Families and Natural History of Lipids in Childhood: An 18-Year Follow-up Study

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The natural history of total cholesterol and lipoprotein cholesterol in offspring was studied in relation to total cholesterol levels in their parents in the Epidemiological Prevention Study of Zoetermeer (EPOZ). All residents of 5 or more years who were living in two districts in the Dutch town of Zoetermeer were invited to participate in a study on indicators for chronic diseases between 1975 and 1978. In a random sample of 483 youngsters who were 5–19 years old, yearly measurements of cardiovascular risk factors were performed during a follow-up period of 18 years (average follow-up, 13.8 years). Total and subtraction cholesterol levels in offspring during follow-up were studied by tertiles of age-adjusted total cholesterol in their parents. Total and low density lipoprotein (LDL) cholesterol levels measured from childhood into young adulthood differed significantly between offspring whose fathers were in the highest total cholesterol tertile compared with those whose fathers were in the lowest tertile, amounting to 0.4 mmol/liter for total cholesterol and 0.5 mmol/liter for LDL cholesterol. Offspring differences by maternal tertiles amounted to 0.5 mmol/liter for total cholesterol and 0.6 mmol/liter for LDL cholesterol. Offspring (n = 53) with both parents in the upper cholesterol tertile had almost 1 mmol/liter higher cholesterol levels compared with offspring (n = 51) with both parents in the lowest tertile, whereas offspring (n = 48) with both parents in the middle tertile had intermediate levels. Differences remained after adjustment for sex, Quetelet index, systolic and diastolic blood pressure, and use of alcohol, cigarettes, and oral contraceptives. Offspring group differences in total and LDL cholesterol were already present in childhood and persisted into young adulthood. There was no clear relation between offspring change in cholesterol levels and parental total cholesterol levels. For high density lipoprotein cholesterol and its subfractions, no relations with parental total cholesterol levels were found. Based on the evidence of a strong positive relation between total cholesterol levels in parents and offspring levels of total and LDL cholesterol measured from childhood into young adulthood, the authors conclude that total and LDL cholesterol levels in offspring may already be characterized from young age and beyond through cholesterol levels in their parents. Am J Epidemiol 1997;145:777–85.

adolescence; family; lipids; natural history

The atherosclerotic process begins early in life, and adverse lipoprotein levels in childhood are associated with the presence of coronary atherosclerosis among adolescents and young adults (1, 2). Studies on the natural history of lipids and lipoproteins in children and adolescents (3–5) suggest that the relative positions of serum lipid and lipoprotein levels within distributions are relatively stable from childhood into young adulthood. In agreement with these findings, a recent prospective study (6) has shown that total cholesterol levels in young adult men are related to coronary heart disease much later in life. If lipid levels at a young age are predictive of lipid levels and disease in adulthood, then preventive measures might already have been taken in childhood and adolescence. Although lipids and lipoproteins tend to aggregate within families (7), much less is known about the relation between the natural history of offspring lipids and total cholesterol levels in their parents. Therefore, in this study we examined whether offspring of parents with high risk factor levels have high levels themselves and to what extent this offspring-parent association persists longitudinally. This was done by assessing parental total cholesterol level as a determinant of offspring lipid level at various ages and as a determinant of the change of lipid levels over time.
MATERIALS AND METHODS

Subjects

All residents of ages 5 or more years who were living in two districts in the Dutch town of Zoetermeer were invited to participate in a study of risk indicators for chronic diseases (Epidemiological Prevention Study of Zoetermeer [EPOZ] (8–10)) between 1975 and 1978. Near The Hague in the Netherlands, Zoetermeer is a suburban residential community of at that time about 55,000 inhabitants. Between 1975 and 1979, 4,649 persons aged 5–19 years (82 percent of those invited) took part in the study. In 1980 and 1981, all migrants moving to Zoetermeer were also asked to participate in the study. From this total group, a random sample of 596 children was selected for annual follow-up in a study on the natural history of cardiovascular risk factors and its determinants. This paper deals with 483 subjects (81 percent), 252 males and 231 females, who took part in the follow-up study. The average follow-up period was 13.8 years (range, 3–18 years). In the total group, there were 36 pairs of siblings; in all other families, only one offspring participated. Data on parents of these 483 subjects were obtained during 1975–1981 at the baseline study. Complete data were available for 425 fathers and 454 mothers. For this study, all 483 individuals who contributed data at any point in time were included in the analysis. Up to 1992, 352 offspring were still taking part in the follow-up study.

Measurements

Starting with the 1975–1981 baseline screening, each offspring was seen yearly and measurements were performed by the same research assistant throughout the entire follow-up period. At each examination, systolic blood pressure and diastolic blood pressure were measured using a random zero sphygmomanometer (Hawksley, Lancing, Sussex, United Kingdom) as described in detail elsewhere (11). Serum blood samples were drawn by antecubital venipuncture for measurement of total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and its subfractions HDL$_2$ and HDL$_3$ cholesterol. Height and weight were measured without shoes and heavy clothing.

At each examination, the offspring answered a questionnaire about use of medication, alcohol intake, coffee consumption, and smoking habits. Additionally, daughters were asked about use of oral contraceptives, menstrual cycle, and pregnancies.

Laboratory analysis

Serum total cholesterol at baseline was measured with an automated enzymatic method (12). Beginning in 1983, we used a modified reagent (CHOD/PAP High Performance, Boehringer Mannheim, Germany). During the transition period, we used both reagents simultaneously in 170 sera in seven runs and obtained a high correlation (beta coefficient 1.02; r > 0.99). The overall coefficient of variation was 2.5 percent at baseline and 2.3 percent during follow-up. Cholesterol determinations at follow-up were performed on serum samples stored at $-20^\circ$C for up to 4 years. Repeated measurements performed by our laboratory in several hundreds of frozen serum samples showed no significant changes from 1 to as many as 4 years after sampling compared with frozen samples measured within 1 week after venipuncture. The standard deviation of these duplicate measurements did not exceed 3.0 percent in all instances and did not show a significant drift.

Age-specific total cholesterol levels measured in 1975–1982 were similar to those measured in 1983–1990. For instance, comparing the first with the second period, in 15 year olds the levels were 4.54 (standard deviation (SD) 0.84) mmol/liter ($n = 210$) and 4.51 (SD 0.71) mmol/liter ($n = 101$), respectively; in 16 year olds levels were 4.52 (SD 0.89) mmol/liter ($n = 203$) and 4.51 (SD 0.75) mmol/liter ($n = 127$), respectively. In 20 year olds levels were 4.81 (SD 0.85) mmol/liter ($n = 168$) and 4.92 (SD 0.90) mmol/liter ($n = 189$), respectively.

HDL cholesterol measurements were started in 1979 and LDL cholesterol measurements, in 1984. High density and low density lipoprotein cholesterol levels were measured by the same method after precipitation. For HDL cholesterol, we used the phosphotungstate method according to Burstein et al. (13), with a minor modification as described by Grove (14). For LDL cholesterol, precipitation was carried out with polyanysulfate (Boehringer Mannheim). All measurements were carried out in the laboratory of the Department of Epidemiology and Biostatistics (Erasmus University Medical School, Rotterdam, the Netherlands), which had participated since 1978 in the lipid standardization program of the World Health Organization (WHO) Regional Lipid Reference Centre in Prague, Czechoslovakia, and since 1977 in the Dutch National Cholesterol standardization program (Stichting Kwaliteitsbewaking Klinisch-chemische Analyses ten behoeve van Epidemiologisch Onderzoek), initiated in analogy to the program of the Centers for Disease Control (CDC) Lipid Standardization Laboratory in Atlanta, Georgia. In addition, during the baseline period, quality control was indirectly checked on the CDC proto-
tertiles as well as the above-mentioned factors as the dependent and an indicator for parental cholesterol characteristics of parents and offspring were calculated. Systolic and diastolic blood pressure, Quetelet index, total cholesterol levels in offspring by parental total cholesterol, was performed to obtain mean (lipoprotein) cholesterol distributions were made for fathers and mothers separately using stepwise precipitation of apolipoprotein B containing lipoproteins with heparin/Mn<sup>2+</sup> in two steps and HDL<sub>2</sub> with dextran sulfate.

**Data analysis**

The data analysis was intended to assess parental total cholesterol level as a determinant of offspring lipid level and change in offspring lipid levels over time. Mean values and standard deviations of baseline characteristics of parents and offspring were calculated. Tertiles of the age-adjusted serum total cholesterol distributions were made for fathers and mothers separately using linear regression. Baseline characteristics of offspring were calculated according to both paternal and maternal tertiles of total cholesterol.

An unbalanced repeated measures analysis (BMDP 5V, BMDP Statistical Software, Berkeley, California) was performed to obtain mean (lipoprotein) cholesterol levels in offspring by parental total cholesterol, adjusted for offspring gender, concurrent levels of systolic and diastolic blood pressure, Quetelet index, and use of cigarettes, alcohol, and oral contraceptives. The model included offspring cholesterol levels as the dependent and an indicator for parental cholesterol tertiles as well as the above-mentioned factors as the independent variables. This analysis was performed separately for the age periods 5-9, 10-14, 15-19, 20-24, 25-29, and 30-37 years. Offspring cholesterol levels were analyzed according to total cholesterol levels in fathers and mothers separately as well as the combination of total cholesterol levels of both parents.

Individual lipid level change with age was determined by calculating slopes of regression lines through total cholesterol and lipoprotein values over 10-year intervals. Subsequently, from these individual slopes, the mean change was calculated using all values within age categories corresponding to the 10-year intervals and by tertiles of parental total cholesterol. Subjects could contribute to values calculated for different age categories. Differences in change of offspring lipid levels between parental tertiles were tested using the Student’s t test with values in the lowest parental tertile as the reference category. Group comparisons of changes in offspring lipid levels were weighed for individual numbers of lipid measurements within age categories.

**RESULTS**

In table 1 are shown anthropometric characteristics, systolic and diastolic blood pressure, and serum total cholesterol levels of fathers (\(n = 425\)) and mothers (\(n = 454\)) overall and by tertiles of their respective total cholesterol levels. The cutoff points of parental total cholesterol corresponding to the tertiles were 5.4 and 6.2 mmol/liter in fathers and 5.2 and 6.0 mmol/liter in mothers.

Baseline characteristics by paternal tertiles of total cholesterol of offspring are shown in table 2. The same characteristics by maternal cholesterol tertiles showed similar results (data not shown). None of the differences between parental tertiles was statistically significant; and other than total cholesterol levels, cardio-

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of fathers and mothers by age-adjusted tertiles of total cholesterol, Epidemiological Prevention Study, Zoetermeer, the Netherlands, 1975-1978</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tertiles of fathers’ total cholesterol</strong></td>
</tr>
<tr>
<td><strong>Low</strong></td>
</tr>
<tr>
<td>(n = 142)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>44.6 (8.2)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td><strong>Quetelet index (kg/m²)</strong></td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/liter)</strong></td>
</tr>
<tr>
<td><strong>Range</strong></td>
</tr>
</tbody>
</table>

* SD, standard deviation.
vascular risk factors, particularly, did not differ materially between offspring groups.

In Table 3, total cholesterol levels are shown for age periods of offspring by parental tertiles of serum total cholesterol levels. With repeated measures analysis, these offspring cholesterol levels were adjusted for sex, Quetelet index, systolic and diastolic blood pressure, and concurrent use of alcohol, cigarettes, and oral contraceptives. Total cholesterol levels in offspring are given according to the tertile of paternal cholesterol, maternal cholesterol, and both parents. Differences in these offspring cholesterol levels were adjusted for maternal cholesterol, and both parents. Differences in

TABLE 3. Total cholesterol and LDL* cholesterol in offspring by tertiles of total cholesterol in fathers and mothers, Epidemiological Prevention Study, Zoetermeer, the Netherlands, 1975–1978

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Tertiles of mothers' total cholesterol</th>
<th>Tertiles of fathers' total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>5-9</td>
<td>4.61</td>
<td>4.74</td>
</tr>
<tr>
<td>10-14</td>
<td>4.51</td>
<td>4.75</td>
</tr>
<tr>
<td>15-19</td>
<td>4.32</td>
<td>4.52</td>
</tr>
<tr>
<td>20-24</td>
<td>5.47</td>
<td>5.50</td>
</tr>
<tr>
<td>25-29</td>
<td>5.17</td>
<td>5.42</td>
</tr>
<tr>
<td>30-37</td>
<td>5.17</td>
<td>5.50</td>
</tr>
</tbody>
</table>

* LDL, low density lipoprotein; Δ, difference between high and low tertile; CI, confidence interval (difference between high and low tertile).
† Values are means (unbalanced repeated measures analysis) adjusted for sex, Quetelet index, systolic and diastolic blood pressure, and concurrent use of alcohol and cigarettes.
FIGURE 1. Mean total cholesterol levels in offspring by tertiles of total cholesterol in fathers (top), mothers (middle), and both parents (bottom), Epidemiological Prevention Study of Zoetermeer, the Netherlands, 1975–1978. Levels were adjusted for gender, Quetelet index, systolic blood pressure, diastolic blood pressure, and concurrent use of cigarettes, alcohol, and oral contraceptives using repeated measures analysis.
offspring total cholesterol levels were highest, amounting to almost 1 mmol/liter, when both parents were taken into account as compared with only the fathers or only the mothers.

In table 4, the relation between the change of total cholesterol in offspring with age and total cholesterol level in mothers and fathers is shown. There was an inverse relation between cholesterol change in offspring and total cholesterol in fathers only in the lowest age period. As expected from the comparative analyses of levels of total cholesterol, there was no relation between absolute change in total cholesterol levels or between the change in position in the distribution and parental total cholesterol levels present in any of the other age periods. HDL cholesterol, HDL₂ cholesterol, and HDL₃ cholesterol levels did not show differences by paternal and maternal tertiles at any age.

In the total group (n = 483), there were 36 pairs of siblings (the others were single offspring) in which observations cannot be assumed to be independent. A separate analysis of the data in which one of each pair of siblings was randomly included and the other excluded yielded similar results (data not shown).

Up to 1992, there were 131 offspring who had terminated their participation in the study, including 44 from the low paternal group, 35 from the medium group, and 33 from the high group. All available data were used on these subjects for whom group differences would be present analyses are based on data obtained in a random sample of youngsters initially examined in a population survey and subsequently invited for longitudinal follow-up. We believe that response rates were high enough to consider this sample representative of the source population. There is the possibility that loss to follow-up has biased our results in the sense that offspring loss to follow-up would be differentially (with regard to offspring groups) associated with risk factor levels. Induced offspring differences would emerge if in the high level parental groups there were more loss to follow-up in the offspring with low total cholesterol levels compared with offspring with parents in the lowest cholesterol tertile. These differences were already present in childhood and persisted into young adulthood, remaining similar in magnitude and direction throughout this period.

No relations between parental total cholesterol levels and offspring HDL cholesterol and its subfractions were found. The relation between maternal total cholesterol levels and offspring total and LDL cholesterol levels appeared slightly more pronounced than for paternal total cholesterol levels. The largest effects were observed when cholesterol levels of both parents were taken into account. The results for total and LDL cholesterol were similar in daughters and sons. Except for an inverse relation between paternal cholesterol and change in offspring total cholesterol in the age period 5–14 years, there was no clear overall relation between parental cholesterol levels and change in cholesterol levels in their offspring.

Before we can interpret the findings, some methodological aspects of the study must be discussed. The present analyses are based on data obtained in a random sample of youngsters initially examined in a population survey and subsequently invited for longitudinal follow-up. We believe that response rates were high enough to consider this sample representative of the source population. There is the possibility that loss to follow-up has biased our results in the sense that offspring loss to follow-up would be differentially (with regard to offspring groups) associated with risk factor levels. Induced offspring differences would emerge if in the high level parental groups there were more loss to follow-up in the offspring with low total cholesterol levels and vice versa for the low level.

### TABLE 4. Relation between change of total cholesterol in offspring with age and total cholesterol level in mothers and fathers in three age categories, Epidemiological Prevention Study, Zoetermeer, the Netherlands, 1975–1978

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mothers</th>
<th>Fathers</th>
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<tbody>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>Absolute change (mmol/liter/year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–14</td>
<td>0.008</td>
<td>-0.007 to 0.032</td>
</tr>
<tr>
<td>15–24</td>
<td>0.002</td>
<td>-0.005 to 0.010</td>
</tr>
<tr>
<td>25–37</td>
<td>0.032</td>
<td>-0.008 to 0.072</td>
</tr>
<tr>
<td>Change in rank (standard normal deviate/year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–14</td>
<td>0.006</td>
<td>-0.026 to 0.038</td>
</tr>
<tr>
<td>15–24</td>
<td>-0.007</td>
<td>-0.020 to 0.006</td>
</tr>
<tr>
<td>25–37</td>
<td>0.035</td>
<td>-0.009 to 0.079</td>
</tr>
</tbody>
</table>

* Regression coefficients show the absolute change per year in total cholesterol (mmol/liter) in offspring and the change per year in total cholesterol rank (expressed as standard normal deviate) for 1-mmol/liter increase in maternal and paternal total cholesterol level.

† CI, confidence interval.
parental groups. However, there was no evidence from the data for such differential loss to follow-up both in terms of numbers of subjects and of total cholesterol levels. In fact, in the offspring that terminated participation, differences according to parental levels were found to be similar to differences in offspring that continued participation. During the entire follow-up period, laboratory measurements quality was controlled by the CDC/WHO (total cholesterol and HDL cholesterol) and by the Stichting Kwaliteitsbewaking Klinisch-chemische Analyses ten behoeve van Epidemiologisch Onderzoek (for total cholesterol measurements) and did not show any drift of measurements over time. Furthermore, a differential effect of such a selective drift for some of the various groups of offspring discussed in the present study is not considered likely by the authors. In the present study, no trends in total cholesterol level were observed that could possibly explain the reported familial differences. The large number of measurements of serum lipids that were performed in each individual precludes underestimation due to measurement error, which may have resulted if only single measurements had been performed (17).

Coronary heart disease risk factors including lipids are known to aggregate within families, and many studies have shown that the variation in lipid levels is determined by genetic as well as environmental factors (7, 18-24). Cross-sectionally, the relation between total cholesterol levels in parents and total cholesterol and lipoprotein cholesterol levels in their offspring throughout childhood and young adulthood has been reported previously (25), including in the present EPOZ study (26). Our data add to the earlier findings that familial aggregation of total and LDL cholesterol has a substantial and lasting effect from a young age until at least young adulthood. In studies on serum lipids in younger and older groups of elderly twins who did and did not share environments, it is shown that there is considerable genetic influence on serum lipid levels, which for many decrease with age. For total cholesterol, it was shown that a shared rearing environment had an important effect particularly in older individuals (27, 28). A recent study on lipids in parents and their twin offspring showed an increasing contribution of environmental variation to lipid levels with increasing age, and the genetic variation was equal in both generations (29). The genetic basis for the differences found in the present study is supported by the fact that familial differences are already present from a young age and beyond. Furthermore, adjustment for other cardiovascular risk factors including systolic and diastolic blood pressure and Quetelet index, but also use of alcohol, cigarettes, and oral contraceptives as environmental factors, had no effect on the results. Therefore, although familial aggregation is also found for other important cardiovascular risk factors such as hypertension (30, 31), obesity (32, 33), diabetes mellitus (34, 35), physical fitness (36), and smoking (37), the findings of the present study appear to be relatively independent of the possible familial aggregation of those other risk factors.

Our data revealed an inverse association between total cholesterol levels in fathers and change of offspring total cholesterol in their children between ages 5 and 15 years. Although this appears to indicate that offspring of fathers with high total cholesterol levels came from higher initial levels before their entry into the study, there is no clear explanation for a difference in this respect between mothers and fathers in relation to their offspring, and we therefore believe that this finding should be interpreted with caution.

With regard to parent-offspring resemblance of lipid levels, stronger mother-offspring than father-offspring correlations for several lipid parameters have been reported (38), although such asymmetry was not found in other studies (29, 39). In our data, differences by maternal total cholesterol appeared to be somewhat greater than differences by parental total cholesterol. There was no asymmetry with regard to offspring gender in relation to parental total cholesterol levels both for total and LDL cholesterol.

Tracking of lipids at a young age, i.e., the stability of ranking in the age- and sex-specific lipids distribution, has been demonstrated in a number of studies in general populations of children and young adults (3-5). Although the longitudinal relation between high lipid levels in childhood and coronary artery disease much later in life has not yet been shown, this relation was already shown for total cholesterol levels in young adult men (6). A number of studies have reported familial aggregation of coronary heart disease (40-42), and cross-sectional studies have shown that the offspring of patients with cardiovascular disease have less favorable (apo)lipoprotein profiles compared with offspring of nonpatients (43-47). Our data provide additional evidence for familiarly determined high risk tracking of total and LDL cholesterol from a young age and beyond as one of the underlying mechanisms leading to excess occurrence of cardiovascular disease in certain families.

Although selective cholesterol screening is, among other indications, recommended for children having a parent with a high blood cholesterol level (48), these recommendations are subject to debate (49). Our data may add to the awareness that a family history of hypercholesterolemia, particularly if it involves both parents, characterizes groups at continuously in-
creased risk during youth, which is possibly maintained in adulthood. In terms of cardiovascular risk, such long-term exposed groups may well differ from individuals who develop hypercholesterolemia later in life.

In summary, a strong positive relation was shown between total cholesterol levels in parents and offspring cholesterol and LDL cholesterol levels measured from childhood into young adulthood. There is no clear relation between parental cholesterol levels and change in offspring cholesterol levels over time. These results suggest that total and LDL cholesterol levels in offspring can already be characterized from young age and beyond through cholesterol levels in their parents.

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