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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.

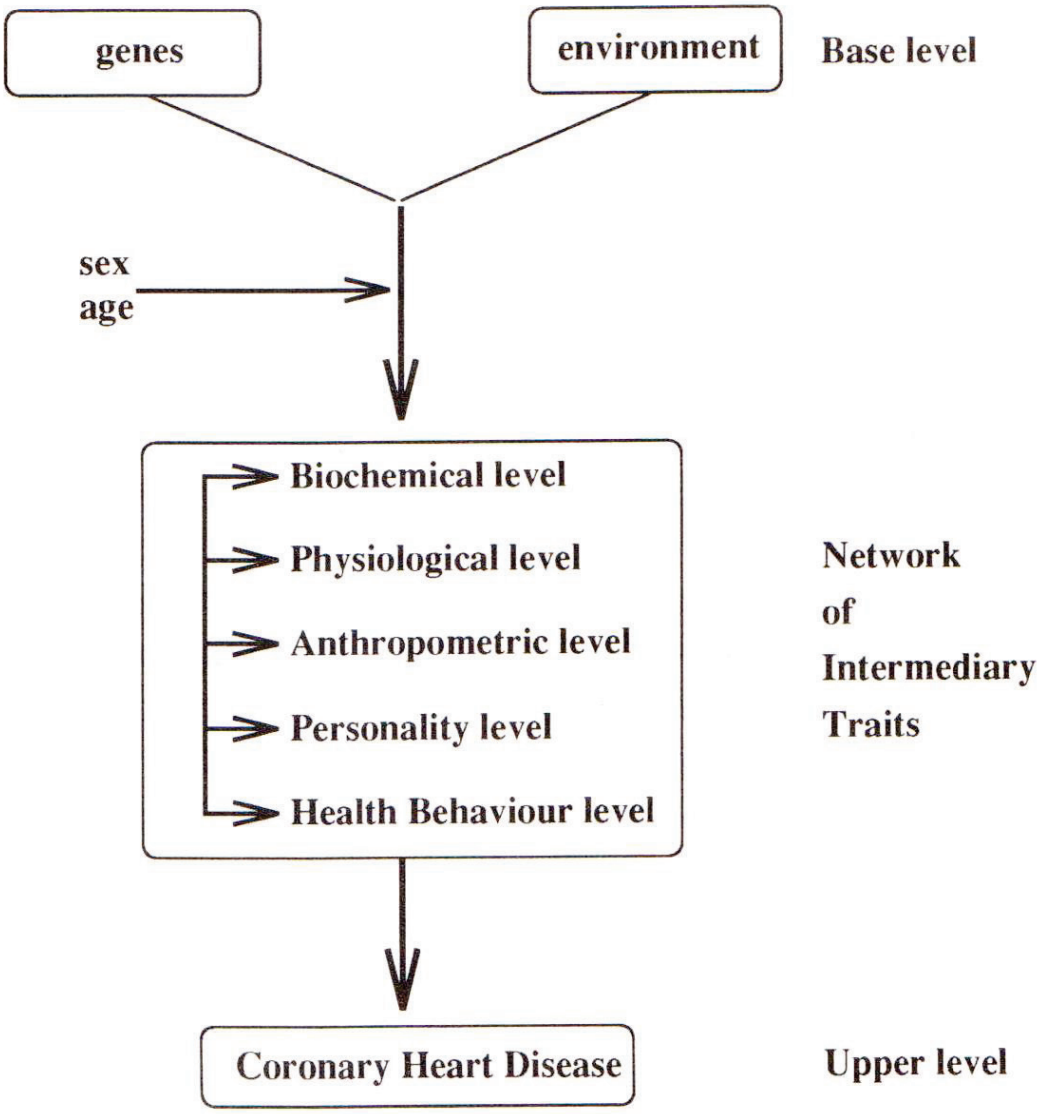

Figure 6.1: Model for linking the influence of genes and environment to endpoints of CHD via a network of intermediary traits.

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.