability. If studies considered sex diffences in h^2, estimates for males and females are listed separately. The studies in Table 4.1 found no evidence for influence of shared family environment (c^2) on blood pressure. With respect to heritability estimates, the results are remarkably consistent: The majority of heritability estimates lie between 0.40 and 0.70 for both males and females and no clear age trend in h^2 can be detected.

Some caution concerning the interpretation of Table 4.1 is required. First, studies used different methods to estimate h^2. Second, there was wide variation in the composition of the twin samples in the studies: The age range within studies differs considerably, and results are more reliable for males, since the majority of studies used only male twins. Furthermore, results from studies with small sample sizes are clearly less reliable. Nevertheless, the absence of an age trend in h^2 in Table 4.1 seems to warrant the conclusion that the relative influence of genes on blood pressure does not change appreciably with age.

Table 4.1: Twin studies estimating heritability (h^2) in systolic (SBP) and diastolic blood pressure (DBP), in ascending order according to age

		Age			h^2	
Investigator	Pairs of twins	Mean(SD)	Range	Sex	SBP	DBP
Levine et al. ('82)	67 MZ, 99 DZ	? (?)	0.5- 1.0	m & f	0.66	0.48
Schieken et al. ('89)	71 MZM, 74 MZF	11.1(0.25)	?	male	0.66	0.64
	23 DZM, 31 DZF, 52 DOS			female	0.66	0.51
McIlhany et al. ('75)	40 MZM, 47 MZF	14.0(6.5)	5.0-50.0	male	0.41	0.56
	32 DZM, 36 DZF, 45 DOS			female	0.78	0.61
Boomsma ('92)	35 MZM, 33 MZF	16.8(2.0)	13.0-22.0	male	0.49	0.69
(see the text)	31 DZM, 29 DZF, 28 DOS			female	0.66	0.50
Sims et al. ('87)	40 MZM, 45 DZM	19.4(3.0)	?		0.68	0.76
Ditto ('93)	20 MZM, 20 MZF	20.0(5.0)	12.0-44.0	male	0.63	0.58
	20 DZM, 20 DZF, 20 DOS			female	0.63	0.58
Bielen et al. ('91)	32 MZM	21.7(3.7)	18.0-31.0		0.69	0.32
	21 DZM	23.8(3.9)				
Hunt et al. ('89)	73 MZM, 81 DZM	34.5(9.5)	?		0.54	0.60
Slattery et al. ('89)	77 MZM, 88 DZM	? (?)	22.0-66.0		0.60	0.66
Snieder et al.	43 MZM, 47 MZF	44.4(6.7)	34.0-63.0	male	0.40	0.42
(see the text)	32 DZM, 39 DZF, 39 DOS			female	0.63	0.61
Feinleib et al. ('77)	250 MZM, 264 DZM	? (?)	42.0-56.0		0.60	0.61
Theorell et al. ('79)	17 MZM, 13 DZM	62.0(?)	51.0-74.0		0.00	0.00

Abbreviations: MZF = Monozygotic Females, MZM = Monozygotic Males, DZF = Dizygotic Females, DZM = Dizygotic Males, DOS = Dizygotic Opposite-Sex, m & f = males and females combined.

Blood pressure reactivity

Studies that have investigated genetic influence on blood pressure reactivity are scarce. Turner and Hewitt (1992) reviewed the twin studies of blood pressure response to psychological stress. They concluded that:

1) Blood pressure reactivity to psychological stress is moderately heritable.
2) A common environmental influence is highly improbable.
3) Sex differences are possible, but as yet unexplored.
4) Genotype-age interactions are probable, but they await systematic evaluation.

Table 4.2: Twin studies estimating heritability (h^2) in systolic (SBP) and diastolic blood pressure (DBP) reactivity, in ascending order according to age

		Age				h^2	
Investigator	Pairs of twins	Mean (SD)	Range	Task	Sex	SBP	DBP
McIlhany et al. ('75)	40 MZM, 47 MZF	14.0(6.5)	5.0-50.0	CP	male	0.36	0.53
	32 DZM, 36 DZF, 45 DOS				female	0.72	0.68
Boomsma ('92)	35 MZM, 33 MZF	16.8(2.0)	13.0-22.0	RT	male	0.00	0.00
(see the text)	31 DZM, 29 DZF, 28 DOS				female	0.00	0.00
				MA	male	0.44	0.38
					female	0.44	0.38
Ditto ('93)	20 MZM, 20 MZF	20.0(5.0)	12.0-44.0	MA	male	0.36	0.47
	20 DZM, 20 DZF, 20 DOS				female	0.36	0.47
				CT	male	0.00	0.34
					female	0.00	0.34
				IH	male	0.00	0.57
					female	0.00	0.57
				CP	male	0.38	0.81
					female	0.38	0.22
Shapiro et al. ('68)	5 MZM, 7 MZF	23.6(?)	?	ST	m & f	0.70(=MAP)	
	4 DZM, 8 DZF	21.9(?)	?				
Smith et al. ('87)	82 MZM, 88 DZM	35.0(?)	21.0-61.0	MA		0.48	0.52
Snieder et al.	43 MZM, 47 MZF	44.4(6.7)	34.0-63.0	RT	male	0.37	0.23
(see the text)	32 DZM, 39 DZF, 39 DOS				female	0.27	0.23
				MA	male	0.36	0.25
					female	0.36	0.25
Theorell et al. ('79)	17 MZM, 13 DZM	62.0(?)	51.0-74.0	SI		0.00	0.00
Carmelli et al. ('91)	47 MZM, 54 DZM	62.4(?)	59.0-69.0	MA		0.80	0.66

Abbreviations: CP = Cold Pressor task, RT = Reaction Time task, MA = Mental Arithmetic task, CT = Concept task, IH = Isometric Handgrip, ST = Stroop task, SI = Structured Interview, MAP = Mean Arterial Pressure.
For further abbreviations see Table 4.1.

A first impression of this genotype-age interaction can be obtained by listing the twin studies in ascending order according to the subjects' ages (Table 4.2).

Heritability estimates of blood pressure reactivity vary between 0.00 and 0.81 and are far less consistent than estimates for blood pressure levels. As in Table 4.1, a trend in h^2 for blood pressure reactivity with age cannot be readily detected. In

addition to the interpretational difficulties mentioned in relation to Table 4.1, interpretation of Table 4.2 is hampered by the use of different stress tasks across studies. Even the use of different stress tasks within the same study leads to different h^2 estimates (Ditto, 1993).

2. PARENT-OFFSPRING STUDIES

Blood pressure levels

Another approach to investigating the age dependency of genetic and environmental effects is to compare parent-offspring data with data from siblings or twins (Eaves, Last, Young & Martin, 1978). If there is an age-dependent genetic or environmental effect on the phenotype, one would expect the parent-offspring correlation to be lower than sibling or dizygotic (DZ) twin correlations, as the latter are measured around the same age. This expectation was confirmed in a review by Iselius, Morton and Rao (1983). They pooled the results from a number of studies and arrived at a mean correlation for 14,553 parent-offspring pairs of 0.165 for SBP and 0.137 for DBP. Corresponding values for 11,839 sibling and DZ twin pairs were 0.235 (SBP) and 0.201 (DBP).

If, on the other hand, parents and their offspring are measured at the same age, a rise in parent-offspring correlations toward levels similar to sibling correlations is to be expected. This expectation was supported by data from Havlik et al. (1979), who measured SBP and DBP for 1141 parent pairs aged 48 to 51. Twenty to 30 years later, blood pressures for 2497 of their offspring were measured. At this time, the offspring were of ages similar to those of their parents when they were measured. Parent-offspring correlations ranged between 0.13 and 0.25 for SBP and between 0.17 and 0.22 for DBP. These ranges were quite similar to the sibling-pair correlations, which were between 0.17 and 0.23 (SBP) and between 0.19 and 0.24 (DBP).

An alternative explanation for the lower parent-offspring correlation compared to the sibling or DZ twin correlation could be the influence of genetic dominance (Tambs et al., 1992). The similarity between parent-offspring and sibling correlations in the study of Havlik et al. (1979) suggests, however, that dominance variation is not important.

Lower values for parent-offspring correlations also lead to lower h^2 estimates for blood pressure in family studies (which usually measure pairs of subjects at different ages) compared with twin studies. Heritability estimates from family studies range from 0.17 to 0.45 for SBP and from 0.15 to 0.52 for DBP (Iselius et al., 1983; Hunt et al., 1989; Rice, Vogler, Perusse, Bouchard, & Rao, 1989; Tambs et al., 1992), while estimates from twin studies range from 0.41 to 0.78 for SBP and from 0.32 to 0.72 for DBP (see Table 4.1)[2].

[2]Results of Theorell et al. (1979) are not considered because of deviant sample characteristics.

Province and Rao (1985) modeled genetic and environmental effects on SBP in nuclear families as a function of age. Before estimating environmental and genetic parameters, they used a standardization method to adjust for the effect of age on the mean and the variance; this standardization leaves temporal trends in familial resemblance intact. They found some evidence for a temporal trend in h^2, reaching maximum values of about 0.45 between 20 and 40 years of age. These results conflict with the results presented in Table 4.1, in which an age trend could not be detected. Thus, parent-offspring studies suggest that age may influence genetic effects on blood pressure, whereas no such effects are clear in twin data.

Two types of age-dependent effect could offer an explanation for the lower parent-offspring correlation compared to the sibling and DZ twin pair correlations. First, the influence of non shared environmental factors could increase with age. Such an increase, however, would lead to a lower h^2. Second, different genes could influence blood pressure in childhood than in adulthood. This possibility is still compatible with the results of Table 4.1, as h^2 can remain stable across time even though different genes are influential at different times. The latter possibility is supported by data from Tambs et al. (1993). In a Norwegian sample with 43,751 parent-offspring pairs, 19,140 pairs of siblings and 169 pairs of twins, correlations between relatives decreased as age differences between these relatives increased. A model specifying age-specific genetic additive effects and unique environmental effects fitted the data well.

This model also estimated the extent to which genetic effects were age-specific. As an example, the expected correlations for SBP and DBP in relatives with an age difference of 40 years were calculated. For SBP, 62% of the genetic variance at, for example, age 20 and at age 60 is explained by genes that are common to both ages, and 38% is explained by age-specific genetic effects. The same values for DBP were 67% and 33%, respectively. The model used by Tambs et al. (1993) assumes invariant heritabilities for blood pressure throughout life. This assumption proved to be valid for SBP, whereas for DBP a very slight increase in h^2 was detected.

On the basis of their results from a study of twins and their parents, Sims, Hewitt, Kelly, Carroll and Turner (1986) found that the assumption that the same genes act in young adulthood and middle-age would require a *decrease* in heritability from 0.68 to 0.38 from young adulthood to middle-age for DBP. This reduction, however, would need to be accompanied by an *increase* in the contribution of individual environmental factors that would account for an increase in phenotypic variance as people grow older. The same pattern of observations was seen for SBP (Sims, Carroll, Hewitt & Turner, 1987). Samples in the studies of Sims et al. (1986, 1987) were relatively small (40 MZ, 45 DZ male twin pairs, and their parents) and thus their results carry less weight than those from the very large Norwegian sample (Tambs et al., 1993). However, on the basis of the relatively small study of Sims et al. (1986, 1987), Hewitt, Carroll, Sims and Eaves (1987) presented an alternative hypothesis for increases in variance of blood pressure across the life span that could be tested in longitudinal studies using genetically informative subjects. They

proposed a developmental model in which genetic effects on blood pressure are largely the same (pleiotropic) at different points in time, but not cumulative throughout adulthood. Specific environmental influences are transient in their occurrence, but their impact is transmitted across occasions (Figure 4.1).

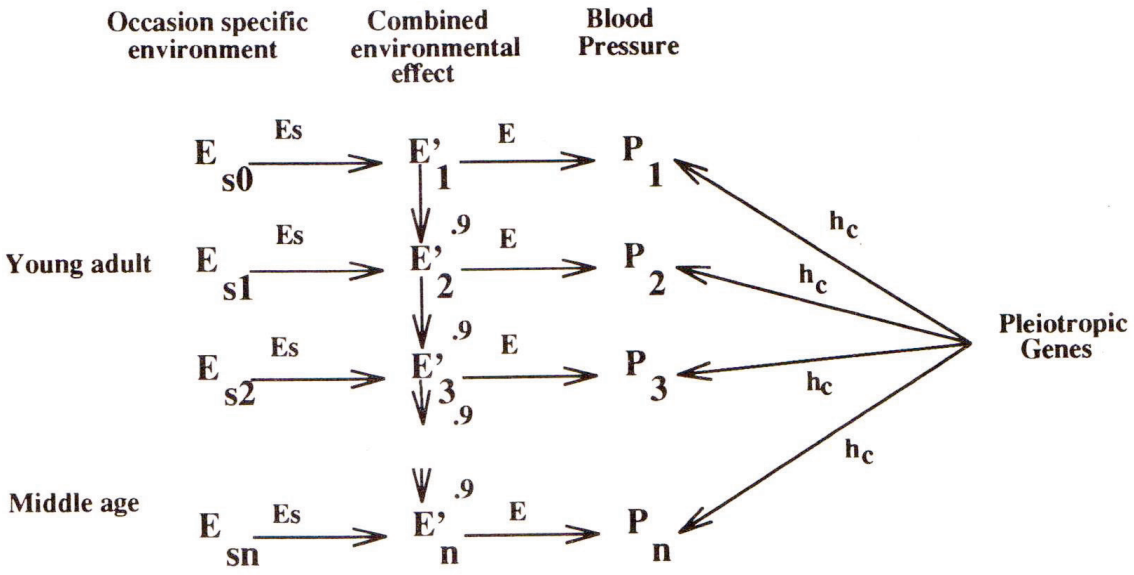

Figure 4.1. A developmental genetic model for adult blood pressure (adapted from Hewitt et al. (1987)).

Although it was restricted to twins aged in their late 40s and older and therefore did not cover the early adult to middle-aged period, the one reported longitudinal twin study (Colletto, Cardon & Fulker, 1993) -which is discussed below- appears to disconfirm this hypothesis, finding little evidence for such a transmission or accumulation of environmental influences.

Blood pressure reactivity

The few parent-offspring studies reported to date will not permit a reliable conclusion about age-dependent genetic effects on blood pressure reactivity, and the comparison of parent-offspring with sibling or DZ twin correlations is difficult because of this paucity of studies. Moreover, the published studies to date have generally found low, or unstable, familial correlations, and quantitative genetic model fitting has not been attempted. Matthews et al. (1988) conducted a study in which 145 families were measured using serial subtraction, mirror image tracing, and an isometric handgrip task. Only the correlation of SBP reactivity to the isometric handgrip was significant for parent-offspring as well as sibling pairs. Ditto (1987) investigated similarities in 36 sibling pairs (aged 18 to 21) in cardiovascular reactivity to four different stress tasks: a conceptual task, a mental arithmetic task, an isometric handgrip task and a cold pressor

task. Only one significant sibling correlation was found: this was for DBP reactivity to the cold pressor task (0.40).

Ditto and France (1990) compared correlations in a group of 30 young (mean age: 26) and 30 middle-aged (mean age: 52) spouse pairs. Spouse correlations in blood pressure reactivity to a mental arithmetic and an isometric handgrip task were small and did not increase as a function of the number of years living together. This suggests that the influence of a common environmental factor is small or even non-existent, thereby offering support for the second conclusion of Turner and Hewitt (1992); that shared environment plays only a minor role in determining individual differences in blood pressure reactivity.

3. LONGITUDINAL STUDIES

While we know of no longitudinal studies of genetic and environmental influences on blood pressure reactivity, two such studies have been reported for blood pressure levels. In such studies, variables are measured on repeated occasions, over an extended period, in the same subjects. A resulting matrix of correlations across occasions often conforms to what is referred to as the simplex pattern, in which correlations are maximal among adjoining occasions and decrease as the time between measurements increases. Such data may be described by autoregressive models in which some random change in the underlying phenotype, distinct from measurement errors, is introduced on each occasion. The underlying phenotype continually changes, making measurements from adjacent time periods more similar than those from more remote ones. This autoregressive simplex model can be generalized to the genetic analysis of longitudinal data, providing information on genetic stability across time (Boomsma & Molenaar, 1987).

Although they did not exploit these modern methods of time series analysis, Hanis, Sing, Clarke and Schrott (1983) studied genetic and environmental influences on familial aggregation, coaggregation and tracking (intraindividual correlation over time) of SBP and weight. The study started with a sample of 998 full-sibs and first cousins from 261 families of whom 601 were measured on 4 occasions. Data from occasions 1 and 2 were analyzed together as Group 1, and those from times 3 and 4 were similarly analyzed as Group 2. Models were fit first to Group 1 and then to Group 2. With respect to tracking, 59% of the SBP tracking correlation (0.33) and 60% of the weight tracking correlation (0.89) were attributable to genetic effects. Relationships between SBP and weight remained stable over time. Two shortcomings of this study, however, should be noted: the short period of follow-up (4 years) and the subjects' young age. Subjects were measured during preadolescence (from 9.2 to 13.3 years), whereas it is likely that the development of blood pressure can be divided into a preadult and an adult phase (Hewitt et al., 1987).

During adulthood, the developmental genetics of blood pressure might be similar to that recently reported for the body mass index; although the overall heritability remains relatively constant from young adulthood on, there are

nevertheless additional genetic influences acting in middle age independent of those that influence young adults (Fabsitz, Carmelli & Hewitt, 1992). This possibility has been given support by the report of Colletto et al. (1993), who analyzed SBP and DBP for 254 MZ and 260 DZ male twin pairs assessed in middle age (mean age: 48 years) and again 9 years and 24 years later. Using a time series analysis of genetic and environmental components of variation, they found that shared family environmental effects were absent and that specific environmental influences were largely occasion-specific. In contrast, genetic influences were in part the same across adulthood (60% of genetic variation at the later ages was already detected in middle age) and in part age-specific (the remaining 40% of the genetic variation at later ages was unrelated to that expressed earlier). Despite these changing genetic influences, the estimated heritabilities remain relatively constant across ages at around 0.5.

4. A COMPROMISE: ESTIMATION OF AGE-DEPENDENT GENETIC AND ENVIRONMENTAL EFFECTS WITHOUT A LONGITUDINAL DESIGN

Though twin-family designs are advantageous in that they yield additional information from the parents of the twins (Sims et al., 1986,1987; Eaves, Fulker & Heath, 1989; Boomsma, 1992), a disadvantage of this design is the underlying assumption that the same genes are expressed in parents and their offspring. Stated differently, the assumption is that the correlation between genetic effects during childhood and adulthood equals unity. To test this assumption rigorously, longitudinal data from genetically informative subjects are needed. However, a less expensive and less time consuming option is to extend the parent-offspring design to include, in addition to younger twins and their parents, a group of middle-aged twins of the same age as those parents (Stallings, Baker & Boomsma, 1989).

This extended design, which includes young twins, their parents and twins of the same age as the parents, can take account of the possibility that the correlation between genetic effects during adolescence and adulthood does not equal unity. The model can be written as:

$$R_{po} = 0.5 \times R_g \times h_1 \times h_2 \quad (1)$$

where R_{po} is the parent-offspring correlation, R_g is the genetic correlation across time (which is 1 if the same genes are expressed during adolescence and adulthood), h_1 is the estimate of genetic influence during adolescence, and h_2 is the estimate of genetic influence during middle-age. h_1 and h_2 are equal to the square root of the corresponding heritabilities and can both be estimated with a univariate analysis for the adolescent and middle-aged twin groups, respectively. As R_{po} is observed, R_g is the only unknown left, which allows one to test whether R_g differs from unity (Figure 4.2). Figure 4.2 depicts the parent-offspring model as outlined by Boomsma, van den Bree, Orlebeke and Molenaar (1989). In this model, the impact of total parental

environment on offspring environment is considered and the spouse correlation [R*e(s)*] is modeled as a correlation between total environments. The total environmental offspring correlation consists of a part that is accounted for by parental influences [through the environmental transmission parameter (*z*)] and a part independent of the resemblance with their parents [R*e(t)*].

In the extended model, parameters are estimated by using information from 10 groups: 5 groups of young twins and their parents and 5 middle-aged twin groups, grouped according to the sex and zygosity of the twins. In this multigroup design, path coefficients for the parents equal those of the middle-aged twins. The genetic transmission from parents to offspring is modeled by the *g* coefficient. This *g* coefficient equals 0.5 R*g* and *g* equals 0.5 if the same genes are expressed in adolescence and adulthood {as R*g* equals 1 in that case [see equation (1)]}. A prerequisite for the application of this model is a significant parent-offspring resemblance, which implies significant heritabilities in both childhood and adulthood and a substantial genetic correlation across time [see equation (1)].

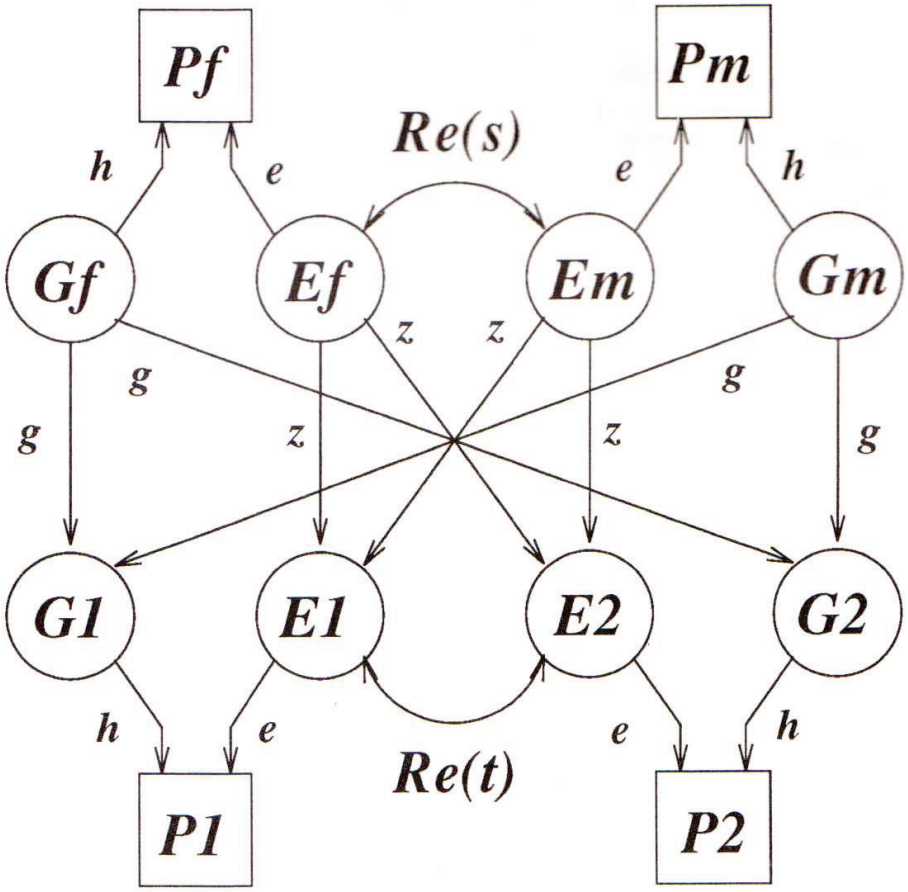

Figure 4.2. Parent-offspring model. P are observed phenotypes of father (*f*), mother (*m*), Twin 1 (*1*) and Twin 2 (*2*). G and E denote latent genotype and total environment. The genetic transmission from parents to offspring was modeled by *g*. Nongenetic transmission (*z*) is from total parental environment to offspring environment and is in this case modeled to be equal for fathers and mothers. The correlation between total environments of spouses is represented by R*e(s)* and the correlation between residual environments of twins by R*e(t)*.

Blood pressure levels

This approach was adopted in the modeling of our own data. Data were combined from two different research projects. In the first project (Boomsma, 1992) SBP and DBP were measured in a group of adolescent twins and their parents. In the other project blood pressure data from twins in the same age range as the parents were collected. Some characteristics of the subject groups are shown in Table 4.3.

Table 4.3: Number of pairs (N), mean age, standard deviation (sd) and age range of included subject groups

		Age		
Subject groups	N	mean (sd)		range
Young twins	156	16.8	(2.0)	13 - 22
Parents	156	46.8	(6.3)	35 - 65
Middle-aged twins	200	44.4	(6.7)	35 - 62

Blood pressure was measured three times by the Dinamap 845XT, using oscillometric techniques, while subjects rested and were comfortably seated in a sound-attenuated cabin for 8.5 minutes. In the first project only, this condition was repeated once. One average SBP and one average DBP value for the rest condition was calculated in all groups. Mean values and standard deviations of SBP and DBP for males and females in the three subject groups are presented in Table 4.4. Both means and standard deviations of SBP and DBP are larger in the parents and the middle-aged twin group compared to the young twin group.

Table 4.4: Number of individuals (n), mean systolic (SBP) and diastolic (DBP) blood pressure and standard deviation (sd) for males and females within the three subject groups

	Males					Females				
Subject groups	n	SBP	(sd)	DBP	(sd)	n	SBP	(sd)	DBP	(sd)
Young twins	160	119.8	(8.8)	65.8	(6.8)	152	114.9	(6.5)	67.6	(5.1)
Parents	156	128.2	(10.6)	80.7	(8.0)	156	125.7	(14.1)	77.6	(9.9)
Middle-aged twins	189	127.4	(10.8)	78.6	(8.7)	211	122.1	(13.5)	74.2	(10.3)

Before genetic modeling using LISREL VII (Jöreskog and Sörbom, 1988), blood pressure data were corrected for body weight. Maximum-likelihood estimates of parent-offspring correlations are shown in Table 4.5.

Table 4.5: Models for systolic (SBP) and diastolic (DBP) blood pressure rendering maximum likelihood estimates of parent-offspring correlations

	R*sp*	R*fs*	R*fd*	R*ms*	R*md*	χ^2	df	p
SBP	0.06	0.17	-0.02	0.22	0.44	39.36	36	0.32
	0	**0.16**	**-0.04**	**-0.21**	**0.44**	**39.88**	**37**	**0.34**
	0	0.19	0.19	0.19	0.19	55.41	40	0.05
	0	0	0	0	0	72.36	41	0.00
DBP	-0.04	0.08	0.13	0.30	0.26	25.54	36	0.90
	0	0.09	0.14	0.30	0.27	25.78	37	0.92
	0	**0.20**	**0.20**	**0.20**	**0.20**	**29.50**	**40**	**0.89**
	0	0	0	0	0	45.94	41	0.28

Abbreviations: R*sp* = spouse correlation, R*fs* = father-son correlation, R*fd* = father-daughter correlation, R*ms* = mother-son correlation, R*md* = mother-daughter correlation, χ^2 = Chi-square value, df = degrees of freedom, p = probability, AIC = Akaike's information criterion. The best-fitting models are in **boldface** type.

For SBP the model in which the spouse correlation was set to zero fitted best. In the best fitting model for DBP the spouse correlation was set to zero and the four different parent-child correlations were set equal. Models in which parent-child correlations were set to zero fitted significantly less well for both SBP and DBP (Table 4.5), which means that there is a significant parent-offspring correlation.

As stated earlier, h*1* and h*2* [equation (1)] can be estimated with a univariate analysis within the adolescent and middle-aged twins groups, respectively. Twin correlations of both groups are shown in Table 4.6. The pattern of correlations as shown in Table 4.6 is indicative of genetic influences on blood pressure, as MZ twin correlations are larger than DZ twin correlations. Standardized estimates from univariate analyses are shown in Table 4.7 for young and middle-aged twins.

Table 4.6: Twin correlations for systolic (SBP) and diastolic (DBP) blood pressure in both young twins and middle-aged twins

	Young twins			Middle-aged twins		
	N	SBP	DBP	N	SBP	DBP
MZM	35	.49	.61	43	.44	.44
DZM	31	.53	.56	32	.16	.23
MZF	33	.51	.49	47	.50	.59
DZF	29	.33	.40	39	.42	.28
DOS	28	.37	.32	39	.23	.21

N = Number of twin pairs. For further abbreviations see Table 4.1.

For both young and middle-aged twins, models estimating additive genetic (G) and environmental (E) factors fitted best. For both groups, a G and E model that allowed heritabilities to be different in males and females gave the best account of the data.

Table 4.7: Standardized estimates of best fitting univariate models for systolic (SBP) and diastolic (DBP) blood pressure in twins

	h^2_m	e^2_m	h^2_f	e^2_f	χ^2	df	p
Young twins							
SBP[a]	.49	.51	.66	.34	7.80	12	.80
DBP[b]	.69	.31	.50	.50	5.81	12	.93
Middle-aged twins							
SBP[c]	.40	.60	.63	.37	23.98	12	.02
DBP[c]	.42	.58	.61	.39	7.84	12	.80

Abbreviations: h^2_m = heritability for males, e^2_m = unique environmental variance for males, h^2_f = heritability for females, e^2_f = unique environmental variance for females. For further abbreviations see Table 4.5.

[a] For SBP, absolute genetic variance is equal for males and females.

[b] For DBP, absolute unique environmental variance is equal for males and females.

[c] For both SBP and DBP, absolute unique environmental variance is equal for males and females.

Under these conditions of a significant parent-offspring resemblance and significant heritabilities both in childhood and adulthood, it is possible to fit the extended model (Figure 4.2) and estimate the value of the genetic transmission coefficient. Results are shown in Table 4.8.

Models that estimated the genetic transmission coefficient fitted slightly better than models in which *g* was fixed at 0.5 for both SBP and DBP. This difference, however, was not significant, which means that a model with a *g* equal to 0.5 cannot be rejected on the basis of these data. The possibility that the same genes are active in parents and their offspring can therefore not be excluded. Although not visible in Table 4.8 (which present only standardized estimates), the increase in SBP and DBP variance with age (see Table 4.4) was explained in the best fitting models by an increase in both genetic and unique environmental variance for females and, for males, an increase in unique environmental variance only. As a consequence, h^2 in males is smaller in the combined parent/middle-aged twin group compared to the h^2 in the young twins.

Table 4.8: Standardized estimates of best fitting extended models for systolic (SBP) and diastolic (DBP) blood pressure

	Parents[a]				Offspring[b]							
	h^2_m	e^2_m	h^2_f	e^2_f	h^2_m	e^2_m	h^2_f	e^2_f	*g*	χ^2	df	p
SBP												
g=.5	.26	.74	.57	.43	.47	.53	.65	.35	.50	81.35	59	.029
g free	.25	.75	.62	.38	.49	.51	.66	.34	.38	79.66	58	.031
DBP												
g=.5	.29	.71	.55	.45	.69	.31	.50	.50	.50	42.31	59	.950
g free	.38	.62	.60	.40	.70	.30	.51	.49	.36	39.56	58	.970

For abbreviations see Table 4.7. The best fitting model in which the genetic transmission coefficient (g) is fixed at 0.5 and the one in which g is estimated are shown.

[a] For both SBP and DBP, absolute unique environmental variance is equal for males and females.

[b] For SBP, absolute genetic variance is equal for males and females; for DBP, absolute unique environmental variance is equal for males and females.

These results accord with data from Sims et al. (1986, 1987), who studied a group of male twins and their parents and also suggested a decrease in h^2 caused by an increase in unique environmental variance from young adulthood to middle-age. In the models in which *g* was estimated rather than fixed at 0.5, *g* was estimated to be 0.38 for SBP and 0.36 for DBP (but not significantly different from .5). This result means that the genetic correlation across time [R*g* from equation (1)] equals 0.76 for SBP and 0.72 for DBP. The slightly lower values found by Tambs et al. (1993) (0.62 for SBP and 0.67 for DBP) might be explained by the larger age difference (40 years) in their example, compared to the age difference between parents and offspring in this study (30 years).

To conclude: the slightly better fit of models estimating a genetic transmission coefficient offered some evidence, but not definitive evidence, that different genes influence blood pressure in childhood and in adulthood. The decrease of h^2 in males with age could be explained by an increase in unique environmental variance. The h^2 in females did not change with age, as unique environmental and genetic variance increased proportionally.

Blood pressure reactivity

Subjects were exposed to two mental stress tasks: a choice reaction time (RT) task and a pressured mental arithmetic (MA) task (for a detailed description of the tasks, see Boomsma, van Baal & Orlebeke, 1990). Each task lasted 8.5 minutes, during which blood pressure was measured three times. In the first project only, tasks were repeated once. For each task, one average SBP and one average DBP value were calculated in all groups. Blood pressure reactivity was calculated as the absolute difference between blood pressure level during the tasks and during the

resting period. Mean values and standard deviations of SBP and DBP reactivity to both tasks are presented in Table 4.9.

Table 4.9: Number of individuals (n), mean systolic (SBP) and diastolic (DBP) blood pressure reactivity to a reaction time (RT) and mental arithmetic (MA) task and standard deviations (sd) for males and females in the three subject groups

	Males					Females				
Subject groups	n	SBP	(sd)	DBP	(sd)	n	SBP	(sd)	DBP	(sd)
Young twins										
RT	160	6.5	(5.4)	4.7	(3.7)	152	5.4	(5.6)	3.8	(3.4)
MA	160	10.0	(6.9)	7.8	(4.7)	152	9.6	(7.9)	7.3	(4.4)
Parents										
RT	156	5.7	(6.5)	3.3	(3.7)	156	4.6	(6.7)	1.8	(3.8)
MA	156	12.5	(8.1)	6.7	(4.1)	156	11.1	(9.0)	5.0	(4.3)
Middle-aged twins										
RT	189	7.9	(8.5)	3.7	(5.9)	211	6.7	(9.0)	3.2	(5.4)
MA	189	11.1	(10.2)	6.5	(7.0)	211	9.7	(10.4)	6.5	(6.8)

In all subject groups and in both sexes SBP and DBP reactivity during the MA task was greater than during the RT task. Blood pressure responses to the tasks in males were slightly higher than in females. DBP reactivity in both sexes and to both tasks was somewhat larger in the young twins compared to the parents and middle-aged twins. Such a pattern was absent for SBP reactivity.

Maximum-likelihood estimates of parent-offspring correlations are shown in Table 4.10. In the best fitting model for SBP reactivity to both tasks, the spouse correlation was set to zero and the four different parent-child correlations were set equal. For DBP reactivity to both RT and MA tasks, models in which spouse and parent-child correlations were set to zero gave the most parsimonious account of the data. This means that there is no significant parent-offspring correlation for DBP reactivity.

Table 4.10: Best fitting models for systolic (SBP) and diastolic (DBP) blood pressure reactivity to a reaction time (RT) and mental arithmetic (MA) task rendering maximum- likelihood estimates of parent-offspring correlations

	R*sp*	R*fs*	R*fd*	R*ms*	R*md*	χ^2	df	p
SBP								
RT	0	.11	.11	.11	.11	29.05	40	.90
MA	0	.13	.13	.13	.13	42.69	40	.36
DBP								
RT	0	0	0	0	0	76.94	41	.00
MA	0	0	0	0	0	53.77	41	.09

For abbreviations see Table 4.5.

Univariate analyses for blood pressure reactivity to both tasks were executed to estimate h*1* and h*2* [equation (1)] within the adolescent and middle-aged twins groups, respectively. Twin correlations of both groups are shown in Table 4.11. No clear overall pattern pointing to either genetic or common environmental influences could be detected in these correlations. Some correlations were even negative.

Table 4.11: Twin correlations for systolic (SBP) and diastolic (DBP) blood pressure reactivity to a reaction time (RT) and mental arithmetic (MA) task in young twins and middle-aged twins

	Young twins					Middle-aged twins				
		SBP		DBP			SBP		DBP	
	N	RT	MA	RT	MA	N	RT	MA	RT	MA
MZM	35	.30	.51	-.04	.33	43	.54	.48	.19	.32
DZM	31	.16	.07	-.07	.06	32	.03	-.38	.19	-.03
MZF	33	.37	.33	.07	.27	47	.15	.33	.36	.16
DZF	29	.41	.49	.25	.41	39	-.23	.00	-.15	.09
DOS	28	.35	.07	.24	.12	39	.29	.29	.04	-.02

For abbreviations see Table 4.6.

Results of univariate analyses are shown in Table 4.12 for young twins and in Table 4.13 for middle-aged twins. For the young twins (Table 4.12), best fitting models for the two stress tasks (RT and MA) and the two reactivity measures (SBP and DBP reactivity) did not show a consistent pattern. For the SBP reactivity to the

RT task, a model invoking shared environment (C) and nonshared environmental (E) factors fitted best. A model estimating E only gave the best fit for the DBP reactivity to the RT task. For SBP and DBP reactivity to the MA task, a G and E model fitted best to the data. Best fitting models were thus different for the two tasks.

Table 4.12: Standardized estimates of best fitting univariate models for systolic (SBP) and diastolic (DBP) blood pressure reactivity to a reaction time (RT) and mental arithmetic (MA) task in young twins

	h^2	c^2	e^2	χ^2	df	p
SBP						
RT	-	.31	.69	3.94	13	.99
MA	.44	-	.56	25.63	13	.02
DBP						
RT	-	-	1.00	23.21	14	.06
MA	.38	-	.62	12.98	13	.45

Abbreviations: h^2 = heritability, c^2 = shared environmental variance, e^2 = unique environmental variance.

Table 4.13: Best fitting univariate models for systolic (SBP) and diastolic (DBP) blood pressure reactivity to a reaction time (RT) and mental arithmetic (MA) task in middle-aged twins

	h^2_m	e^2_m	h^2_f	e^2_f	χ^2	df	p
SBP							
RT[a]	.37	.63	.27	.73	27.58	12	.01
MA	.36	.64	.36	.64	26.63	13	.01
DBP							
RT	.23	.77	.23	.77	23.12	13	.04
MA	.25	.75	.25	.75	10.78	13	.63

For abbreviations see Table 4.7.

[a] For SBP reactivity to the RT task, absolute unique environmental variance is equal for males and females.

For middle-aged twins (Table 4.13), a G and E model gave the best fit in all four conditions. For SBP reactivity to the RT task, this model allowed heritabilities to be different in males and females.

As mentioned earlier, two conditions have to be met to be able to fit the extended model (Figure 4.2) and estimate a genetic transmission coefficient. These requirements were not met: No significant parent-offspring correlations were found for DBP reactivity to RT and MA tasks, and no heritabilities were found for SBP and DBP reactivity to the RT task in the young twins. Only for SBP reactivity to the MA

task was the parent-offspring correlation greater than zero and heritabilities found in childhood and in adulthood.

The picture is thus very far from clear for blood pressure reactivity. For SBP reactivity, a significant parent-offspring correlation was found; for DBP reactivity, no such correlation was found. Different models fitted best for different tasks, and for the same task, different models fitted best in young and in middle-aged twins.

DISCUSSION

This chapter has examined whether and how underlying genetic or environmental influences, or both, lead to stability or change across the life span in individual differences in blood pressure level and blood pressure reactivity to stress. Different types of genetic studies and models investigating changes with age of heritability of blood pressure level and reactivity were discussed to shed some light on this question.

In twin studies of blood pressure level, no age trend in h^2 could be detected. Findings in family studies of lower parent-offspring compared to sibling and DZ twin correlations indicate, however, that age may influence genetic or environmental effects on blood pressure level. This age-dependency could take two forms: the influence of unique environmental factors could increase with age, or different genes could influence blood pressure in children and adults. For both possibities, some evidence was found in the literature (Sims et al., 1986, 1987; Tambs et al., 1993). However, an increase of unique environmental variance in adulthood, without a commensurate increase in genetic variance, would lower the heritability estimate, and the lack of an age trend in h^2 in twin studies is inconsistent with this prediction. On the other hand, the twin data are not inconsistent with the hypothesis of genes switching on and off with age, because the overall influence of genes can remain stable even though different genes are responsible for the effect.

Modeling of our own data gave some evidence, but not definitive evidence, that different genes are active during childhood and adulthood. However, a fall in h^2 with age in males could be explained by a rise in unique environmental variance, thus supporting the other possible age effect. In females, because environmental and genetic variance increased proportionally, heritability did not change with age. It seems that at least in males, both age-dependent effects on SBP and DBP level could act simultaneously: Different genes influence blood pressure in children and adults, and unique environmental variance increases with age.

As for blood pressure reactivity, in twin studies, no age trend in h^2 could not be detected either, and the few family studies reported did not allow any conclusions about age-dependency.

Furthermore, the picture that arises from modeling of our own data is far from clear. A significant parent-offspring correlation was found for SBP reactivity, but not for DBP reactivity. Different models fitted best in young and middle-aged twins, and in young twins, the best fitting models for both SBP and DBP reactivity were different for the two tasks. This finding is somewhat unexpected if it is assumed that

responses to mental tasks represent the underlying general propensity of subjects to be reactive or not. The same task dependency of heritability estimates was observed by Ditto (1993): the SBP response to a concept formation task showed no heritability, whereas the response to a mental arithmetic task did.

A reason for these differences might be that the blood pressure response is a composite of several contributing mechanisms that may differ between tasks (see Turner, 1994). In one task, vascular processes may be the main determinant of the blood pressure elevation, while cardiac involvement may be more prominent in another task. If the genetic contribution to cardiac and vascular reactivity is different, this difference will lead to different heritability estimates of the blood pressure response to these tasks. Studying the genetic contribution to blood pressure reactivity is, in fact, studying the combined genetic effects on several intermediate phenotypes such as vascular reactivity and baroreflex sensitivity. Future studies will have to measure the genetic contributions to the response of cardiac sympathetic (pre-ejection period) and parasympathetic indices [respiratory sinus arrythmia (Boomsma et al., 1990)] and of indices of peripheral resistance and cardiac output. Such studies may reveal why heritability estimates for reactivity differ between stressors. Moreover, more knowledge of the genetics of these intermediate phenotypes will bring the genetic approach closer to the crucial questions in hypertension research.

There is growing evidence that in contrast to earlier ideas, baroreceptor sensitivity and cardiac and vascular structural changes (all of which influence reactivity) have a genetic component (Weinstock, Weksler-Zangen & Schorer-Apelbaum, 1986; Harrap, Van der Merwe, Griffin, MacPherson & Lever, 1990; Unger & Rettig, 1990; Parmer, Cervenka & Stone, 1992) and, moreover, are not merely a consequence of elevated pressure but may precede a rise in pressure and thus have an etiological role. The study of the genetics of blood pressure levels and its age and sex dependency will benefit from measuring intermediate phenotypes in addition to blood pressure. Clearly, the genetic variance in blood pressure reflects the genetic variance of its determinants. A genetic contribution to stroke volume and peripheral resistance was shown by Bielen, Fagard and Amery (1991). The age dependency of the genetic contribution to these parameters is of special interest because the contribution of the vasculature to high blood pressure is greater in older than in younger people (Lund-Johansen, 1977). By looking only at the age dependency of the genetic contribution to blood pressure, one may miss information about the genetics of mechanisms hypothesized to be involved in the etiology of high blood pressure. Our own data on these underlying mechanisms will become available in the near future.

A general problem in hypertension research, which applies to the study of age dependency of blood pressure, is the difficulty of determining whether abnormalities in blood- pressure-regulating mechanisms (in this case measured in different age groups) are causal factors for, or mere consequences of, elevated pressures. Moreover, conditions that have played a causal role at an early age may have disappeared when measured at a later age. For example an elevated cardiac output is a characteristic of some individuals with early borderline hypertension. With

increasing age, the role of peripheral resistance as a determinant of blood pressure becomes more pronounced. Thus, in older people, one might be assessing the genetics of a consequence of elevated pressure instead of the genetics of a causal factor. To properly interpret the age dependency of the genetic contribution to blood pressure and its determinants, therefore, one must place the data in the framework of our knowledge of the etiology of hypertension development.

The study of the genetics of mechanisms involved in blood pressure regulation in young children might bring us closer to causal mechanisms. There is a considerable tracking of blood pressure levels from early to later childhood (Szklo, 1979), and blood pressure at young age is an important predictor of adult levels (Lauer & Clarke, 1989). Though moderately elevated blood pressure is a precursor of essential hypertension, only 20-30% of individuals will eventually reach the hypertensive range. A longitudinal twin study approach will allow examination of the difference in patterning of genetic and environmental factors between those who return to the normotensive range and those who develop hypertension.

The genetic autoregressive simplex model could be used in this respect to construct individual genetic and environmental profiles across time by means of a statistical technique known as Kalman filtering (Boomsma, Molenaar & Dolan, 1991). Such individual profiles allow us to attribute individual phenotypic change to changes in the underlying genetic or environmental processes. Simulations have shown that these individual estimates can be reliably obtained. Estimation of genetic and environmental profiles across time would permit identification of sources of underlying deviant development in blood pressure for individual subjects.

Williams et al. (1990) have reviewed possible candidates for genetic and environmental causes of the development of hypertension. They expressed the hope that in the near future biochemical tests and application of gene markers may provide methods for quantitatively assessing a person's risk for hypertension. A possible genetic marker for high blood pressure was identified in the parents of twins from our study: Individuals carrying the α-1-antitrypsin deficiency alleles S and Z had lower blood pressure during rest and stress. This finding replicates that from an Australian twin sample (Boomsma, Orlebeke, Martin, Frants & Clark, 1991). Determining the balance between environmental and such predisposing genetic factors as early as possible is of utmost importance for preventive purposes because high blood pressure, once established, is not easily reversed.

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5

The age-dependency of gene expression for plasma lipids, lipoproteins and apolipoproteins

Harold Snieder, Lorenz J.P. van Doornen and Dorret I. Boomsma

INTRODUCTION

Lipids, including cholesterol and triglycerides, are transported in the circulation by a number of different lipoproteins of which very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) are the most important. Apolipoproteins, are bound to the surface of the lipoprotein particles and play an important role in lipid metabolism: through apolipoproteins, lipoproteins are actively recognized, bound and absorbed by specific receptors (Rader & Brewer, 1994). Apolipoprotein A1 (ApoA1) is part of the HDL, and apolipoprotein B (ApoB) is the sole protein of LDL.

Numerous epidemiological studies have established the association between separate constituents of the lipid system, and the risk of coronary heart disease. High levels of total cholesterol, LDL and ApoB and low levels of HDL and ApoA1 are all predictors of atherosclerotic coronary heart disease. Apolipoproteins are of great interest in this respect, as several studies indicate that they are better discriminators than plasma lipids for atherosclerosis (Avogaro, Cazzolato, Bittolo Bon & Quinci, 1979; Durrington, Ishola, Hunt, Arrol & Bhatnagar, 1988). An independent association between triglyceride levels and cardiovascular risk remains to be established, although triglycerides have prognostic value in combination with LDL and HDL (Schulte, Rothman & Austin, 1994) and as one of the components of the insulin resistance syndrome (Reaven, 1988). Another particle of the lipid system, lipoprotein(a) [Lp(a)], is also considered an important risk factor for coronary heart disease (Rhoads, Dahlen, Berg, Morton & Dannenberg, 1986; Durrington et al., 1988). Lp(a) was first described by Berg in 1963, and is structurally related to LDL (Utermann, 1989).

Developmental trends in mean levels

Plasma lipid and (apo)lipoprotein levels vary considerably with age and the changes of the different lipid components do not run in parallel. Moreover, the age trends differ for males and females. Superimposed on the global change during the whole life span four more specific periods are associated with more dramatic changes: the first years after birth, adolescence, the menopausal period in females, and old age.

The most dramatic changes in lipids, lipoproteins and Lp(a) occur during the first years of life. During the first 6 months concentrations double or triple (Berenson et al., 1979; Wang, Wilcken & Dudman, 1992). Lp(a) concentrations already reach adult levels at 8.5 months (Wang, Wilcken & Dudman, 1992). Levels of other lipid parameters rise further at a slower rate until about 2-3 years when they approach young adult levels. Cholesterol and HDL increase slightly between 3 and 5 years. No clear sex differences exist yet (Berenson et al., 1978). In preadolescents (5-10 years) cholesterol and triglyceride levels remain fairly constant (Frerichs, Srinivasan, Webber & Berenson, 1976).

During puberty (12-16 years) cholesterol declines by 10 to 20% in boys after which it starts to rise, reaching preadolescent levels again by the end of teenage. In girls the picture is less clear, a drop is sometimes reported (Berenson, Srinivasan, Cresanta, Foster & Webberet, 1981) but a recent longitudinal study observed no change (Twisk, Kemper & Mellenbergh, 1995). HDL in boys goes down in this period but remains unchanged in girls (Berenson et al., 1981; Twisk et al., 1995). Triglyceride levels show an increase in both sexes during adolescence, but relatively more in boys than in girls (Berenson et al., 1981). By the end of puberty girls have equal cholesterol and triglyceride levels but higher HDL levels than boys. The ApoA1 and ApoB changes during puberty parallel those in HDL and total cholesterol respectively. Lp(a) shows no changes during this period and does not differ between sexes (Boomsma, Kaptein, Kempen, Gevers Leuven & Princen, 1993; Bergstrom, Hernell, Persson & Vessbyet, 1995).

The development in young males, between 18 and 30 years, shows an increase of cholesterol, LDL, triglycerides and ApoB and no change in HDL. In women little change (Donahue et al., 1989) or a rise are observed for cholesterol and LDL (Twisk et al., 1995), a decline in triglycerides and a rise in HDL and ApoA1 (Donahue et al., 1989). This results in the typical sex difference in lipid profile as observed in the middle-aged population. Up to 50 years of age, cholesterol, LDL and triglycerides continue to rise and HDL decreases slightly in both sexes (Verschuren et al., 1993). In this period males have higher cholesterol, triglyceride, LDL and ApoB levels, but lower HDL and ApoA1 levels than females (Schaefer et al., 1994; Brunner et al., 1993). After the age of 50, female cholesterol levels exceed the male levels (Schaefer et al., 1994). Lp(a) increases slightly between 20 and 59 years and has the same level in both sexes in all age groups (Jenner et al., 1993).

In women menopause influences some lipid parameters. Matthews et al. (1989) did a follow-up study in premenopausal women. In some women menopause occurred during the follow-up period. Menopause led to a significant decrease in

HDL. The increase in LDL was twice as large in menopausal compared to premenopausal women of the same age during follow-up. Total cholesterol, triglycerides, ApoA1 and ApoB changed to the same extent in both groups. Thus apart from an age effect no specific influence of menopause on these parameters was evident. Lp(a) levels are a bit higher in post-menopausal women but the effect disappears after age correction (Jenner et al., 1993).

In elderly people, from about 65 years on, cholesterol levels go down, especially in males. HDL remains stable (Kromhout et al., 1990; Kronmal, Cain, Ye & Omenn, 1993). The fall in total cholesterol with old age, however, may represent cohort, period or survivorship effects or a combination of these effects, rather than a true decline (Newschaffer, Bush & Hale, 1992).

As lipid and (apo)lipoprotein levels vary considerably with age, it is important to realise that the factors that determine lipid levels at a certain age will probably partly differ from those involved at another age. Moreover, these lipid determining factors may partly differ for males and females. For example, the drop in cholesterol level during puberty may have another origin than the rise during middle age, which in turn may have a different cause than the change in lipid levels due to menopause. These (sex-specific) age trends may indicate the involvement of different genetic or environmental factors or both, at different ages.

Developmental trends in variances

Generation and sex influences on the variances of components of the lipid system are less well understood than mean trends. Most studies did not have an explicit interest in age and sex trends in variances. Nevertheless, the large population studies on age and sex trends in mean levels, also provide standard deviations. From these, it can be deduced that variances increase in most lipids and (apo)lipoproteins between early adulthood (20-29) and middle-age (40-59) in both males and females (Verschuren et al., 1993; Schaefer et al., 1994). During middle-age, variances in lipids and (apo)lipoproteins remain relatively stable (Pooling Project Research Group, 1978; Kronmal et al., 1993; Verschuren et al., 1993; Schaefer et al., 1994). Only in old age (70+) there are indications for a decline (Schaefer et al., 1994). These results pertain to both sexes. Except for the drop during old age, variance trends in Lp(a) were similar (Jenner et al., 1993).

In contrast to abovementioned studies, Reilly, Kottke and Sing (1990) specifically investigated effects of sex and generation on variances in lipids and (apo)lipoproteins. To this goal lipids were measured in grandparents (mean age: 68), parents (mean age: 42) and children (mean age: 15) of both sexes. Significant differences in variances between generations were found for total cholesterol and LDL in both males and females, and for triglycerides, HDL and ApoA1 in males only. Differences were attributable to an increase in variance with increasing age. In a study of 160 twin pairs and their parents, Boomsma et al. (1996) largely confirmed results of Reilly, Kottke and Sing (1990). Variances of total cholesterol, triglycerides, HDL, LDL, ApoA1 and ApoB were all significantly higher in the parental generation. Only for Lp(a) no generation effect was observed.

In summary, most studies report an increase in variance with age for most lipid parameters in both sexes. Such an increase in variance from adolescence to adulthood in lipids and (apo)lipoproteins may be due to an increase in the amount of genetic variance, nongenetic variance, or both.

Genetic developmental trends

Genetic factors have been shown to exert considerable influence on levels of lipids, lipoproteins and apolipoproteins. A large number of monogenic disorders have been described (Schonfeld & Krul, 1994), but they can only account for a relatively small portion of the population variance in lipid levels and rates of coronary heart disease. Twin and family studies, using quantitative genetic approaches, indicate that a considerable part of the variation in lipids, lipoproteins and apolipoproteins results from polygenic influences (reviewed in Iselius, 1979; Iselius, 1988; O'Connell et al., 1988; Lamon-Fava et al., 1991; Heller, de Faire, Pedersen, Dahlén & McClearn, 1993; Rao & Vogler, 1994; Brenn, 1994). The proportion of the variance explained by genetic factors can be expressed as heritability.

Currently we know very little about developmental trends in the heritability of lipid parameters, and we know even less about causes of stability and change in these variables. As stated, the sex specific age trends in levels and variances of lipid parameters may indicate that different genetic and environmental factors may be influential in different sex-age cohorts. Changes in variance components with age can imply changes in heritabilities with age, as well as differences in correlations among relatives of different ages. Both twin and parent-offspring studies may be informative in this respect.

Twin studies

If twins within a specific age range are measured in studies estimating the genetic influence on lipids, lipoproteins and apolipoproteins, heritabilities for this specific age range are obtained. To get an impression of possible age trends in heritability, we have listed recent twin studies (since 1977) in ascending order according to the age of the twin sample. Twin studies before 1977 were reviewed by Iselius (1979). Estimates for males and females are given separately. Heritability, estimated as $2(r_{MZ} - r_{DZ})$ (Falconer, 1989), was listed for all studies that did not use model fitting techniques. Results for lipids and lipoproteins are shown in Table 5.1. Although studies used different methods to estimate heritability, results were relatively consistent. Most studies found that in both males and females, over 50% of the total variance in total cholesterol, LDL, HDL and triglycerides could be explained by genetic factors. Furthermore, the studies listed did not find much support for a considerable influence of the shared family environment. No obvious age trend in heritability estimates can be detected from Table 5.1. Heritabilities measured before (Bodurtha et al., 1991) and during puberty (Boomsma et al., 1996) are highly similar, and they remain fairly constant during early adulthood and middle-age. Only in the elderly, heritabilities seem to decrease (Heller et al., 1993).

Table 5.2 shows the results for apolipoproteins and lipoprotein(a). Since less studies, with mostly smaller twin samples were done for apolipoproteins, estimates were somewhat more variable compared to those in Table 5.1. With the exception of some null results for ApoA1, observed heritabilities were roughly within the same range as for lipids and lipoproteins. High heritabilities for Lp(a) are in accordance with findings, that the apo(a) gene accounts for more than 90% of the variation in Lp(a) concentration (Boerwinkle et al., 1992). Again a clear age trend cannot be detected, although Heller et al. (1993) found a significantly smaller heritability for ApoB in their older as compared to their younger twin sample.

Parent-offspring and longitudinal studies

Another approach with which the age dependency of genetic and environmental effects can be investigated, is to compare different familial correlations (Eaves, Last, Young & Martin, 1978). If there is an age-dependent genetic or environmental effect on the phenotype, one would expect the parent-offspring correlation to be lower than the sibling correlation, and the sibling correlation to be lower than the dizygotic (DZ) twin correlations, as the age difference within those pairs decreases. This expectation was confirmed for cholesterol, as shown in a review by Iselius (1979) of earlier twin and family studies. For 4,716 parent-offspring pairs a mean correlation of 0.26 was found, whereas for 2,056 sibling pairs and 622 pairs of dizygotic twins, these values were 0.34 and 0.44 respectively. Although the observed correlations are in accordance with the hypothesized age effect. Iselius (1979) attributed the differences in familial correlations to a DZ twin specific shared environmental effect in addition to a specific shared environmental factor for siblings. In accordance with Iselius (1979), Boomsma et al. (1996) also observed lower parent-offspring than DZ twin correlations for lipids, lipoproteins and apolipoproteins. The earlier mentioned higher phenotypic variances in parents, compared to that of their offspring, could best be explained by a genetic model that specified an increase in unique environmental variance with increasing age. This increase in unique environmental variance subsequently led to smaller heritabilities in the parental generation. However, the parent-offspring model used by Boomsma et al. (1996) assumed that the same genes are expressed in parents and their offspring. This assumption may not be valid. Recent studies on body mass index (Fabsitz, Carmelli & Hewitt, 1992) and blood pressure (Colletto, Cardon & Fulker, 1993; Tambs et al., 1993; Snieder, van Doornen & Boomsma, 1995) indicate that it may well be possible that the same phenotype is influenced by different genes in different periods of life. Longitudinal studies, in which the same genetically informative subjects are measured repeatedly, are needed to test such an assumption rigorously. Williams and Wijesiri (1993) analyzed longitudinal data on total cholesterol, LDL, HDL and triglycerides from the NHLBI veteran twin study.

Table 5.1: Twin studies estimating heritability (h^2) in lipids and lipoproteins, in ascending order according to age

		Age					h^2			
Investigator	Pairs of twins	Mean	(SD)	Range	Sex	Adjusted	TC	LDL	HDL	TRG
Bodurtha et al. ('91)	65 MZM, 68 MZF 23 DZM, 27 DZF, 50 DOS	11.0	(?)	?	male female		0.71 0.80	0.85 0.85	0.76 0.76	0.68 0.68
Boomsma et al. ('96)	35 MZM, 35 MZF 31 DZM, 30 DZF, 29 DOS	16.7	(2.0)	13.0-22.0	male female		0.80 0.80	0.82 0.82	0.71 0.71	0.60 0.60
Whitfield & Martin ('83)	42 MZM, 42 MZF 38 DZM, 44 DZF, 39 DOS	23.1	(?)	18.0-34.0	male female		0.54 0.54		0.24 0.24	0.53 0.51
Hunt et al. ('89)	73 MZM, 81 DZM	34.5	(9.5)	?		age age & env	0.61 0.65		0.74 0.51	0.81 0.75
O'Connell et al. ('88)	39 MZM, 67 MZF 25 DZM, 69 DZF	36.3	(12.4)	17.0-66.0		sex sex & env	0.58 0.79	0.89 0.63	0.60 1.00	
Williams et al. ('93)	44 MZF, 31 DZF	37.6	(?)	17.0-64.0			0.64	0.67	0.71	
Austin et al. ('87)	233 MZF, 170 DZF	42.0	(?)	?		age age & env		0.98 0.92	0.68 0.82	0.80 0.63
Berg ('83)	35 MZM, 43 MZF 33 DZM, 47 DZF	?	(?)	33.0-61.0	male female		0.40 0.40			0.00 0.72
Snieder et al.(see the text)	39 MZM, 50 MZF 36 DZM, 40 DZF, 38 DOS	44.1	(6.7)	34.0-63.0	male female		0.68 0.68	0.69 0.69	0.71 0.71	0.59 0.59
Feinleib et al. ('77)	250 MZM, 264 DZM	?	(?)	42.0-56.0			0.43	0.57	0.46	0.56
Heller et al. ('93)	57 MZ, 94 DZ 56 MZ, 95 DZ	? ?	(?) (?)	52.0-65.0 66.0-86.0		age & sex age & sex	0.63 0.32		0.76 0.55	0.72 0.28

Abbreviations: MZM = Monozygotic Males, MZF = Monozygotic Females, DZF = Dizygotic Females, DZM = Dizygotic Males, DOS = Dizygotic Opposite-Sex, TC = Total Cholesterol, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, TRG = Triglyceride, env = environment.

Table 5.2: Twin studies estimating heritability (h^2) in apolipoproteins and lipoprotein(a), in ascending order according to age

		Age					h^2		
Investigator	Pairs of twins	Mean	(SD)	Range	Sex	Adjusted	ApoA1	ApoB	Lp(a)
Boomsma et al. ('93,'96)	35 MZM, 35 MZF 31 DZM, 30 DZF, 29 DOS	16.7	(2.0)	13.0-22.0	male female		0.78 0.78	0.48 0.48	0.98[a] 0.93[a]
Sistonen & Ehnholm ('80)	25 MZM, 40 MZF 34 DZM, 36 DZF	36.0	(?)	20.0-69.0	male female		0.00 0.00		
Berg ('83,'84)	44 MZM, 54 MZF 46 DZM, 54 DZF	?	(?)	33.0-61.0	male female		0.53 0.53	0.52[b] 0.74[b]	
Snieder et al. (see the text)	39 MZM, 50 MZF 36 DZM, 40 DZF, 38 DOS	44.1	(6.7)	34.0-63.0	male female		0.58 0.58	0.73 0.73	0.87 0.87
Austin et al. ('92)	338 MZF, 250 DZF	51.0	(?)	?					0.94
Kuusi et al. ('87)	17 MZM, 18 DZM	?	(?)	48.0-63.0			0.66		
Hong et al. ('95)	63 MZ, 108 DZ	?	(?)	50.0-64.0		age & sex			0.94
Heller et al. ('93)	57 MZ, 94 DZ	?	(?)	52.0-65.0		age & sex	0.69	0.78	
Hayakawa et al. ('87)	42 MZM, 22 MZF 17 DZM, 2 DZF	?	(?)	50.0-74.0	m & f		0.48	0.80	
Lamon-Fava et al. ('91)	109 MZM, 113 DZM	?	(?)	60.0-70.0		 env	0.38 0.38	0.91 0.85	0.67
Hong et al. ('95)	55 MZ, 78 DZ	?	(?)	65.0-86.0		age & sex			0.94
Heller et al. ('93)	56 MZ, 95 DZ	?	(?)	66.0-86.0		age & sex	0.52	0.51	

Abbreviations: ApoA1 = Apolipoprotein-A1, ApoB = Apolipoprotein-B, Lp(a) = Lipoprotein(a), m & f=males and females combined.
For further abbreviations see Table 5.1.
[a] Estimates are the same for parents and their twin offspring [152 families, see Boomsma et al. (1993)].
[b] Estimates for ApoB are based on a smaller number of twin pairs, as listed in Table 5.1.

Between 48 and 63 years of age a high stability in the expression of genetic factors was found. However, the age range of the veteran sample is not directly comparable to the age range of adolescents and their parents participating in the study of Boomsma et al. (1996).

An alternative to a longitudinal study is to augment the parent-twin design with a different group of twins of the same age as the parents of the adolescent twins. This allows the estimation of heritabilities for each generation separately, based on the information available from adolescent and adult twins. If these heritabilities are known, the observed parent-offspring correlation can be used to estimate the correlation between genes effective in adolescence and adulthood. Likewise, the stability of environmental effects can be examined (Stallings, Baker & Boomsma, 1989; Snieder, van Doornen & Boomsma, 1995).

Aim of the study

The principal aim of this study was to disentangle the genetic and non-genetic causes of stability and change in lipid, lipoprotein and apolipoprotein levels that occur during the lifespan. To that end, a quantitative genetic model was defined in which the regular twin-family design, including parents and their twin-offspring, was extended with a group of middle-aged twins of the same age as those parents.

METHODS

Subjects

Data were combined from two different research projects. In the first project cardiovascular risk factors were measured in a group of 160 adolescent twin pairs and their parents. Detailed information on recruitment, zygosity determination and exclusion criteria is given elsewhere (Boomsma et al., 1993: Boomsma et al., 1996). The adolescent twin sample consisted of 35 pairs of monozygotic males (MZM), 31 pairs of dizygotic males (DZM), 35 pairs of monozygotic females (MZF), 30 pairs of dizygotic females (DZF) and 29 dizygotic pairs of opposite sex (DOS). For Lp(a) group sizes were slightly smaller: 33 pairs of MZM, 30 pairs of DZM, 33 pairs of MZF, 30 pairs of DZF and 26 pairs of DOS.

In a second project cardiovascular risk factors were studied in a group of 213 middle-aged twins (Snieder, van Doornen & Boomsma, 1995; Snieder, Boomsma, van Doornen & de Geus, 1996). Middle-aged twins were in the same age range (34-63 years) as the parents of the adolescent twin sample (35-65 years). Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry and sollicitation through the Dutch Twin Club. In addition, a small number of twins who heard from the study in another way volunteered to participate. Informed consent was obtained from all subjects. Data from 10 twins were excluded from the sample. In 8 twins one or both members of the pair used lipid lowering medication (HMG-CoA reductase inhibitors). In one subject no blood could be obtained. Data from another subject were discarded because of an extremely high triglyceride value (5.96 mmol/l). One

(monozygotic) triplet was included in the sample by discarding the data from the second born subject. In total, 188 males and 218 females were included in the study. As it is known that other lipid parameters and HMG-CoA reductase inhibitors hardly affect Lp(a) levels (Sundell, Nilsson, Hallmans, Hellsten & Dahlén, 1989; Austin et al., 1992; Kostner et al., 1993), exclusion criteria for Lp(a) were different: only twins in which one or both members had an extremely high value (>128 mg/dl; 3 pairs) were excluded.

In 76 same-sex twin pairs zygosity was determined by DNA fingerprinting (Jeffreys, Wilson & Thein, 1985; Meulenbelt, Droog, Trommelen, Boomsma & Slagboom, 1995). In the remaining 89 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 39 pairs of MZM, 36 pairs of DZM, 50 pairs of MZF, 40 pairs of DZF and 38 dizygotic pairs of DOS. For Lp(a) group sizes were: 42 pairs of MZM, 39 pairs of DZM, 49 pairs of MZF, 40 pairs of DZF and 39 pairs of DOS.

Blood sampling and biochemical assays

In the first project EDTA blood was obtained between 8:30 and 10:30 a.m. by venipuncture after overnight fasting. Plasma was separated from cells after centrifugation for 10 minutes at 3000 rpm. Part of the plasma was kept at 4^{o}C for lipid determinations within the next 5 days. The remainder was frozen at -20^{o}C for later use. Total cholesterol and HDL were determined by the enzymatic CHOD-PAP method (Boehringer, Mannheim, Germany; Cat. No. 236691). HDL was determined in the supernatant after precipitation of VLDL and LDL with $MgCl_2$ and sodium-phosphowolframate (Lopes-Virella, Stone, Ellis & Coldwell, 1977). LDL was calculated using the formula of Friedewald (Friedewald, Levy & Fredrickson, 1972). With this formula triglyceride concentrations must not exceed 4.52 mmol/l (Rifai et al., 1992). There were no subjects with triglycerides above 4.07 mmol/l. Triglycerides were measured by the enzymatic GPO-PAP method (Boehringer, Mannheim, Germany; Cat.No. 701904). Apolipoproteins A1 and B were quantified by radial immunodiffusion as described by Albers, Wahl, Cabana, Hazzard and Hoover (1976) and Havekes, Hemmink and de Wit (1981). Lp(a) levels were measured with a "bi-site" sandwich ELISA using polystyrene microtiter plates ("Nunc Immuno" plates, "high binding" quality, Intermed, Roshilde, Denmark). For a more detailed description of Lp(a) determination see Boomsma et al. (1993).

In the second project twins arrived at the laboratory at about 10.00 a.m. They were requested to fast, refrain from smoking and the use of alcohol, coffee and tea after 11.00 p.m. of the preceding night. Blood was collected by venipuncture, using Vacutainer tubes (Becton-Dickinson) containing sodium-EDTA. The tubes were

placed on ice and centrifuged promptly (30 minutes, 2000g) at 4 ^{o}C to separate plasma from cells. Aliquots of plasma were snap-frozen using liquid nitrogen and stored at -20 ^{o}C until processing. Total cholesterol, HDL and triglycerides were determined in the same way as in the first project. LDL was again calculated with Friedewald's formula. One subject showed a higher triglyceride value than 4.52 mmol/l (5.92 mmol/l) and was therefore removed from further analysis. Apolipoproteins A1 and B were quantified with the method of Beckman using the "Array Protein System" (Beckman Instruments, Inc., Fullerton, California, U.S.A.) (Maciejko et al., 1987). The Beckman calibrator (standardized to IUIS/WHO reference material CDC #1883) was used as standard reference material. Monospecific goat-antihuman ApoA1 and ApoB antisera were used (Beckman). Lp(a) was measured with the same "Array Protein System" (Beckman). The calibrator from Immuno AG (Vienna GmbH, Heidelberg) was used for the calibration curve, which was calculated as a polynomal-5 with the Flexisoft software (Beckman). Goat-antihuman Lp(a) antiserum was used (Immuno AG, Vienna GmbH). The measuring range of the test lies between 2 and 128 mg/dl.

Genetic analysis of lipids, lipoproteins and apolipoproteins

As an alternative to a longitudinal study the parent-twin design used by Boomsma et al. (1996) was augmented with twins of the same age as the parents. A prerequisite for the application of this extended model is a significant parent-offspring resemblance, which implies significant heritabilities in both childhood and adulthood and a substantial genetic correlation across time. Therefore, preceding the application of the extended model, univariate model fitting to adolescent and middle-aged twin data was performed to estimate heritabilities for each generation separately. Parent-offspring correlations were already estimated by Boomsma et al. (1996). Before we describe the univariate twin model and the extended parent-twin model that were used in the data analysis, some general features of the genetic model fitting procedure are discussed.

Model fitting procedure

Both univariate and extended models were fitted to variance-covariance matrices by the method of maximum likelihood, using the program Mx (Neale, 1994), which was specifically developed for analysis of genetically informative subjects. Mx provides parameter estimates (h, d, c, e, a), a chi-square test of the goodness-of-fit of the model and Akaike's information criterion (AIC). The overall χ^2 tests the agreement between the observed and predicted variances and covariances in the different zygosity groups. A large chi-square indicates a poor fit (low probability), while a small chi-square indicates that the model is consistent with the data (high probability). Submodels were compared by hierarchic χ^2 tests, in which the χ^2 for a reduced model is subtracted from that of the full model. The degrees of freedom (df) for this test are equal to the difference between the df for the full and the reduced model. The eventual purpose of the model fitting procedure is to explain the

pattern of covariances and variances by using as few parameters as possible. To evaluate the fit of the models, AIC (calculated as χ^2-2df) was also used. The model with the lowest AIC reflects the best balance of goodness-of-fit and parsimony. Besides AIC, Jöreskog's χ^2/df was used to evaluate the fit of the extended models. This measure can be used when fitting many models with large degrees of freedom. A value of less than 2 for Jöreskog's χ^2/df, indicates an adequate fit of the model to the data (Finkel, Pedersen, McGue & McClearn, 1995).

Univariate analyses

Separate univariate analyses within the adolescent and middle-aged twins groups were executed. Lipid phenotypes (and age) in twin and co-twin of both twin groups, were summarized into 3x3 variance-covariance matrices for each of the 5 zygosity groups. Age can spuriously introduce a common environmental effect if there is a significant correlation between the phenotype and age, which is the case for many lipid parameters. By incorporating age into the model, the influence of age on the phenotype can be quantified (Neale & Martin, 1989).

Sex differences were examined 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. In addition to those models a scalar model was tested. In a scalar model heritabilities are constrained to be equal across sexes, but total variances may be different. All (non-standardized) variance components for males are constrained to be equal to a scalar multiple, k^2, of the female variance components, such that $h_m^2 = k^2 h_f^2$, $c_m^2 = k^2 c_f^2$, $e_m^2 = k^2 e_f^2$, $d_m^2 = k^2 d_f^2$ and $a_m^2 = k^2 a_f^2$. As a result, the standardized variance components such as heritabilities are equal across sexes, even though the unstandardized components differ (Neale & Cardon, 1992). Sex-specific genetic effects can be tested by fixing the correlation between the latent genetic factors (R_G) in the DOS twin group to zero, or estimating it (allowing it to be smaller than 0.5).

The applied univariate model that includes age, is presented in Figure 5.1. The path diagram shows an opposite-sex twin pair. Twin 1 is a female, twin 2 a male. The observed phenotypes for twin and co-twin are shown in squares, and latent factors are shown in circles. Correlations between the latent genetic factors (R_G) are 1 in MZ twins, 0.5 in DZ same-sex twins. R_G can be estimated in DOS twin pairs. Factor loadings of observed variables on the different latent factors are also shown. *k* is the scalar factor.

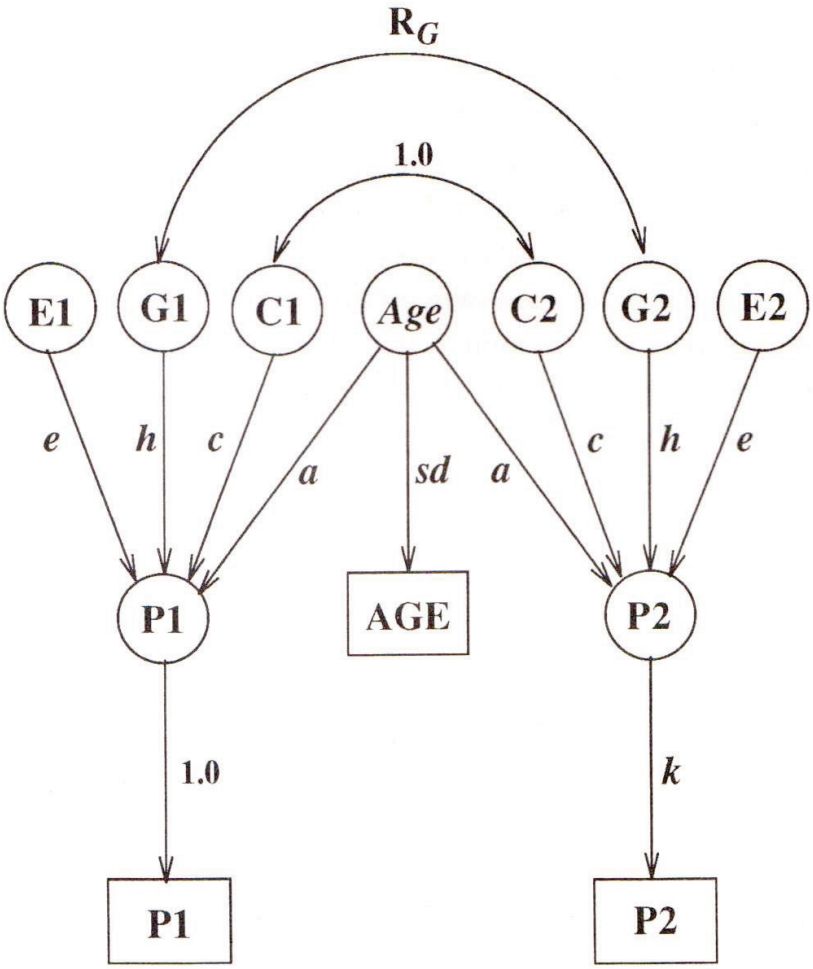

Figure 5.1: Path diagram for a univariate scalar model including age. An opposite-sex twin pair is shown, twin 1 being female and twin 2 being male. Observed phenotypes (P) for twin 1 and twin 2 are shown in squares, latent factors are shown in circles. Correlations between additive genetic factors (R_G) are 1 in MZ twins and 0.5 in DZ same-sex twins, and can be estimated for DOS twins. Factor loadings of observed variables on the different latent factors are also shown: h = additive genetic effect, c = shared environmental effect, e = unique environmental effect, a = influence attributed to age, sd = standard deviation of age, *k* = scalar factor.

Extended parent-twin design

An underlying assumption of the parent-twin design is, that the same genes are expressed in parents and their offspring. Stated differently, it is assumed that the correlation between genetic effects during childhood and adulthood equals unity. With the extended parent-twin design, which includes young twins, their parents and twins of the same age as the parents, the hypothesis can be tested that the correlation between genetic effects during adolescence and adulthood does *not* equal unity. The model can be written as:

$$R_{po} = 0.5 \times R_g \times h_1 \times h_2 \quad (1)$$

where R*po* is the parent-offspring correlation, R*g* is the genetic correlation across time (which is 1 if the same genes are expressed during adolescence and adulthood), h*1* is the estimate of genetic influence during adolescence, and h*2* is the estimate of genetic influence during middle-age. h*1* and h*2* are equal to the square root of the corresponding heritabilities and can both be estimated with a univariate analysis for the adolescent and middle-aged twin groups, respectively. As R*po* is observed, R*g* is the only unknown left (assuming that shared environmental influences are negligible), which allows the test whether R*g* differs from unity. If there is shared environment between parent and offspring, it would explain part of the correlation between them (R*po*). Therefore, the model we used considered both genetic (*g*) and environmental transmission (*z*) as a source of covariance between parents and offspring (Figure 5.2).

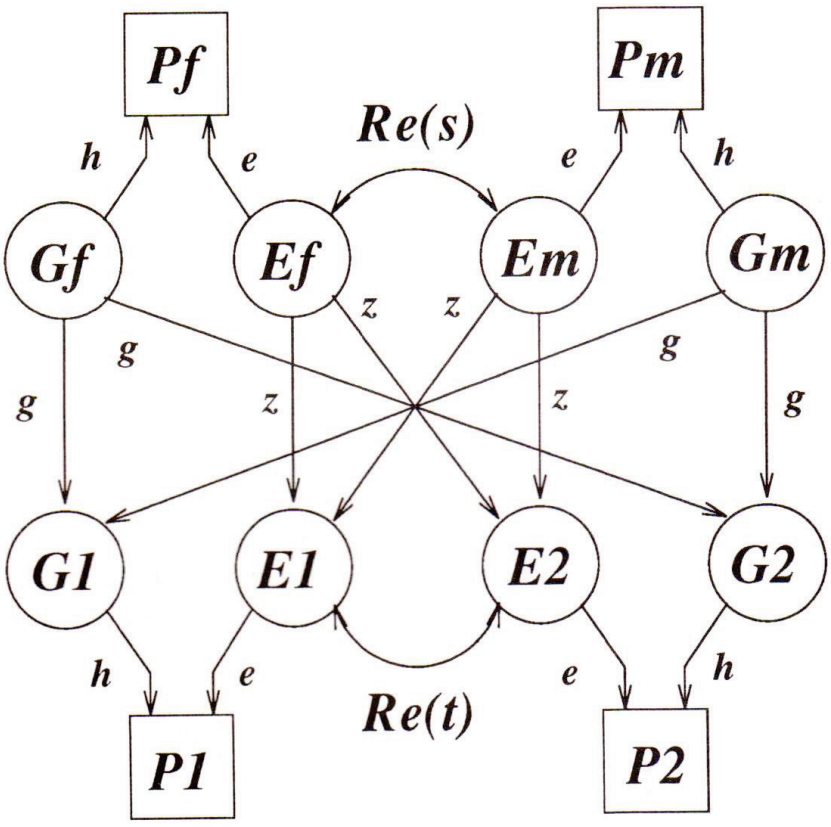

Figure 5.2. Parent-offspring model. P are observed phenotypes of father (*f*), mother (*m*), Twin 1 (*1*) and Twin 2 (*2*). G and E denote latent genotype and total environment. *g* is the genetic transmission from parents to offspring. *z* is the nongenetic transmission from total parental environment to offspring environment, in this case modeled to be equal for fathers and mothers. The correlation between total environments of spouses is represented by R*e(s)* and the correlation between residual environments of twins by R*e(t)*.

Figure 5.2 depicts the parent-offspring model as it was first outlined by Boomsma, van den Bree, Orlebeke and Molenaar (1989). In this model, the impact of total parental environment on offspring environment is considered and the spouse correlation [R*e(s)*] is modeled as a correlation between total environments. The total environmental offspring correlation consists of a part that is accounted for by parental influences [through the environmental transmission parameter (*z*)] and a part independent of the resemblance with their parents [R*e(t)*]. Although not shown, age was also incorporated into the model to control for its influence on parameter estimates of the model.

In the extended model, parameters are estimated by using information from 10 groups: 5 groups of young twins and their parents and 5 middle-aged twin groups, grouped according to the sex and zygosity of the twins. In this multigroup design, path coefficients for the parents equal those of the middle-aged twins. The genetic transmission from parents to offspring is modeled by the *g* coefficient. This *g* coefficient equals 0.5 R*g* and *g* equals 0.5 if the same genes are expressed in adolescence and adulthood {as R*g* equals 1 in that case [see equation (1)]}.

Statistical analysis

Because the distributions of triglyceride and Lp(a) levels were skewed, these data were logarithmically transformed to obtain a more normal distribution. All data were corrected for any between occasions variation in mean levels. The effects of sex and zygosity on the means were tested by likelihood ratio χ^2 tests, using Mx (Neale, 1994).

RESULTS

Table 5.3 shows means and standard deviations of age, lipids, lipoproteins and apolipoproteins in young twins, their parents and middle-aged twins. Significance of sex differences in mean values within each of these groups, evaluated by likelihood ratio χ^2 tests, are also shown. Females had a higher HDL and ApoA1 in all three groups. In general, middle-aged males and fathers had higher mean values than middle-aged females and mothers for risk enhancing factors like triglycerides, total cholesterol, LDL and ApoB. Furthermore, significance of generation differences in mean values between parents and offspring are listed. Means were significantly higher in parents than in their offspring for most of the variables except Lp(a), which showed the same mean across generations, and HDL, whose mean value was lower in fathers than in their sons. Effects of zygosity on the means were absent. Boomsma et al. (1993, 1996) also reported on generation differences in standard deviations.

Table 5.3: Number of individuals (n), means, standard deviations (sd) and significance of sex and generation differences[a] of age, lipids, lipoproteins and apolipoproteins for males and females within the three subject groups

	Young Twins			Parents			Pa-Off	Middle-aged Twins		
	males	females	sex diff.	males	females	sex diff.	gen. diff.	males	females	sex diff.
n	161	159		160	160			188	218	
Age (years)	16.77 (1.78)	16.71 (2.20)	ns	48.10 (6.29)	45.60 (5.89)	<.001		43.48 (6.53)	44.70 (6.79)	ns
TC (mmol/l)	4.11 (0.63)	4.41 (0.77)	<.01	5.83 (1.00)	5.63 (1.09)	<.025	<.001	5.38 (1.03)	5.46 (1.04)	ns
TRG (mmol/l)	0.67 (0.30)	0.71 (0.28)	ns	1.39 (0.71)	0.95 (0.45)	<.001	<.001	1.32 (0.69)	1.04 (0.45)	<.001
HDL (mmol/l)	1.24 (0.21)	1.37 (0.27)	<.001	1.14 (0.27)	1.41 (0.30)	<.001	<.001	1.07 (0.28)	1.39 (0.33)	<.001
LDL (mmol/l)	2.56 (0.62)	2.72 (0.70)	ns	4.07 (0.94)	3.79 (1.02)	<.001	<.001	3.72 (0.97)	3.60 (0.97)	ns
ApoA1 (g/l)	1.34 (0.14)	1.43 (0.21)	<.001	1.40 (0.22)	1.56 (0.20)	<.001	<.001	1.50 (0.35)	1.93 (0.38)	<.001
ApoB (g/l)	0.78 (0.14)	0.80 (0.18)	ns	1.12 (0.21)	1.02 (0.21)	<.001	<.001	1.27 (0.34)	1.19 (0.34)	<.001
Lp(a) (g/l)	0.14 (0.16)	0.12 (0.13)	ns	0.13 (0.16)	0.13 (0.16)	ns	ns	0.17 (0.24)	0.20 (0.25)	ns

[a] Sex and generation differences are tested by hierarchic χ^2 tests (degrees of freedom=1).
Abbreviations: Pa-Off = parent-offspring, ns = non significant. For further abbreviations see Table 5.1 and 5.2.

Except for Lp(a), which had the same standard deviation, parental standard deviations were significantly higher for all lipid parameters.

Table 5.4: Familial correlations for measured lipids, lipoproteins and apolipoproteins

	N[a]	TC	TRG[b]	HDL	LDL	ApoA1	ApoB	Lp(a)[b]
Young twins								
MZM	35	.86	.57	.69	.85	.84	.83	.98
DZM	31	.28	.46	.47	.35	.40	.56	.48
MZF	35	.74	.77	.74	.79	.73	.79	.93
DZF	30	.57	.21	.38	.46	.52	.50	.43
DOS	29	.30	.01	.45	.40	.30	.67	.63
Spouses								
Fa-Mo	160	.21	.07	.07	.21	.12	.31	.10
Parent-child								
Fa-So	161	.27	.15	.15	.33	.24	.24	.56
Fa-Da	159	.22	.09	.23	.23	.36	.30	.52
Mo-So	161	.37	.14	.37	.35	.35	.35	.50
Mo-Da	159	.23	.20	.24	.27	.30	.34	.37
Pa-Ch	640	.28	.13	.23	.30	.28	.31	.46
Middle-aged twins								
MZM	39	.75	.62	.62	.78	.60	.79	.91
DZM	36	.55	.40	.23	.64	.06	.68	.21
MZF	50	.79	.59	.65	.77	.64	.78	.90
DZF	40	.41	.40	.24	.30	.26	.30	.58
DOS	38	.46	.44	.21	.41	.37	.35	.54

Abbreviations: N = Number of pairs, Fa-Mo = Spouse correlation, Fa-Son = Father-Son correlation, Fa-Da = Father-Daughter correlation, Mo-So = Mother-Son correlation, Mo-Da = Mother-Daughter correlation, Pa-Ch = Parent-Child correlation estimated over all pairs. For further abbreviations see Table 5.1 and 5.2.

[a] For Lp(a), number of pairs were different (see the text).

[b] For triglyceride and lipoprotein(a), logarithmically transformed values were used.

Familial correlation for twins, spouses and parents and offspring are presented in Table 5.4. MZ correlations were consistently larger than DZ correlations for all variables in both young and middle-aged twins, which points to the influence of genes. For triglycerides in young twins, correlations in the DOS group are much smaller than in the same-sex DZ groups, which suggests sex-specific genetic effects. This suggestion was not supported, however, by the results from parent-offspring correlations. Parent-offspring correlations were estimated separately for fathers and mothers with their sons and daughters, but χ^2 tests showed that these correlations did not depend on either the sex of the parent or the offspring. Constraining the different parent-offspring correlations to be equal rendered one total parent-child correlation, which was significantly different from zero for all

variables. Spouse correlations were not significantly different from zero except for total cholesterol, LDL and apoB.

Table 5.5a: Standardized parameter estimates of best fitting univariate models for measured lipids, lipoproteins and apolipoproteins in young twins

	Young twins								
	h^2	c^2	e^2	a^2	*k*	χ^2	df	p	AIC
TC									
males	.87		.13						
females	.68		.27	.05		18.20	24	0.79	-29.80
TRG[a]									
males	.63		.34	.03					
females	.63		.34	.03		35.43	26	0.10	-16.57
HDL									
males	.70		.30						
females	.70		.26	.04		32.66	24	0.11	-15.34
LDL									
males	.82		.18						
females	.82		.18			24.66	27	0.59	-29.34
ApoA1									
males	.84		.16						
females	.61		.31	.08		35.87	24	0.06	-12.13
ApoB									
males	.47	.34	.19						
females	.47	.34	.19		.82	29.21	25	0.26	-20.79
Lp(a)									
males	.98		.02						
females	.92		.08			26.92	25	0.36	-23.08

Abbreviations: h^2 = heritability, e^2 = proportion of variance explained by unique environment, a^2 = proportion of variance explained by age, c^2 = proportion of variance explained by shared environment, *k* = scalar factor, χ^2 = Chi-square value, df = degrees of freedom, p = probability, AIC = Akaike's information criterion. For further abbreviations see Table 5.1 and 5.2.

[a] For TRG, R*g* in the DOS group (see Figure 5.1) could be set to zero.

Univariate model fitting results within the adolescent and middle-aged twins groups are presented in Tables 5.5a and 5.5b respectively. For all variables best fitting models and standardized parameter estimates are shown. In young twins, best fitting models included additive genetic influence for all variables, with heritabilities from .47 to .98. Only for ApoB a significant influence of shared environment was found. For triglycerides, LDL and ApoB, parameter estimates were equal in males and females. For total cholesterol, HDL and ApoA1 sex differences in parameter estimates were found. These sex differences can be attributed to the larger influence of age in females. When age was not considered, a scalar model in which

heritabilities are constrained to be equal for males and females gave the best fit to the data (Boomsma et al., 1996).

Table 5.5b: Standardized parameter estimates of best fitting univariate models for measured lipids, lipoproteins and apolipoproteins in middle aged twins

	Middle-aged twins								
	h^2	c^2	e^2	a^2	*k*	χ^2	df	p	AIC
TC									
males	.68		.21	.11					
females	.68		.21	.11		29.75	26	0.28	-22.25
TRG									
males	.59		.39	.02					
females	.59		.39	.02	1.26	21.93	25	0.64	-28.07
HDL[a]									
males	.71		.29						
females	.71		.29		.89	33.00	25	0.13	-17.00
LDL									
males	.69		.21	.10					
females	.69		.21	.10		31.34	26	0.22	-20.66
ApoA1									
males	.58		.41	.01					
females	.58		.41	.01	.83	28.63	25	0.28	-21.37
ApoB									
males	.73		.20	.07					
females	.73		.20	.07		30.77	26	0.24	-21.23
Lp(a)									
males	.87		.09	.04					
females	.87		.09	.04		27.68	26	0.37	-24.32

For abbreviations see Table 5.5a.

[a] For HDL in male and female twins, h^2 reflects broad sense heritability, as the best fitting model contained dominance variance: $d^2 = 0.63$.

In the group of middle-aged twins (see Table 5.5b), models without sex differences gave the best account of the data for all variables. A straightforward model including only additive genes (G), unique environment (E) and age fitted best for total cholesterol, LDL, ApoB and Lp(a). For Triglycerides and ApoA1 a scalar model including G, E and age gave the best fit. Also for HDL a scalar model turned out to be best. For HDL, dominant genetic influences were significant, besides the influence of additive genes and unique environment. In HDL the influence of age was absent. Overall, genetic influence was relatively high with (broad sense) heritabilities ranging from .58 to .73.

Thus, significant heritabilities were found for all measured elements of the lipid system in both young and middle-aged twins. Furthermore, parent-offspring correlations for all variables were significantly different from zero. As stated earlier, such results enable the estimation of the genetic transmission from parent to offspring by fitting the extended parent-twin model, thereby rendering insight into the genetic correlation across time.

Results of fitting the extended model are shown in Table 5.6a for lipids and lipoproteins and in Table 5.6b for apolipoproteins and Lp(a). Non-standardized parameter estimates of best fitting models in which the genetic transmission coefficient (*g*) is fixed at 0.5 ánd in which *g* is estimated are listed for all variables. Jöreskog's χ^2/df was less than 2 for all fitted models, which indicates that all models showed an adequate fit to the data (Finkel et al., 1995). For total cholesterol, triglycerides, HDL and LDL, there was evidence for g to be smaller than 0.5. For total cholesterol, the improvement in fit for a model in which g was estimated instead of fixed to 0.5, just failed to reach significance. This means that a model with *g* fixed to 0.5 cannot be rejected, although AIC and Jöreskog's χ^2/df were smaller for the model in which *g* was estimated. For triglycerides, HDL and LDL models estimating *g* fitted significantly better. These results indicate that in measured lipids and lipoproteins partly different genes are expressed in childhood than in adulthood. Computation of the genetic correlation across time [R*g*=2**g*, see equation 1], showed that it ranged from 0.46 in triglycerides to 0.80 in total cholesterol, with intermediate values for HDL and LDL.

For Apolipoproteins and Lp(a) a different picture emerges. Models for ApoA1, ApoB and Lp(a), in which *g* is fixed to 0.5 show the same [ApoA1 and Lp(a)] or almost the same fit (ApoB) as the model in which *g* is estimated. In addition to that, values of AIC and Jöreskog's χ^2/df were lower. A model in which *g* is fixed to 0.5, thus gives the most parsimonious account of the data in these variables, i.e. the same genes influence apolipoproteins and Lp(a) in childhood and in adulthood. Estimation of *g* also yielded a value of 0.5 for ApoA1 and Lp(a). ApoB is the only variable for which environmental transmission (*z*) turned out to be significant. This means that for ApoB, a combination of both genetic and environmental transmission explains the parent-offspring correlation. The small value of g (0.12) when it was estimated, went together with a relatively large value of *z* (0.41), whereas z was only 0.18 in the model in which g was fixed at 0.5.

Table 5.6a: Non-standardized variance components of best fitting extended models for lipids and lipoproteins

	Parents			Offspring							
	V_G	V_E	V_A	V_G	V_E	V_A	*g*	χ^2	df	AIC	χ^2/df
TC											
g=.5											
males	.734	.262		.363	.054						
females	.734	.262	.165	.363	.168	.042	.50	237.83	159	-80.17	1.50
g free											
males	.758	.231		.354	.053						
females	.758	.231	.159	.354	.157	.039	.40	234.02	158	-81.98	1.48
TRG											
g=.5											
males	.029	.016		.015	.009	.001					
females	.013	.016	.001	.015	.009	.001	.50	223.78	159	-94.22	1.41
g free											
males	.031	.014		.015	.008	.001					
females	.015	.014	.001	.015	.008	.001	.23	211.03	158	-106.07	1.34
HDL											
g=.5											
males	.051	.029		.027	.016	.001					
females	.051	.029		.058	.016	.001	.50	258.84	159	-59.17	1.63
g free											
males	.055	.024		.027	.016	.001					
females	.055	.024		.054	.016	.002	.33	251.82	158	-64.18	1.59
LDL											
g=.5											
males	.649	.233		.359	.082						
females	.649	.233	.110	.359	.082		.50	245.39	161	-76.61	1.52
g free											
males	.677	.197		.344	.079						
females	.677	.197	.108	.344	.079		.38	239.69	160	-80.31	1.50

Abbreviations: V_G = additive genetic variance, V_E = unique environmental variance, V_A = variance due to age, *g* = genetic transmission coefficient, χ^2/df = Jöreskog's χ^2/df.
The best fitting model in which *g* is fixed at 0.5 and the one in which *g* is estimated are shown.
For further abbreviations see Table 5.1 and Table 5.5a.

Table 5.6b: Non-standardized variance components of best fitting extended models for apolipoproteins and lipoprotein(a)

	Parents			Offspring							
	V_G	V_E	V_A	V_G	V_E	V_A	*g*	χ^2	df	AIC	χ^2/df
ApoA1											
g=.5											
males	.027	.033		.021	.003						
females	.043	.033	.003	.021	.016	.004	.50	287.92	159	-30.08	1.81
g free											
males	.027	.033		.021	.003						
females	.043	.033	.003	.021	.016	.004	.50	287.92	158	-28.08	1.82
ApoB[a]											
g=.5											
males	.048	.026		.013	.011						
females	.048	.026	.007	.013	.020	.002	.50	298.89	157	-15.11	1.90
g free											
males	.048	.026		.012	.011						
females	.048	.026	.007	.012	.021	.002	.12	298.41	156	-13.59	1.91
Lp(a)											
g=.5											
males	.272	.031	.004	.296	.006						
females	.272	.031	.004	.253	.023		.50	235.38	160	-84.62	1.47
g free											
males	.272	.031	.004	.296	.006						
females	.272	.031	.004	.253	.023		.50	235.38	159	-82.62	1.48

Abbreviations: *g* = genetic transmission coefficient, χ^2/df = Jöreskog's χ^2/df.
The best fitting model in which *g* is fixed at 0.5 and the one in which g is estimated are shown.
For further abbreviations see Table 5.6a.
[a] For ApoB, z=0.18 in the model in which *g* is fixed at 0.5, and z=0.41 in the model in which g is estimated.

As mentioned earlier, variances were higher in the parental generation for all variables except Lp(a). As can be seen from Tables 5.6a and 5.6b, the higher variance in the parents could in most cases be attributed to a rise in both additive genetic and unique environmental variance from childhood to adulthood. For total cholesterol, LDL and ApoB in females, a rise in variance due to age also played a role. For most variables standardized estimates of heritability did not differ very much across generations (Table 5.7), because increases in additive genetic variance and increases in the sum of environmental and age-variance were roughly proportional. Only for ApoA1 in males, heritabilities were substantially lower in adults due to a greater increase in unique environmental variance. For ApoB, heritability was larger in adults, due to the disappearance of the influence of shared environment.

Table 5.7: Standardized parameter estimates of best fitting extended models for lipids, lipoproteins, apolipoproteins and Lp(a)

	Lipids and lipoproteins					Apolipoproteins and Lp(a)				
	Parents		Offspring			Parents		Offspring		
	h^2	a^2	h^2	a^2		h^2	a^2	h^2	a^2	
TC					ApoA1					
males	.77		.87		males	.46		.87		
females	.66	.14	.64	.07	females	.55	.03	.51	.09	
TRG					ApoB					
males	.69		.61	.04	males	.65		.52		
females	.51	.03	.61	.04	females	.59	.08	.37	.05	
HDL					Lp(a)					
males	.69		.61	.03	males	.89	.01	.98	.02	
females	.69		.76	.02	females	.89	.01	.92	.08	
LDL										
males	.77		.81							
females	.69	.11	.81							

Proportions of variance explained by additive genes (h^2) and age (a^2) are shown. The remaining proportion (not shown) is explained by unique environment (e^2). For lipids and lipoproteins standardized estimates of models in which g is estimated are shown. For apolipoproteins and Lp(a) standardized estimates of models in which *g* is fixed at 0.5 are shown. For abbreviations see Table 5.1.

DISCUSSION

The main aim of this study was to investigate and disentangle the genetic and non-genetic causes of stability and change in lipid, lipoprotein and apolipoprotein levels that occur during the lifespan. To achieve this goal, data from two different research projects were combined. In the first project parents with their twin offspring were measured. In the second project, data from middle-aged twins were collected. Combining the data of both projects enabled the specification of an extended quantitative genetic model in which the genetic transmission from parent to offspring could be estimated. Evidence was found for the genetic transmission coefficient (*g*) to be smaller than 0.5 for total cholesterol, triglycerides, HDL and LDL, which indicates that partly different genes influence these variables in childhood compared to adulthood. Doubling the genetic transmission coefficient gives the genetic correlation across time, which is a measure of the part of the genetic variance that is common to both age groups. These values ranged from 46% in triglycerides to 80% in total cholesterol, with intermediate values for HDL and LDL. The rest of the

genetic variance can thus be attributed to age-specific genetic effects. In contrast to the results for lipids and lipoproteins, for apolipoproteins and Lp(a) evidence was found that the *same* genes act in childhood and in adulthood.

Our study compared middle-aged parents (and twins of similar age) with their adolescent offspring. As described above, both adolescence and the menopausal period in females, are associated with substantial changes in lipid concentrations. In these periods large changes take place in production of sex hormones, which may cause lipid changes through their effect on LDL receptors and important lipid enzymes like hepatic lipase, lipoprotein lipase and lecithin:cholesterol acyltransferase (LCAT) (Lobo, 1990; Seed, 1991; Krauss, 1991). The effect of female sex hormones on lipid metabolism can be illustrated by two findings from our own data: Postmenopausal women in the middle-aged twin group showed significantly higher levels of total cholesterol, LDL and ApoB. Women using contraceptives had higher levels of HDL, ApoA1 and triglycerides. Differences remained significant after correction for age. These effects are in accordance with the literature (Lobo, 1990; Seed, 1991). It is not unthinkable that timing as well as magnitude of changes in sex-hormones are controled by specific genes. It might therefore not be too surprising that a substantial part of the variance in lipids and lipoproteins was specific to either adolescence or middle age. Some evidence for different genetic mechanisms affecting HDL level in pre- and postmenopausal women was found by Harris, Falk, Goldstein and Park (1993). A higher MZ twin correlation was found in the postmenopausal group, which suggests that nonadditive genetic influences might become more important after the menopause. Our group of female middle-aged twins was too small to test this hypothesis.

In contrast to our study, which compared children around their puberty with parents in their middle age, Williams and Wijesiri (1993) studied stability of genetic influences during middle-age. They analyzed longitudinal data of male veteran twins on total cholesterol, LDL, HDL and triglycerides. Between 48 and 63 years of age, subjects were measured three times. As evidenced by the large correlations between genetic effects at successive examinations, similar genetic effects on lipids appear to be present throughout this segment of the life span.

In accordance with the literature we found larger variances in the parental generation (Pooling Project Research Group, 1978; Reilly, Kottke & Sing, 1990; Kronmal et al., 1993; Verschuren et al., 1993; Schaefer et al., 1994). The observed generation effect on the variance of lipid parameters, may reflect the weakening of homeostatic control mechanisms of the lipid system with aging (Reilly, Kottke & Sing, 1990). Larger variances in the parental generation could be ascribed to increases in both unique environmental and additive genetic variance from childhood to adulthood. An explanation for the larger unique environmental variance in adults may be that they are exposed to a larger range of environmental variations like variation in diet, exercise, stress, and alcohol or cigarette consumption, each of which may affect lipid metabolism. An increase in additive genetic influence on the other hand, could have been caused by a less tight gene regulation of homeostatic control with increasing age (Reilly, Kottke & Sing, 1990). Increases were roughly

proportional for both sources of variance, which led to heritabilities of similar magnitude in the parental generation. Furthermore, observed heritabilities for most lipid variables did not differ much between males and females. However, considering age within the model fitting, uncovered some sex differences in parameter estimates not found by Boomsma et al. (1996), because in females a larger part of the variance was due to age in most variables. Also, part of the resemblance in young twins could now be attributed to age, which led to slightly lower heritability estimates in the variables concerned. Both the magnitude of observed heritabilities, and the lack of a clear age trend in heritabilities are in accordance with the literature as summarized in Tables 1 and 2. Only the study of Heller et al. (1993) seems to be an exception with respect to the effect of age. Heller et al. (1993) compared an older (66-86 years) with a younger (52-65 years) Swedish twin cohort and observed a marked decreased with age in heritabilities of total cholesterol, triglycerides and apoB, but not for HDL and apoA1. Heritability estimates, thus may only begin to decline from about 65 years on. It remains unknown to what extent survivorship effects or the concurrent fall in total cholesterol of elderly people might affect the drop in heritabilities. The lack of an age dependency in other periods of life, implicates that relative influence of additive genes on total cholesterol, LDL, HDL and triglycerides remains more or less the same with age, although partly different genes are responsible for the effects at different ages.

Boomsma et al. (1996) found a large decrease in heritabilities of parents compared to their offspring for all measured lipid variables. Thus, conclusions based on data from parents and their twin offspring only, were quite different from the results of our study that included extra information from middle-aged twins. If only data from parents and offspring are available, one has to assume that the same genes are expressed in parents and their offspring. This assumption proved to be false for total cholesterol, LDL, HDL and triglycerides, which probably partly explains observed differences in heritability estimates of the parental generation.

Using the same parent-offspring model, no intergenerational differences in heritability were observed in the same sample for plasma Lp(a) concentrations (Boomsma et al., 1993). Our study showed that the same genes influence Lp(a) in childhood and in adulthood, which means that assuming a perfect genetic correlation across time, was valid in this case. This finding did not come as surprise, as it is in accordance with evidence that variation in Lp(a) is probably determined by a single gene. Boerwinkle and colleagues (1992) found that more than 90% of the variation in Lp(a) concentration could be ascribed to the apo(a) gene, which is located on the tip of the long arm of chromosome 6 (Utermann, 1989; Schulte, Rothman & Austin, 1994). Wang, Wilcken and Dudman (1992) concluded that the apo(a) gene is fully expressed before the age of 1 year. In their study of 5-13 months old babies and their parents, they observed parent-offspring correlations that already were of the same magnitude as the parent-offspring and DZ twin correlations in this study.

For apolipoproteins, there also was no evidence of age-specific genetic influences. ApoB was the only variable for which (a small) environmental transmission proved to be significant. In combination with the significant spouse

correlation, the shared environment in young twins and the lack of its influence in middle-aged twins, these data point to one or more environmental factors that have their influence on family members, but only for as long as they live together.

The difference in age dependency of genes influencing total cholesterol, LDL, HDL and triglycerides, compared to those that influence ApoA1 and ApoB is an interesting finding, which offers ground for further speculation. Apolipoproteins serve some basic physiological functions in metabolism of other lipoprotein particles, for example by acting as a ligand for cell-surface receptors. Their more basic function is supported by findings which indicate that apolipoproteins better discriminate atherosclerotic patients from controls than plasma lipids and lipoproteins (Avogaro, Cazzolato, Bittolo Bon & Quinci, 1979; Durrington, Ishola, Hunt, Arrol & Bhatnagar, 1988). Complex segregation studies found evidence for major gene effects on ApoA1 and ApoB (Hopkins & Williams, 1989; Schulte, Rothman & Austin, 1993), probably pointing to the influence of the apolipoprotein genes themselves, which have been mapped to chromosome 11 (ApoA1) and chromosome 2 (ApoB) (Weiss, 1993). The difference in age-specific genetic influence in lipids and lipoproteins compared to the apolipoproteins might point to a simpler genetic architecture for the apolipoproteins. In contrast to the genetic architecture of cholesterol, triglycerides, LDL and HDL in which, besides some major gene effects, most genetic variation is polygenic (Sing & Moll, 1989,1990; Hopkins & Williams, 1989; Weiss, 1993), genetic variation in apolipoproteins may be determined by one [just like in Lp(a)] or a few major genes. Moreover, expression of these genes is not dependent on age, as is apparent from this study.

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6

Summary and discussion

The occurence of premature coronary heart disease (CHD) runs in families. This familial risk of premature CHD could in principal be due to (a combination of) two effects: genetic influences or environmental influences that are shared by family members. Results from twin studies suggest, that shared genes are more important than shared family environment in determining CHD mortality. Further evidence indicates that the genetic risk for CHD is only partly mediated by the well known traditional risk factors like hypertension, smoking and cholesterol. Other risk factors that represent intermediate steps in the causal path from genes to the CHD endpoint have thus to be considered. Candidates discussed in this thesis are stress reactivity, insulin and vagal control of the heart.

Two other important factors that may influence CHD risk are sex and age. The marked difference in CHD incidence between males and females and changes of the CHD risk profile with age, implicates that the expression of genetic and environmental influences on CHD risk factors may be dependent on sex and age.

The main focus of this thesis therefore involved two issues. The relative contributions of genetic and environmental factors on intermediate and traditional risk factors and the age- and sex-dependency of these relative contributions. To resolve these issues, risk factors for CHD were measured in a sample of middle-aged twins that included opposite sex pairs in addition to male and female same-sex twin pairs. The stability of genetic influences across age was tested by combining these data with data from an earlier project in which parents with their twin offspring were measured (Boomsma, 1992).

In this general discussion, I will first concentrate on intermediate risk factors; after discussing results on Respiratory Sinus Arrhythmia (RSA) and fasting insulin, I will question the status of stress reactivity as an intermediate risk factor. Next, I will focus on the age-dependency of gene expression for lipids and blood pressure, which are more traditional risk factors, before I discuss sex differences. The overview of results is concluded with the presentation of a model that describes the causal path from genes and environment to coronary heart disease. Finally, I shift the attention to the practical implications of the results for the treatment and prevention of CHD, and the theoretical implications for future research.

Genetic epidemiology of risk factors for coronary heart disease

Respiratory Sinus Arrhythmia

Respiratory Sinus Arrhythmia is a sensitive, noninvasive index of the vagal control of the heart. A strong RSA, which is characterized by a large variability in heart rate, is regarded an index of good cardiovascular health. A reduced heart rate variability, on the other hand, is associated with cardiac disease and hypertension. RSA can therefore be considered a tentative intermediate risk factor for cardiovascular disease. Furthermore, RSA decreases with increasing age and under conditions of psychological stress. In the second chapter, estimates of genetic and environmental influences on RSA were compared between rest, mental and physical stress conditions, while simultaneously taking account of the influence of age and respiration rate on RSA. In all experimental conditions, individual differences in RSA were best explained by additive genetic and unique environmental factors, independent from age and respiration rate. These factors influenced RSA to the same extent in both sexes. Total genetic influence on RSA varied between 28% and 43% across the experimental conditions. This variation in heritability estimates was not systematically related to the magnitude of the stress response to the tasks. Correction for influence of respiration rate yielded RSA-heritabilities of similar size. The covariance between RR and RSA, which was seen in all conditions, could be attributed to a combination of correlated unique environmental and correlated additive genetic factors.

Fasting insulin

The third chapter addressed the question to what extent genetic and environmental factors influence another tentative intermediate risk factor: fasting insulin. In population based studies fasting insulin is considered the best marker of insulin resistance, which in turn is a precursor of both non-insulin-dependent diabetes mellitus (NIDDM) and, via the insulin resistance syndrome (syndrome X), of cardiovascular disease. In addition to the first question, it was tested whether the homeostatic regulation of the blood glucose concentration by insulin could best be explained by a unidirectional causation from insulin to glucose (or vice versa), or a reciprocal causation of insulin and glucose (i.e. a negative feedback loop). Just as for RSA, a model specifying additive genetic and unique environmental factors, showed the best fit to the data. Heritability for fasting insulin was small (.21) and the same for males and females. The interaction between insulin and glucose was best described by a reciprocal causation.

Stress reactivity

The concept of stress reactivity has a long history in cardiovascular psychophysiology. An exaggerated cardiovascular response to a physical or psychological challenge has been promoted as a potential risk factor for

cardiovascular disease (Menkes et al., 1989; Matthews, Woodall & Allen, 1993). An idea which is known as "the reactivity hypothesis".

Results concerning the heritability of reactivity to laboratory stress tasks were inconsistent in our study. In chapter 2 we described that the pattern of twin correlations for RSA reactivity was incompatible to a biologically plausible model. In chapter 4, a review of twin studies and the analysis of our own data indicated that blood pressure reactivity might be moderately heritable, but different results were observed for different tasks. The reason for the inconsistent findings could lie in the less reliable determination of reactivity measures compared to the reliability of levels. Reactivity is calculated as the difference between two levels, which increases the error term. Another possibility is that someone's reactivity simply is a less reliable person characteristic. Such limited reliabilities could lead to inconsistent patterns of twin correlations and thus to variability in estimates of genetic and environmental influences.

In addition to the inconsistencies of our results concerning stress reactivity, its questionable status as an intermediate risk factor has also attributed to the modest attention it has been given in this thesis. The prevailing assumption within stress research has always been that an exaggerated physiological response to stress is deleterious to health. In recent years, this assumption has been challenged because prospective evidence relating a heightened stress reactivity to hypertension or CHD, remains scarce (Pickering & Gerin, 1994; Carroll, Davey Smith, Sheffield, Shipley & Marmot, 1995). Some authors even regard an exaggerated stress reactivity as a normal sign of adaptive coping (e.g. Dienstbier, 1989, 1991). The latter view is in line with results from our own laboratory: de Geus, van Doornen and Orlebeke (1993) observed an even higher reactivity of both systolic and diastolic blood pressure in subjects with a high level of physical fitness.

The main reason for the lack of evidence linking an exaggerated stress reactivity to pathophysiological consequences in later life, may be the limited predictive value of reactivity to laboratory stress for reactivity to stress in *real* life. In general, correlations between laboratory and real-life reactivity are moderate or even absent (van Doornen & Turner, 1992). This is most probably related to the difference in psychological and physiological nature of the two stress situations (van Doornen, Knol, Willemsen & de Geus, 1994). Above considerations point to the importance of ambulatory measurements of the cardiovascular system during daily life. Therefore, application of ambulatory measurements is also strongly advocated for the quantitative genetic investigation of stress reactivity (Turner & Hewitt, 1992; Hewitt & Turner, 1995b).

Further reserves against the idea that an exaggerated stress reactivity can be viewed as an intermediate risk factor for CHD grew from analyses of subsets of our own data in which the relation between stress reactivity and more established risk factors was explored. In line with the shift in attention from lab to real life, Holdstock (1995), for example, measured ambulatory parameters in 6 MZM and 6 DZM twin pairs from our sample during a 24 hour period. These measurements were related to an index of the "insulin resistance syndrome" (IRS), calculated from the available

data. In the laboratory, sympathetic activation as measured by the pre-ejection period (PEP) did not differ significantly between subjects with a high or low IRS index, whereas results from the ambulatory measurements showed that subjects with a higher IRS score also showed a higher sympathetic activation during the day. These results would be in line with evidence pointing to a role for sympathetic activity in the IRS (Deibert & DeFronzo, 1980; Julius & Jamerson, 1994; Reaven, Lithell & Landsberg, 1996). In a larger subset of our middle-aged twin data, Luykx (1994) investigated the relation between the abovementioned IRS index and stress reactivity to laboratory tasks. In contrast to the ambulatory findings in men, a significant negative correlation of the IRS score with heart rate reactivity and diastolic blood pressure reactivity was observed, only however in women. This is a paradoxical finding in the framework of the reactivity hypothesis, which would predict higher reactivity to be associated with this risk cluster.

Several studies have reported a relation between serum lipid levels and cardiovascular reactivity to laboratory stressors (see for references, van Doornen, Snieder & Boomsma, 1996), which points to the possibility that part of the risk enhancing effect of stress reactivity might be mediated by lipids. However, previous studies used small sample sizes and did not control for age and medication use. These shortcomings were resolved by van Doornen, Snieder and Boomsma (1996) who studied the association between stress reactivity and plasma lipids in the middle-aged twins from our sample and in parents and their twin offspring from an earlier project (Boomsma, 1992). It has been hypothesized that the common mechanism of sympathoadrenal activity may explain the possible association between stress reactivity and lipid levels. However, in middle-aged subjects, correlations between lipids and stress reactivity not only were small, they also differed between the sexes, and were inconsistent across tasks. In youngsters correlations were virtually absent. It was therefore concluded that previously reported associations may have been chance findings or due to publication bias, and that if any relationship exists, it is very small and of doubtful relevance for the study of the effects of stress reactivity on the risk for CHD.

To summarize, it can be stated that stress reactivity to laboratory tasks has a doubtful status as an intermediary risk factor. Therefore, ambulatory measurement of the cardiovascular system is prefered as a means to gain insight into adverse effects on health of stress in real life. In spite of the justified doubt concerning the risk factor status of stress reactivity to laboratory stressors, these tasks may remain a useful tool for providing insight into the dynamics of the cardiovascular system and its relation to other systems that play a role in cardiovascular functioning.

Age-dependency of gene expression for lipids and blood pressure

In chapters 4 and 5 it was investigated whether the expression of genes is different in childhood and adulthood for phenotypes related to blood pressure and lipid metabolism. In both chapters the literature on twin studies was reviewed and heritability estimates from these studies ordered according to the age of the various twin samples. From these data it can be concluded that heritabilities for systolic and

diastolic blood pressure, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides do not show a clear age trend: estimates were consistently high and stayed relatively stable with age. For apolipoprotein A1 (ApoA1) and B (ApoB) and blood pressure reactivity to stress tasks, heritability estimates were more variable. Also for these traits, no clear age trend could be detected. For our own analyses, we combined the data from our middle-aged twins with data from an earlier project in which parents with their twin offspring were measured (Boomsma, 1992). This unique combination of data enabled the specification of an extended parent-twin model, with which we could estimate genetic stability despite the absence of longitudinal data. For blood pressure, lipids and lipoproteins, results indicated that partly different genes are expressed in childhood and adulthood. For ApoA1, ApoB and Lipoprotein(a), the same genes are expressed during these different periods of life. The blood pressure reactivity data did not allow the application of this extended model. These results indicate that blood pressure, lipids and lipoproteins are probably influenced by a different combination of multiple genes in different periods of life.

Sex differences

A remarkable finding in our study was the almost complete lack of sex differences in genetic and environmental influences on the various investigated risk factors. Through the inclusion of opposite sex pairs in addition to male and female same-sex twin pairs we were not only able to determine whether genetic and environmental influences differed in magnitude between the sexes, but we also tested whether different genes were expressed in males and females. Since we did not uncover major sex differences in the influence of genes and environment on risk factors for CHD, our results cannot provide a direct explanation for the large difference in CHD incidence and mortality between the sexes. Theoretically, the excess CHD incidence in men could result from less favourable levels of risk factors, or a more severe response of men to these levels, or both (Goldbourt, 1994). Our results are consistent, with the idea that the protection against CHD in females is due to one or more intermediary factors on a phenotypic level, which moderate the risk factors themselves and the deleterious effects of these risk factors on the atherosclerotic process. Oestrogen maybe a likely candidate as this female sex hormone not only has a positive effect on the lipid spectrum, but also acts favourably on the arterial wall (Lobo, 1990; Seed, 1991). On the other hand, after reviewing the evidence Goldbourt (1994) comes to the conclusion that hormonal differences as a single entity are insufficient to explain the observed differences in disease susceptibility. The exact nature of the origin of sex differences in CHD incidence and mortality therefore remains unclear.

The complex etiology of coronary heart disease

In general, moderate to high heritabilities were found for most risk factors that were studied in young and older twins. The low heritability for fasting insulin was the only exception. Genetic variance was mostly of an additive nature, dominant

genetic variance appeared to be unimportant. As far as environmental factors are concerned, influences on all traits were specific to the individual; none of the traits was influenced by environmental factors that were shared by family members. These results indicate that by far the largest part of differences between individuals for quantitative risk factor traits can be explained by a combination of unique environmental exposures and multiple genes that act in an additive fashion. These results are in agreement with expectations concerning the genetic architecture of most continuously distributed traits that determine the susceptibility to CHD (Sing & Moll, 1989,1990).

This knowledge on the genetic and environmental architecture of risk factors for CHD provides an important first step in understanding the underlying causes of CHD. It has to be realized however, that most CHD results from a complex interplay of multiple etiological factors. This complex etiology of CHD was recognized in our study by the explicit focus on the effects of age and sex and the use of multivariate (RSA, respiration rate and age) and bivariate (fasting insulin and glucose) model fitting. Sing, Haviland, Templeton, Zerba and Reilly (1992) tried to capture this complexity in a model that regards risk factors for CHD as a coherent network of intermediate traits consisting of different subsystems, which mediate the influence of genes (and environment) on the eventual disease outcome. According to the authors, a useful hierarchical organization for the study of genetic influences on CHD discriminates three levels and assigns DNA to the base level, biochemical, physiological and anatomical traits to the intermediate level and the clinical diagnosis of CHD to the upper level. This model assumes that genotypic variation is translated through variation in the network of intermediate agents to the upper level of clinical manifestations. The network of intermediate traits can be decomposed into subsystems of strongly intercorrelated traits. These subsystems, for example, include lipid metabolism, haemostasis, carbohydrate metabolism and blood pressure regulation. Sing et al. (1992) further assert that correlations between traits in different subsystems are typically weaker than within subsystems. The clustering of risk factors from different subsystems within the insulin resistance syndrome, shows however, that this is not always the case.

We extended our basic model as discussed in the Introduction, with some elements from Sing's model to give a better reflection of the complex etiology of CHD (Figure 6.1). In Figure 6.1, the subdivision of risk into intermediate and traditional factors, has been substituted by a network of intermediate traits.

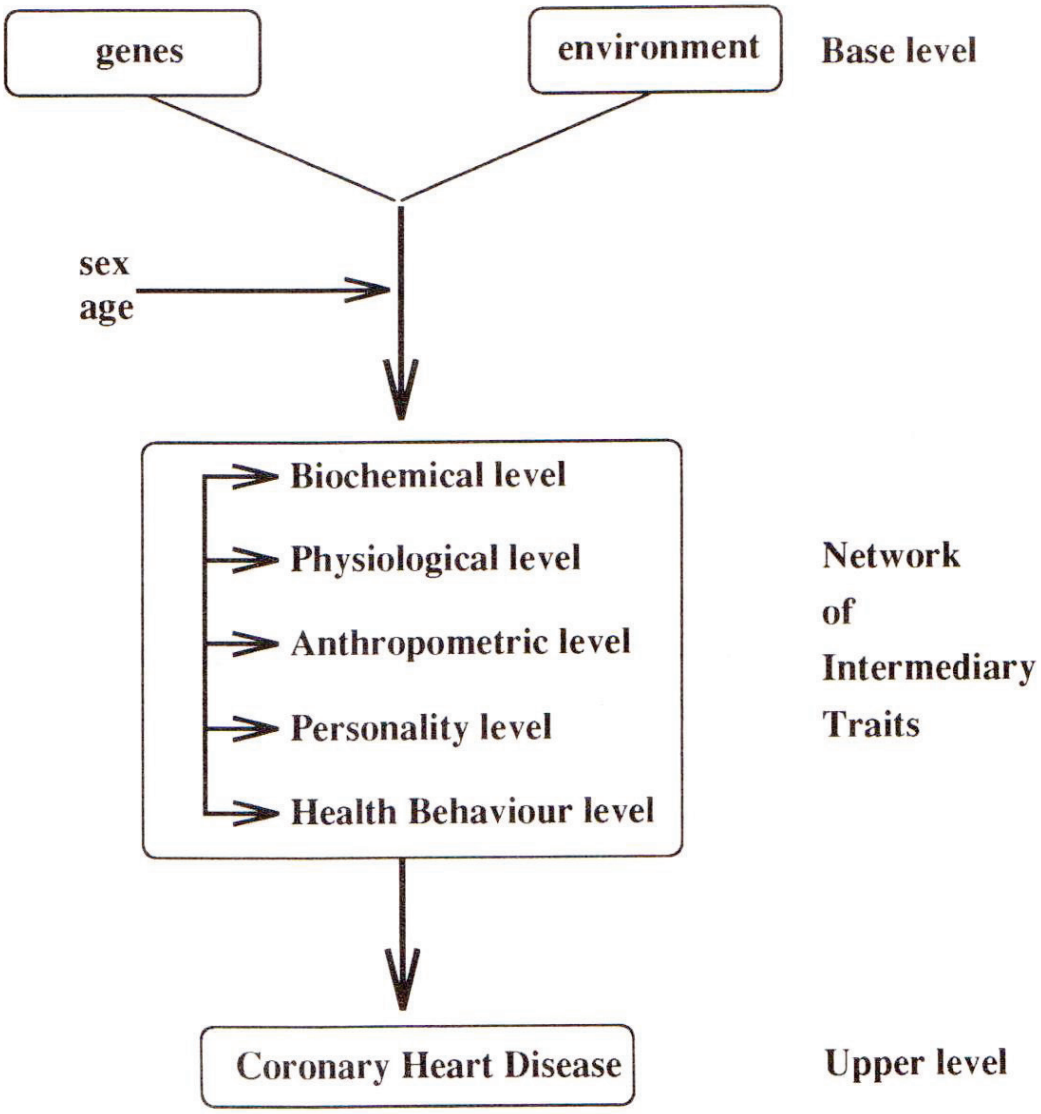

Figure 6.1: Model for linking the influence of genes and environment to endpoints of CHD via a network of intermediary traits.

Furthermore, instead of subdividing the network into subsystems like Sing et al. (1992) did, we divide the network into different levels on which risk factors for CHD can be measured to get a closer resemblance to the design of our study (see Table 1.1 of the Introduction). Intermediate traits within this network interact with each other both within and across levels in a complex way. The incorporation of age and sex in the model indicates that these factors may mediate the expression of genetic or environmental influences on CHD risk.

Practical implications for treatment and prevention of CHD

As mentioned earlier, our results suggest that individual differences in most quantitative risk factor traits can be explained by a combination of unique environmental and additive genetic factors. These results have important implications for the prevention and intervention of CHD on a public health level.

The substantial influence of unique environmental factors implies that successful intervention is mostly possible. The experience of an adverse environment in early life forms an exception to this rule. That such an influence can be important with respect to CHD is illustrated by evidence which indicates that exposure to a poor maternal environment in early life is related to cardiovascular risk factors (Barker et al., 1993) and cardiovascular mortality (Barker, Osmond, Simmonds & Wield, 1993) in adult life. Although in this case intervention is not possible, future adverse effects can be prevented by education of expectant mothers.

The lack of shared environmental influences indicates, that for example health related behaviour which may have been learned while growing up within the family, does not have a large influence on risk factors for CHD at middle-age. There is, however, one case in which shared environment could be important for CHD risk. This is the case in which shared environment induces the expression of a certain genetic susceptibility. Such an G x C interaction (for example, use of salt in a salt sensitive family) would namely show up as additive genetic influence within our twin design. Nevertheless, it seems wise to mainly aim interventions at influences that are not shared within families, but are specific to an individual.

Furthermore, contrary to popular belief, the observed moderate to high heritabilities on most risk factor traits do not imply untreatability. Evidence of genetic effects on risk factors for CHD might suggest pharmacological intervention to reduce morbidity, like for example the use of lipid-lowering medication for individuals with elevated lipid levels (Rao & Vogler, 1994). Evidence further shows that an environmental factor like diet is also able to mitigate the severity of genetically determined disorders. A striking illustration of this fact is provided by patients with heterozygous familial hypercholesterolaemia in China. These patients do not express CHD and are only recognized when they are parents of homozygous children, although their mutations in the LDL-receptor have no less deleterious effects on receptor function than in other patients. This suggests that some other factor, probably their diet, protects them from CHD (Soutar, 1995).

Throughout this thesis it has been discussed that the expression of genes and environment may be age- and sex-dependent. The latter example also illustrates that the expression of genetic and environmental influences may be dependent on the investigated population. The same is true for estimates of genetic and environmental components of variance as based on quantitative genetic techniques. Heritabilities for example, are not absolute properties of physical or behavioral characteristics, but only give an impression of the variance explained by genetic factors for a given population against its particular environmental background (Hewitt & Turner, 1995a). This environmental background can be investigated by measuring health behaviour related to CHD risk like smoking, diet and physical exercise. Although even these seemingly

environmental traits have a heritable component (De Castro, 1993; Koopmans, van Doornen & Boomsma, 1994), information on these traits is highly important as it may offer indications for the available room for intervention within this specific population. The Dutch Twin/Family Study of Health-Related Behaviour collected data on smoking and sports participation in a sample of over 1300 adolescent twin pairs and their middle-aged parents (Koopmans, van Doornen & Boomsma, 1994). The parents came from the same age stratum and the same (Dutch) population as our sample of middle-aged twins. Within the Dutch Twin/Family Study, 50% of the fathers and 53% of the mothers reported to participate in sports. These values were somewhat higher in our study (59% for males and 60% for females), probably due to the incorporation of recreational biking in our definition of sports. Nevertheless, this still means that in our sample around 40% of the subjects did hardly engage in any physical exercise at all. For smoking, data were similar in both studies. Thirtysix percent of the males in our study reported current smoking, against 38% in the study of Koopmans, van Doornen and Boomsma (1994). Values for females were 30% and 29% respectively. These figures for both physical inactivity and smoking indicate that by influencing these behavioural risk factors the potential gain in health on the population level could be enormous.

Another health behaviour eligible to intervention is diet. Within our study, daily nutrient intake was measured with a 2-day dietary record method. Subjects reported their intake of foodstuffs on one representative weekday and one representative weekendday. The same method was used in two studies of dietary habits in the Netherlands: the first (Wat eet Nederland, 1988) and second (Zo eet Nederland, 1992) Dutch National Food Consumption Survey (DNFCS) [See Appendix for a summary of results]. Comparison of the mean daily nutrient intake from our study and the two population surveys with the recommended dietary allowances as determined by the Dutch Nutrition Council (Voedingsraad, 1986,1990,1991) indicated that the most important recommended change in nutrient intake is a decrease in consumption of total fat (especially saturated fat) in favour of carbohydrate intake. Such a change in dietary habits would presumably lead to a substantial improvement of public health (Hopkins & Williams, 1989).

Theoretical implications and future research

In accordance with other recent models (e.g. Krauss, 1991; Ferrannini, 1991; Sing et al., 1992), the model as presented in Figure 6.1 recognizes the complex etiology of CHD by putting a stronger emphasis on the complex interrelations of the multiple risk factors for CHD. By using multivariate genetic modeling techniques it can be investigated whether the sources of these relations are of a genetic or environmental origin. Multivariate genetic modeling of twin or family data not only involves a decomposition of phenotypic variances into its various components, it also makes it possible to determine to what extent the covariation between multiple measures is due to correlated genetic and/or environmental factors (Heath, Neale, Hewitt, Eaves & Fulker, 1989; Neale & Cardon, 1992). Multivariate models can thus be an important aid in unraveling the sources of interrelations between multiple risk factors of CHD. Information on the genetic or environmental sources of coaggregation can also be

applied in designing intervention strategies. The most cost-effective use of resources is namely to focus on intervention which will have the largest impact on overall risk. Lifestyle or pharmacological intervention which affect multiple risk factors will have a greater effect on public health than interventions which focus only on a single risk factor (Rao & Vogler, 1994). In future studies multivariate techniques can for example be applied to the exploration of relations within the lipid system or within the insulin resistance syndrome.

Evidence of genetic effects on risk factor traits for CHD as observed in our study, justifies a further search for the genes that influence CHD risk. Besides the identification of common genetic or environmental infuences on multiple risk factors of CHD, multivariate genetic modeling can also be important for the detection of loci that influence quantitative risk factor traits. With multivariate modeling, power to detect quantitative trait loci (QTLs) can be increased substantially.

After an overview of the lipid system and the IRS and some suggestions for applications of multivariate genetic modeling in future studies of these risk clusters for CHD, I will discuss the extension of our present study to a project aimed at mapping QTLs of risk factors for CHD and the role of multivariate modeling therein.

Multivariate genetic modeling of the lipid system

The majority of quantitative genetic studies of the lipid system have used univariate analysis to decompose the variation of single lipid variables into its constituent parts. However, as the multiple factors of the lipid system most probably act in concert to cause an increased cardiovascular risk, it is also important to acquire insight into the sources of their covariation. Only a few studies have perfomed quantitative genetic analyses on multiple components of the lipid system simultaneously. Colletto, Krieger and Magalhães (1981) used a bivariate path model to analyse lipid data (cholesterol, triglycerides, very low density lipoprotein (VLDL), LDL and HDL) from 105 pairs of Brazilian twins of both sexes. This approach enabled them to estimate the genetic correlation between the genes for the different lipid components. For all possible pairings significant genetic correlations were found with the highest values for total cholesterol and LDL (.76) and triglycerides and VLDL (.83). Genetic correlations between HDL and both triglycerides and VLDL were negative (-.24 and -.26 respectively). On the basis of the high genetic correlations for all lipid variables the authors suggest that there may be one common genetic factor for all lipid components. Vogler, Rao, Laskarzewski, Glueck and Russel (1987) analyzed family data of VLDL, LDL and HDL within a multivariate path model. Genetic correlations between HDL and VLDL (-0.22) and VLDL and LDL (0.35) were found, but phenotypic covariation of all three lipoproteins could not be ascribed to a single genotype. There were also strong and consistent contributions of unique environment to the HDL-VLDL covariation in parents and offspring, the VLDL-LDL covariation in both parents and the HDL-LDL covariation in mothers. The contribution of common family environment was fairly small. Mosteller (1993) employed a triangular (Cholesky) decomposition on data of 381 female twin pairs, including body mass index, HDL, LDL and age. Dominance genetic effects were found to be most

important in bringing about the phenotypic correlation between HDL and LDL (-0.15). Heller, Pedersen, de Faire and McClearn (1995) employed multivariate model fitting (a Cholesky decomposition) on data of younger (<65 years) and older (>65 years) twins reared apart and together, to partition phenotypic correlations between total serum cholesterol, triglycerides, HDL, ApoA1 and ApoB into their genetic and environmental sources. Both genetic and unique environmental factors were found to be important in mediating the phenotypic correlation, but there was no evidence for a single genetic factor common to all five lipids. The most remarkable finding was that unique environmental factors appeared to become more important as mediators of phenotypic correlations between serum lipids in the older age group. This finding might have implications for preventive actions in the elderly that are directed at two or more lipids at the same time.

Abovementioned studies have yielded important information on the genetic and environmental origin of correlations within the lipid system. Nevertheless, only exploratory quantitative genetic models like the triangular (Cholesky) decomposition were applied. These models have limited explanatory power because they do not allow tests of the direction of causation between different phenotypes. Future studies therefore have to apply models which try to incorporate prior physiological knowledge on the causal relationships between different components of lipid metabolism (Duffy, O'Connel, Heller & Martin, 1993). One example of such a relation is the basic role apolipoproteins play in the metabolism of other lipoprotein particles like VLDL, LDL and HDL, by acting as a structural protein and a ligand for cell-surface receptors (Rader & Brewer, 1994).

Multivariate genetic modeling of the insulin resistance syndrome

It is increasingly recognized that a number of risk factors for coronary heart disease tend to cluster within the same individual. These interrelated risk factors comprise a syndrome which is known as "syndrome X" (Reaven, 1988, 1993, 1994) or "insulin resistance syndrome" (Haffner et al., 1992; Wajchenberg, Malerbi, Rocha, Lerario & Santomauro, 1994; Grootenhuis, 1994). In Reaven's (1988) original definition, the syndrome consisted of insulin resistance as the key element, compensatory hyperinsulinaemia, glucose intolerance, hypertriglyceridemia, low levels of high density cholesterol and hypertension. Although the clinical features of the IRS can develop independently of obesity (Reaven, 1993), it has been known for many years that obesity and an abdominal distribution of body fat are associated with insulin resistance, hyperinsulinaemia and other elements of the IRS (Evans, Hoffmann, Kalkhoff & Kissebah, 1984; Peiris, Mueller, Smith, Struve & Kissebah, 1986; Kissebah, Evans, Peiris & Evans, 1988; Kissebah, 1991). Both obesity and an abdominal distribution of body fat can therefore also be considered part of the syndrome (Wajchenberg et al., 1994; Grootenhuis, 1994). Recently attention has been drawn to the high correlations between aspects of the IRS and one of the main inhibitors of the fibrinolytic system: Plasminogen Activator Inhibitor type 1 (PAI-1). Therefore Juhan-Vague and coworkers (1990, 1991, 1993) and also Reaven in later publications (1993, 1994) advocate that an increased level of PAI-1 in the blood plasma, is included

in the syndrome. This claim was supported by a recent study that reported a highly significant correlation (.87) between PAI-1 and insulin resistance (Kluft, Potter van Loon & de Maat, 1992; Potter van Loon, Kluft, Radder, Blankenstein & Meinders, 1993).

Following Ferrannini (1991) the physiological functions responsible for the interrelations within the IRS can be visualized as a network of carbohydrate and lipoprotein metabolism, body weight, blood pressure, and haemostasis with insulin action occupying a central position in this network. The nature of the connections (physiological mechanisms) is well known for some paths in the network, imperfectly understood for others. Factors causing a disturbance of the network leading to the clinical features of the IRS could be of genetic or environmental origin.

By fitting a multivariate genetic model on questionnaire data of 2508 male twins, Carmelli, Cardon and Fabsitz (1994) found evidence for a common latent factor mediating the clustering of hypertension, NIDDM and obesity. This common factor was influenced by both genetic (59%) and environmental (41%) effects. In this study, incidence of hypertension and diabetes was based on self report of a physician's diagnosis and/or the use of prescription medications. The body mass index (weight/-height^2) was also calculated from self reports. Although the identity of the latent common factor could not be determined from the available data, insulin resistance was proposed by Carmelli et al. (1994) as a possible candidate.

In contrast to the latter study which was dependent on discrete self report data of NIDDM and hypertension and on self reports of weight and height, within our project all phenotypic elements of the IRS were measured quantitatively within the laboratory. Future multivariate genetic analyses of these data offer the prospect of providing insight into the question to what extent genetic or environmental factors are responsible for the clustering of risk factors within the insulin resistance syndrome.

Mapping genes of risk factors for coronary heart disease

As is evident from the results in this thesis, most risk factors for CHD show a large heritable component. This is a strong argument in favour of further research with the ultimate goal to locate the genes and subsequently uncover their function. Identification of these genes and their fuction will increase our insight into the pathogenesis of risk factors, which would enable the development of interventions tailored to subjects with specific genetic predispositions (Lifton, 1995).

Different approaches can be chosen in the search for genes that influence (risk factors for) CHD (Sing & Moll, 1989, 1990; Weiss, 1993; McCarthy, Froguel & Hitman, 1994; Williams, 1994; Cardon, 1995; Lifton, 1995). Williams and coworkers (1994) have followed a useful scientific strategy in their studies of the genetic basis of hypertension. After conducting twin and family studies to estimate the heritability of traits related to hypertension, they performed appropriate segregation analyses to determine the mode of transmission, leading to linkage studies of pedigrees and sib-pairs, and, finally, identifying several functional mutations that contribute to hypertension, like the mutations responsible for glucocorticoid remediable

aldosteronism (GRA), Liddle syndrome and high angiotensinogen (see also, Lifton, 1995).

Most risk factors for CHD are continuously distributed and influenced by multiple genes, each with a relatively small effect. As the transmission of these complex polygenic traits do not follow simple Mendelian rules, identification of underlying genes is difficult. Through recent progress in molecular genetics, the genetic mapping of such complex traits by way of highly polymorphic markers, has become within reach. Several methods have been developed to map loci that influence quantitative traits in data from sibling pairs (Penrose, 1938; Haseman & Elston, 1972). These methods suppose that if a marker is cosegregating with a quantitative trait, siblings whose trait values are more alike, are more likely to receive the same alleles identical by descent (IBD) at a closely linked marker locus than siblings whose resemblance for the trait is less. Sib-pair strategies have several advantages compared to other methods (Cardon, 1995). Trait and genetic marker data only have to be obtained from siblings, rather than from large multigenerational pedigrees. Furthermore, sib-pair methods do not involve any assumptions concerning the mode of transmission, which implies that the intermediate step of segregation analysis between twin/family studies and linkage analysis, is no longer neccesary.

However, one major drawback that sharply contrasts to the abovementioned conveniences of the sib-pair method, is that even with large numbers of highly polymorphic markers that enable the determination of IBD status of siblings, the power to detect a single locus that influences quantitative traits in humans remains low (e.g. Blackwelder and Elston, 1982). One strategy to increase power to detect QTLs is to make use of multivariate genetic modeling of family data. Many risk factors for CHD, like components of the lipid system and the IRS, show strong intercorrelations. Ample evidence shows that correlations between these traits are at least partly due to common genetic factors. Multivariate analysis can be used to test whether the same genetic factor, or QTL, pleiotropically influences multiple, phenotypically correlated measures. If a common genetic factor is found, scores on this factor can be constructed for an individual by standard methods for the estimation of factor scores (Boomsma, Molenaar & Orlebeke, 1990; Boomsma, Molenaar & Dolan, 1991). This approach to estimate an individuals genotypic value at a QTL, not only reduces environmental variance, but also the background genetic variance not associated with the QTL. In a simulation study, Boomsma (1996) has shown that with this application of multivariate modeling the power to detect QTLs in a sibling analysis of quantitative traits can be increased substantially.

Since DZ twins are, genetically, full siblings, all sib-pair methodology can be applied equally well to DZ twin data. If measurements of the phenotypes of interest (e.g. risk factors for CHD) are available, all that is additionally needed are DNA marker data. Exactly such a sib-pair project to detect QTLs, based on the extension of the classical twin study with DNA marker data, is currently underway (Vogler, 1996). Both phenotypic and DNA marker data from a number of twin studies of risk factors for CHD, including data from our present study, will be combined with the aim to investigate the effects of a series of candidate loci and to test for the effects of previously

unknown QTLs in a genomic search. Multivariate modeling techniques will probably prove to be an important tool in increasing power to detect those QTLs.

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APPENDIX

Table 6.1: Mean daily nutrient intake of males and females in two age groups. Data from our study are compared to recommended dietary allowances and data from the first (1987-1988) and second (1992) Dutch National Food Consumption Survey (DNFCS)

		Age between 35 and 50					
		Males			Females		
	Recommended dietary allowance	Our Study	1st DNFCS	2nd DNFCS[a]	Our Study	1st DNFCS	2nd DNFCS[a]
Subjects (n)		150	639	1306	161	609	1493
Energy (MJ)	males: 11.1 females: 8.7	10.8	11.5	11.3	8.3	8.6	8.4
Protein (en%)	15	14.2	13.1	14.8	16.1	14.2	15.6
Fat total (en%)	30-35	36.5	39.8	37.5	36.5	40.9	37.8
saturated	10	15.1	16.1	14.1	15.7	17.1	14.5
monounsaturated	10	14.1	15.4	14.0	14.2	15.8	14.2
polyunsaturated	10	6.9	7.1	7.1	6.4	6.8	6.8
Carbohydrates (en%)	50-55	43.3	41.3	43.0	43.8	41.2	43.9
Alcohol (en%)		6.0	5.9	4.9	3.6	3.7	2.6
Cholesterol (mg/MJ)	33	25.7	30.5	26.7	28.9	34.1	28.1
Vitamin C (mg)	70	85.2	73.7	69.0	88.0	72.8	72.0
Dietary fiber (g/MJ)	3	2.9	2.4	1.6	3.1	2.6	1.8
		Age between 50 and 65					
		Males			Females		
	Recommended dietary allowance	Our Study	1st DNFCS	2nd DNFCS	Our Study	1st DNFCS	2nd DNFCS
Subjects (n)		31	431	405	46	460	545
Energy (MJ)	males: 10.1 females: 8.2	9.1	10.7	10.5	8.2	8.1	7.9
Protein (en%)	15	15.2	13.7	15.4	16.6	14.9	16.9
Fat total (en%)	30-35	37.3	41.2	37.1	36.3	41.1	37.3
saturated	10	16.0	17.2	14.3	15.8	17.5	14.8
monounsaturated	10	14.2	15.7	13.3	13.7	15.6	13.5
polyunsaturated	10	6.5	7.0	7.1	6.0	6.7	6.8
Carbohydrates (en%)	50-55	43.5	40.2	41.4	42.0	41.2	42.8
Alcohol (en%)		4.1	5.0	6.1	5.3	2.7	3.0

Cholesterol (mg/MJ)	33	30.7	33.0	29.2	32.5	35.9	30.7
Vitamin C (mg)	70	83.8	79.9	83.0	102.3	88.1	86.0
Dietary fiber (g/MJ)	3	3.0	2.5	1.8	3.2	2.9	2.0

Abbreviations: n = number, MJ = MegaJoule, en% = energy percentage, mg = milligram, g = gram.
[a] These results from the second DNFCS pertain to a somewhat broader age category (between 22 and 49), as results from the category between 35 and 50 were not given (Zo eet Nederland, 1992).

Samenvatting

Inleiding

Het is een bekend gegeven dat hart- en vaatziekten (HVZ) vaak in dezelfde families voorkomen. Dit familiale risico op HVZ zou theoretisch veroorzaakt kunnen worden door (een combinatie van) twee effecten: erfelijke factoren of gemeenschappelijke factoren in de omgeving (bijv. gezinsinvloeden). Uit tweeling-studies blijkt dat gemeenschappelijke genen een grotere invloed hebben op sterfte aan HVZ dan een gedeelde (gezins)omgeving. Resultaten van verdere studies wijzen erop dat het genetisch risico op HVZ slechts gedeeltelijk verklaard kan worden door de erfelijkheid van de bekende (traditionele) risicofactoren zoals hypertensie, roken en cholesterol. Andere risicofactoren, die tussenliggende stappen representeren in het causale pad van genen tot het uiteindelijke ontstaan van HVZ, moeten daarom in overweging worden genomen. Kandidaten voor deze intermediaire risicofactoren welke zijn besproken in deze dissertatie, zijn stress-reactiviteit, insuline en de vagale controle van het hart.

Het duidelijke verschil in HVZ-incidentie tussen mannen en vrouwen en veranderingen in het risicoprofiel met de leeftijd, impliceren dat de invloed van genen en omgeving op risicofactoren voor HVZ ook van sexe en leeftijd afhankelijk zouden kunnen zijn. Deze dissertatie heeft zich dan ook op twee vragen geconcentreerd: 1) Wat is de relatieve bijdrage van genetische en omgevingsinvloeden op intermediaire en traditionele risicofactoren? en 2) Zijn deze bijdragen afhankelijk van sexe en leeftijd? Om dit te kunnen onderzoeken werden risicofactoren gemeten in vijf groepen tweelingen van middelbare leeftijd, onderverdeeld naar zygositeit en sexe. Naast groepen mannelijke en vrouwelijke tweelingen van gelijke sexe omvatte de steekproef ook een groep tweelingen van ongelijke sexe. De stabiliteit van genetische invloed over de leeftijd kon worden getoetst door de gegevens van de tweelingen van middelbare leeftijd in dit project te combineren met gegevens van een eerder project waarin ouders tezamen met hun tweelingkinderen werden gemeten.

Respiratoire Sinus Aritmie

De Respiratoire Sinus Aritmie (RSA) is de variatie in hartslag zoals deze door de ademhaling wordt veroorzaakt. Het is een gevoelige, non-invasieve index van de vagale controle van het hart. Een sterke RSA, gekarakteriseerd door een grote hartslagvariabiliteit, wordt gezien als een indicatie voor de gezondheid van het hart. Een verminderde hartslagvariabiliteit hangt samen met hartziekte en hypertensie. Een zwakke RSA kan daarom worden beschouwd als een intermediaire risicofactor voor HVZ. Verder is het zo dat de RSA afneemt met de leeftijd en onder invloed van psychologische stress. In het tweede hoofdstuk werden schattingen van de genetische en omgevingsinvloeden op de RSA vergeleken tussen een rustconditie en condities van mentale en fysieke stress, terwijl rekening werd gehouden met de invloed van leeftijd en ademfrequentie op de RSA. In alle experimentele condities

konden individuele verschillen in de RSA het beste worden verklaard door additief genetische en unieke omgevingsfactoren, onafhankelijk van leeftijd en ademfrequentie. Deze factoren beïnvloedden de RSA in gelijke mate in beide sexen. De totale genetische invloed op de RSA varieerde tussen de 28% en de 43% over de experimentele condities. Hoewel er een grotere afname in de invloed van unieke omgevingsfactoren op de RSA verwacht werd bij stressvollere taken, werd dit door de resultaten niet bevestigd. De erfelijkheidsschattingen van de RSA tijdens de verschillende stresscondities bleken namelijk niet systematisch groter te worden met de grootte van de stressrespons op de taken. Correctie voor de invloed van de ademfrequentie bleek nauwelijks effect te hebben op de grootte van de RSA-erfelijkheidsschattingen. De covariantie tussen de ademfrequentie en de RSA, welke in alle condities werd gevonden, kon worden toegeschreven aan een combinatie van gecorreleerde unieke omgevingsfactoren en gecorreleerde additief genetische factoren.

Nuchtere insuline

Het derde hoofdstuk stelde de vraag aan de orde in welke mate genetische en omgevingsfactoren een andere intermediaire risicofactor, namelijk nuchtere insuline, beïnvloeden. In populatiestudies wordt nuchtere insuline beschouwd als de beste maat voor insulineresistentie, welke op zijn beurt een voorloper is van zowel niet-insuline-afhankelijke diabetes mellitus als ook, via het insulineresistentie syndroom (syndroom X), van HVZ. Bovendien werd in hoofdstuk 3 getest of de homeostatische regulatie door insuline van de glucoseconcentratie in het bloed, het beste verklaard kon worden door een enkelvoudig causaal pad van insuline naar glucose (of omgekeerd), of door twee reciproke paden (dat wil zeggen een negatieve terugkoppeling tussen insuline en glucose). Net als voor de RSA liet een model met additief genetische en unieke omgevingsfactoren de beste overeenkomst met de data zien. De invloed van erfelijkheid op nuchtere insuline was klein (21%) en gelijk voor mannen en vrouwen. De interactie tussen insuline en glucose werd het beste beschreven door een model met reciproke paden.

Stress-reactiviteit

Resultaten betreffende de erfelijkheid van reactiviteit op laboratorium stresstaken waren inconsistent in deze studie. In hoofdstuk 2 werd beschreven dat het patroon van tweelingcorrelaties van RSA-reactiviteit onverenigbaar was met een biologisch plausibel model. In hoofdstuk 4 bleek uit een overzicht van tweelingstudies en de analyse van onze eigen data dat bloeddrukreactiviteit matig erfelijk zou kunnen zijn. Er werden echter verschillende resultaten gevonden voor verschillende stresstaken. De reden voor de inconsistentie in de bevindingen zou kunnen liggen in de minder betrouwbare bepaling van reactiviteitsmaten vergeleken met de betrouwbaarheid van de nivo's van bijvoorbeeld RSA en bloeddruk. Reactiviteit wordt berekend als het verschil tussen twee nivo's, wat de errorterm doet toenemen. Een andere mogelijkheid is dat iemands reactiviteit eenvoudigweg een minder betrouwbare persoonseigenschap is. Zulke beperkte betrouwbaarheden

zouden kunnen leiden tot inconsistente patronen in tweelingcorrelaties en zo tot variabiliteit in schattingen van genetische en omgevingsinvloeden.

Leeftijdsafhankelijke genexpressie voor lipiden en bloeddruk

In hoofdstuk 4 en 5 werd onderzocht of de expressie van genen in de kindertijd verschilt met die tijdens volwassenheid voor fenotypen gerelateerd aan bloeddruk en metabolisme van lipiden. In beide hoofdstukken werd een overzicht van de literatuur van tweelingstudies gegeven en erfelijkheidsschattingen van deze studies werden geordend naar leeftijd van de onderzochte tweelingsteekproeven. Uit deze gegevens kon worden geconcludeerd dat erfelijkheidschattingen voor systolische en diastolische bloeddruk, totaal cholesterol, low density lipoproteine, high density lipoproteine en triglyceriden geen duidelijke leeftijdstrend laten zien: schattingen waren telkens hoog en bleven redelijk stabiel met de leeftijd. Erfelijkheidsschattingen waren wat variabeler voor apolipoproteinen en bloeddrukreactiviteit op stresstaken. Ook voor deze variabelen kon geen duidelijke leeftijdstrend worden ontdekt. Voor onze eigen analyses combineerden we de data van de tweelingen van middelbare leeftijd met data van een eerder project waarin ouders en hun tweelingkinderen werden gemeten. Deze unieke combinatie van gegevens maakte het mogelijk een uitgebreid ouder-tweeling model te specificeren, waarmee we genetische stabiliteit konden schatten zonder de beschikking te hebben over longitudinale data. De resultaten voor bloeddruk, lipiden en lipoproteinen lieten zien dat gedeeltelijk verschillende genen tot expressie komen tijdens de kindertijd en de volwassenheid. Bij apolipoproteinen-A1 en -B en lipoproteine(a) zijn het daarentegen dezelfde genen die tot uiting komen tijdens deze verschillende levensperioden. De bloeddrukreactiviteitsdata lieten de toepassing van het uitgebreide ouder-tweeling model niet toe. Bovenstaande resultaten wijzen erop dat bloeddruk, lipiden en lipoproteinen waarschijnlijk beïnvloed worden door een andere combinatie van genen in verschillende perioden van het leven.

Sexe verschillen

Door het opnemen van paren van ongelijke sexe samen met mannelijke en vrouwelijke paren van gelijke sexe waren we niet alleen in staat te bepalen of de grootte van genetische en omgevingsinvloeden verschilden tussen de sexen, maar konden we bovendien testen of verschillende genen tot uiting kwamen in mannen en vrouwen. Een opmerkelijke bevinding in onze studie was de bijna complete afwezigheid van sexeverschillen in genetische en omgevingsinvloeden op de verschillende onderzochte risicofactoren. De grote verschillen in incidentie en mortaliteit van HVZ tussen de sexen lijkt dan ook niet verklaard te kunnen worden door sexeverschillen in genetische invloed op de gemeten risicofactoren.

Conclusies

Over het algemeen werden matig tot hoge erfelijkheidsschattingen gevonden voor de meeste risicofactoren die werden onderzocht in zowel jonge als oudere tweelingen. De lage schatting voor nuchtere insuline was de enige uitzondering. De

genetische variantie was meestal van additieve aard, dominantie variantie bleek onbelangrijk. Wat betreft omgevingsfactoren: deze waren altijd specifiek voor het individu. Geen enkele van de onderzochte variabelen werd door gemeenschappelijke gezinsfactoren beïnvloed. Voor personen van middelbare leeftijd zoals de tweelingen in dit onderzoek, is zo'n resultaat enigzins te verwachten aangezien ze niet meer in het gezin wonen waarin ze zijn opgegroeid. Samenvattend wijzen de resultaten erop dat individuele verschillen in risicofactoren voor HVZ voor het overgrote deel verklaard kunnen worden door een combinatie van blootstelling aan unieke omgeving en multipele genen die op een additieve manier hun invloed uitoefenen. Met enig voorbehoud kan daarom gesteld worden dat preventie van HVZ zich voornamelijk dient te richten op het beïnvloeden van unieke omgevingsfactoren. Anderzijds betekent de grote invloed van erfelijkheid zoals die voor de meeste risicofactoren gevonden werd, in geen geval dat daar niets meer aan te doen zou zijn. Een erfelijke aanleg komt namelijk pas tot uiting bij bepaalde leefgewoonten. Gedragsinterventies gericht op (niet) roken, regelmatige lichaamsbeweging en gezonde voeding blijven daarom van groot belang. Toekomstig onderzoek zal zich meer moeten gaan richten op de complexiteit van het ontstaan van HVZ welke gekenmerkt wordt door de interactie tussen meerdere risicofactoren tegelijkertijd. Met behulp van multivariate modellen van tweelingdata zal onderzocht kunnen gaan worden of de oorzaak van de relatie tussen verschillende risicofactoren aan genetische dan wel omgevingsinvloeden kan worden toegeschreven. Verder duidt de grote invloed van erfelijkheid op de meeste risicofactoren erop, dat het wellicht de moeite loont in toekomstig onderzoek op zoek te gaan naar de specifieke genen die de verschillende risicofactoren beïnvloeden. Door tweelingstudies naar risicofactoren van HVZ uit te breiden met DNA marker gegevens, wordt het mogelijk de verschillende genen in kaart te brengen die van invloed zijn op bijvoorbeeld bloeddruk of componenten van het lipidenmetabolisme. Een dergelijke studie is onlangs van start gegaan. Multivariate analysetechnieken kunnen hierbij gebruikt worden om te testen of gecorreleerde risicofactoren beïnvloed worden door dezelfde genetische factor. Individuele scores voor zo'n gemeenschappelijke genetische factor kunnen geschat worden, waardoor het vermogen een bepaald gen te detecteren aanzienlijk toeneemt.

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Curriculum vitae

Harold Snieder was born at May 30, 1965, in Bussum, The Netherlands. He attended VWO-ß at the Willem de Zwijger college in Bussum from 1977 till 1983. From 1983 till 1989 he studied at the Faculty of Human Movement Science at the Vrije Universiteit in Amsterdam. His main subjects were exercise physiology and psychology of human movement. During his military service he worked as a research assistant at the Sportsmedical Centre of the Royal Dutch Army. The research leading to this thesis started in 1991 and was carried out at the Department of Psychophysiology, Vrije Universiteit, Amsterdam. The project was funded by the Netherlands Heart Foundation (NHS: 90-313). As part of his research training, Harold Snieder took a number of courses on genetic epidemiology and statistics and methodology (e.g., the Twin Course of 1991 in Leuven, Belgium).