LIPID AND LIPOPROTEIN TRACKING IN CHILDREN

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SUMMARY

This study was designed to assess tracking of plasma cholesterol, triglyceride, high and low density lipoprotein cholesterol (C-HDL, C-LDL), in 108 children followed over a 4 year period in the Cincinnati Lipid Research Clinic's Princeton School Study, with focus on those children initially in the lowest decile for lipids and lipoproteins. For children whose initial lipid-lipoprotein levels were in the lowest decile, 60% (for cholesterol), 33% (for triglyceride), 56% (for C-LDL), and 13% (for C-HDL) remained in the lowest decile after 4 years of follow-up. The difference in total plasma cholesterol, triglyceride, C-LDL, and C-HDL between those children initially in the lowest decile and all others was retained for these specific children during the 4 year follow-up, $p < .003$. A considerable proportion of the increase in group mean lipid-lipoprotein levels over 4 years could be accounted for by the phenomenon of regression toward the mean, with the exception of C-LDL where the observed means did not increase as one would expect due to regression towards the mean ($< .05$), a further example of tight tracking at low C-LDL levels. For children whose initial total plasma cholesterol, triglyceride, C-LDL, and C-HDL were in the highest decile, 46% (for cholesterol), 27% (for triglyceride), 27% (for C-LDL), and 45% (for C-HDL) remained in the highest decile after 4 years follow-up. For all 108 children, initial plasma cholesterol, triglyceride, C-LDL, and C-HDL were all closely correlated with subsequent measurements 1, 2, and 3 years apart, $p < .001$. Such findings raise the possibility that children who tend to track in the lowest and highest deciles for cholesterol and C-LDL may mature to become adults respectively at reduced and increased coronary heart disease (CHD) risk. Children who tend to track in the lowest and highest deciles for C-HDL may also mature to become adults respectively at increased and reduced CHD risk. Although the degree of tracking at either extreme of the distribution and for the group as a whole appears to have cohesiveness and may be useful for prediction of future lipid and lipoprotein levels, individual children at the extremes of the distributions will need to be followed over longer periods of time, to identify the continuity of childhood and adult values, and eventual CHD risk.

Key Words and Abbreviations

Tracking
Cholesterol
Triglyceride
High Density Lipoprotein Cholesterol (C-HDL)
Low Density Lipoprotein Cholesterol (C-LDL)
Coronary Heart Disease (CHD)
Lipid Research Clinics (LRC)

SPECULATION

Children who tend to track in the lowest and highest deciles for cholesterol and C-LDL may mature to become adults respectively at reduced and increased coronary heart disease (CHD) risk. Children who tend to track in the lowest and highest deciles for C-HDL may also mature to become adults respectively at increased and reduced CHD risk.

INTRODUCTION

In both population (1) and family studies (5) low and high density lipoprotein cholesterol levels (C-LDL, C-HDL) respectively are positively and inversely associated with risk for coronary heart disease (CHD). C-HDL's relationship with CHD appears to be independent of other lipoproteins (1). If children with low levels of C-LDL and/or low levels of C-HDL were to persist at these lower levels over time and into adulthood, then quantitation of lipoproteins in children might permit the early identification of persons at diminished risk to future CHD (low C-LDL) (1,5,15) or relatively increased risk to future CHD (low C-HDL) (1). The phenomenon of individuals maintaining their rank position within lipid and lipoprotein distributions is referred to as tracking (2).

The current study was designed to assess tracking of plasma cholesterol, triglyceride, C-HDL and C-LDL in children initially in the lowest and highest deciles for these variables, with follow-up over a four-year period in the Cincinnati Lipid Research Clinic's (LRC) Princeton School Study (11,13,14).

MATERIALS AND METHODS

Subjects

Following the collaborative NHLBI-LRC protocol (11,13,14) fasting plasma cholesterol and triglyceride were measured at a first visit, (Visit 1), in 7,297 school children, ages 6-17 years. Of these children, 1,689 were studied a second time about six weeks later with measurements of lipids and lipoproteins (Visit 2) (11,13,14). These 1,689 children included those with elevated cholesterol (≥ 205 mg/dl), and elevated triglyceride (≥ 150 mg/dl), as well as fifteen percent random subset of all children seen at Visit 1 (11,14).

Following Visits 1 and 2, additional follow-up studies were carried out in 1976 (Visit 3), with repeat sampling in 1977 (Visit 4) and 1978 (Visit 5). In the first year of follow-up (1976, Visit 3), students in grades 5 through 12 randomly drawn from the prevalence population were restudied; 529 participated. Fasting plasma cholesterol and triglyceride but not lipoproteins were measured at Visit 3 in 1976. In 1977 (Visit 4), and in 1978 (Visit 5) fasting plasma cholesterol, triglyceride, C-HDL, and C-LDL levels were measured in 203 and 141 students respectively.

Blood Sampling Techniques, Lipid and Lipoprotein Quantitation.

Blood was taken after a 12-14 hour fast, the plasma separated in a refrigerated centrifuge, and levels of cholesterol, C-HDL, C-LDL, and triglycerides measured following the methods of the Lipid Research Clinic's Laboratory Methods Manual (7). Using the Technicon AutoAnalyzer II, the Lipid Research Clinic's manual involves a Liebermann-Burchard method for cholesterol, a

modified fluorimetric method for triglycerides, and a heparin-Mn⁺⁺ precipitation for C-HDL (with a final Mn⁺⁺ concentration of 0.046 M). Interday coefficients of variation for determination of plasma cholesterol levels in plasma pools containing 158 and 281 mg/dl were 2.0% and 1.2%. Interday coefficients of variation for determination of plasma triglyceride levels in plasma pools containing 66 and 153 mg/dl were 2.3% and 1.7%. Interday coefficients of variation for determination of plasma C-HDL levels in plasma pools containing 50 and 44 mg/dl were 3.7% and 4.2% respectively. The interday coefficient of variation of plasma C-LDL levels in a plasma pool containing 129 mg/dl was 3.5%.

Statistical Analysis

Complete data was available for 108 children having blood samples taken at Visits 1 and 2 in 1974-1975 and in each of the three follow-up study years, 1976, 1977, and 1978. The children were ranked by each separate lipid and lipoprotein measurement into deciles. In the event of ties, all children in the tied group were assigned the same rank.

Those children initially in the lowest and highest deciles of the frequency distributions for cholesterol, triglyceride, C-HDL, or C-LDL when first measured (Visits 1 and 2), were then followed at later visits (Visits 2-5), to determine where in the lipid and lipoprotein distributions they fell (Tables 1 and 2). This report primarily focuses upon tracking of lipids and lipoproteins in children initially in the lowest decile (Tables 1, 2, Figure 1), while a detailed and separate report (9) assessed children initially in the highest decile. All of the material relative to the lowest decile children (and much of that on the top decile) is entirely original to this report.

The ranks of those individuals (initially in the lowest decile) were compared to the ranks of the remainder of individuals. A Van der Waerden scores test (10) was performed to determine whether the difference between the two groups (lowest decile group, [low group]) and other deciles (other group) at Visit 1, for cholesterol and triglyceride, or Visit 2, for C-HDL and C-LDL, remained significant for these specific children at subsequent visits, Table 3. For comparison of tracking in the lowest and highest deciles for lipids and lipoproteins, data are also presented for children initially in the highest deciles of the lipid-lipoprotein distributions, and their subsequent positions in the distribution at Visit 5 (Tables 1,2).

Frequency distributions for the "low group" at Visits 1 and 2 were obtained for subsequent visits (Figure 1). As a graphic means of showing the degree of tracking for children initially in the lowest decile for each lipid and lipoprotein, the decile location over the four-year time span of follow-up for these specific children is presented (Figure 1). This allows determination of the percentage of these children in the bottom decile at their first visit who remain in the bottom decile or were in other deciles at each subsequent visit.

The expected effect, or increase in the mean value for those initially in the lowest decile, due to regression toward the mean at later visits, was calculated using the method of Davis (3), and compared to the observed mean of the group in the lowest decile, Table 4. The only assumption needed to use the method described by Davis (3) is normality, which is supported below.

Plasma cholesterol, C-HDL and C-LDL distributions were tested for normality and were found to be normal by the Kolmogorov-Smirnov test (7). Plasma triglyceride values were normally distributed after a natural log transformation.

RESULTS

Rank Order Correlations: Initial and Subsequent Measurements of Lipids and Lipoproteins.

For all 108 children, the relationship between initial and subsequent measurements of lipids and lipoproteins, using Spearman rank order (16) correlations, were highly significant (9).

Plasma cholesterol at Visit 1 was significantly correlated with cholesterol at Visits 2, 3, 4, and 5, $p < .001$, with the correlation coefficients for visits 1, 2, and 3 years apart (Visit 1 to Visits 3, 4, and 5) being similar, $r = 0.65, 0.66$, and 0.68 . For plasma triglycerides, correlation coefficients for visits 1, 2, and 3 years apart were $r = 0.47, 0.37$, and 0.39 , $p < .001$. C-HDL at Visit 2 was closely associated with C-HDL at Visits 4 and 5, $r = 0.60, 0.53$ ($p < .001$); correlations between Visit 2 C-LDL and C-HDL at Visits 4 and 5 were $r = 0.67, 0.61$, $p < .001$.

Longitudinal Tracking of Lipids and Lipoproteins for those Children Initially in the Lowest Decile for Cholesterol, Triglyceride, C-HDL and C-LDL.

Frequency distributions (at first sampling over time), for those children initially in the bottom decile for cholesterol, triglyceride, C-HDL, and C-LDL are summarized in Figure 1. The ten members of the bottom decile group of children at Visit 1 for cholesterol became dispersed among higher deciles at later visits. The mode of the distribution, however, remained in the bottom decile at later visits. The highest decile attained by a member of the initial "low group" was the 5th at Visits 2 and 4, the 4th at Visit 3, and the 6th at Visit 5 (Figure 1).

Triglyceride values for children initially in the bottom decile were more dispersed at later visits than were cholesterol values at later visits (Figure 1). However, the mode of the frequency distributions for triglyceride remained in the bottom decile at later visits. Children initially in the bottom decile at Visit 1 attained values as high as the 5th decile at Visit 2, the 6th decile at Visits 3 and 4, and the 9th decile at Visit 5.

The mode of the frequency distribution for C-HDL remained in the lowest decile at Visit 4, and rose to the second decile at Visit 5. The highest decile attained by a member of the "low group" for C-HDL was the 7th at Visit 4 and the 6th at Visit 5 (Figure 1). Dispersion was greater for C-HDL than for C-LDL. The mode of the frequency distributions for C-LDL always occurred in the bottom decile. All individuals remained in the bottom two deciles for C-LDL throughout all visits except two children, at Visit 5, who rose to the 5th and 6th decile (Figure 1).

Longitudinal Tracking of Lipids and Lipoproteins for those Children Initially in the Highest Decile for Cholesterol, Triglyceride, C-HDL, and C-LDL.

As summarized by Table 1, at Visit 5, six of the thirteen (46%) children initially having plasma cholesterol in the top decile remained in the top decile; 3 of 11 (27%) children initially having plasma triglyceride and C-LDL in the top decile remained there. At Visit 5, five of the eleven (45%) children initially having C-HDL levels in the top decile remained in the top decile.

As shown in Table 1, after four years of follow-up, for triglyceride, only about one third of children remained in their initial decile groups, either low or high. For C-LDL, 56% of those children initially in the lowest decile group remained there, but only 27% of the children initially in the highest decile group for C-LDL remained there. Conversely, while only 13% of children initially in the lowest decile for C-HDL remained there, 45% of those in the top decile remained there after four years of follow-up, Table 1. It should be noted, however, that 55% to 91% of children initially in the lowest and highest deciles for lipids and lipoproteins remained in the lowest and highest three deciles respectively after four years, indicating relatively persistent rank position over time, Table 2.

Changes in Mean Levels of Plasma Cholesterol, Triglyceride, C-HDL, and C-LDL for those Children in the Bottom Decile at the Initial Visits and with follow-up Over Time.

The degree of tracking that occurred in the bottom decile of the distribution for lipid and lipoprotein measurements (as summarized in Figure 1) was also assessed by collating the mean values of the variables found in the children in the lowest decile at Visit 1 for cholesterol and triglyceride, and at Visit 2 for C-HDL and C-LDL, and examining the mean levels of lipids and lipoproteins from these specific children at subsequent Visits 2, 3, 4, and 5 for cholesterol and triglyceride, and 4 and 5 for C-HDL and C-LDL (Table 3). The difference in mean cholesterol levels between those children initially in the bottom decile at Visit 1 and all others was 44.8 mg/dl at Visit 1, 34.5 at Visit 2, 36.2 at Visit 3, 35.6 at Visit 4, and 35.1 at Visit 5. Comparison of the ranks of the two groups for their cholesterol levels showed a highly significant difference ($p < .0001$) at all visits.

Similarly, the ranks of the two groups for triglyceride levels were also significantly different at all visits, with p values ranging from .0001 to .006, Table 3. The difference in mean triglyceride levels was 35.2 at Visit 1, 27.2 at Visit 2, 27.5 at Visit 3, 29.5 at Visit 4, and 20.0 at Visit 5 (Table 3).

The difference in mean C-HDL levels of the two groups fell from 21.0 at Visit 2 to 13.8 at Visit 4 and to 11.5 at Visit 5. The ranks of the individuals initially in the bottom decile for C-HDL values remained significantly lower (p at least $\leq .003$) than those in the other children at subsequent visits.

The difference in mean C-LDL levels of the two groups (lowest decile and other) fell from 44.0 at Visit 2 to 38.8 at Visit 4, and to 32.7 at Visit 5, following the same decreasing trend as the differences did for C-HDL levels, Table 3. Those individuals ranked in the bottom decile at Visit 2 for C-LDL values retained significantly lower values at Visits 4 and 5 than those ranked in the "other" groups ($p < .0001$), Table 3.

Regression Towards the Mean

The expected means for the low decile group for cholesterol, triglyceride, C-HDL, and C-LDL levels at all visits were calculated (3) and compared to the observed means, to determine the proportion of the increase in the low group means at later visits attributable to the phenomenon of regression towards the mean, Table 4. The observed increase in mean cholesterol in the "low group" from Visit 1 to Visit 2, 10.8 mg/dl, was more than was expected, 6.0 mg/dl. The observed increases in mean cholesterol in the "low" group from Visit 1 to Visits 3-5, were 5.8, 3.0, and 1.5 mg/dl respectively, (Table 4). These increases were all less than those expected by regression towards the mean. None of the above comparisons (observed vs. expected) were significant, however, $p > .1$.

The observed mean triglyceride levels for the "low" group increased less than expected except at Visit 5, where the observed increase in the mean level was greater than that which was attributable to regression towards the mean. There was no significant difference, however, $p > .1$, between the expected and observed means for triglyceride at each visit.

Mean C-HDL levels increased about as much as expected at Visits 4 and 5, Table 4, but increments in mean C-LDL were less than expected. Thus, the mean C-LDL level for the "low" group was 4.7 mg/dl lower at Visit 4 than at Visit 2. The mean level did rise 1.4 mg/dl from Visit 2 to Visit 5. The observed means did not increase as one would expect due to regression towards the mean. The difference between the observed and expected mean levels at Visit 4 was significant ($p < .05$), Table 4.

DISCUSSION

In a fashion similar to that observed by Clarke et al, (2) but at the lowest decile of the lipid-lipoprotein distribution, a majority (60%) of children initially in the bottom decile for plasma cholesterol remained in that decile over a four-year period. Their mean plasma cholesterol levels also remained lower than those of children initially in the upper nine deciles during the four-year follow-up. In a similar fashion, the mode of the frequency distributions for triglyceride remained in the bottom decile at later visits, and the ranks for the mean triglyceride levels also remained lower than those of children initially in the upper nine deciles during the four-year follow-up.

Although there was considerably more dispersion for the lowest decile of C-HDL than for C-LDL, the ranks of the individuals initially in the bottom decile for C-HDL values remained significantly lower than those in the other children at subsequent visits. Perhaps the greatest degree of tracking was observed for C-LDL, in children initially in the lowest decile. The mode of frequency distributions for C-LDL persisted in the bottom decile, and those individuals ranked in the bottom decile at Visit 2 for C-LDL values retained significantly lower values at Visits 4 and 5 than those ranked in the other groups ($p < .0001$). Moreover, mean C-LDL levels for children initially in the lowest decile failed to increase to the degree expected by regression towards the mean, a further exemplar of "tracking" at low C-LDL levels.

A considerable portion of the increase in group mean levels for both cholesterol, triglyceride, and C-HDL could be accounted for by the phenomenon of regression towards the mean, (3) since the more "extreme" values were "less extreme" (i.e. above the lowest decile) on repetitive sampling.

In comparison of the dispersion from the lowest decile and that from the highest decile for lipids and lipoproteins, there were some appreciable differences. Whereas the greatest degree of tracking was observed for C-LDL in children initially in the lowest decile for C-LDL, C-LDL in children initially in the highest decile was considerably dispersed four years later, with only 27% of children retaining C-LDL levels in the highest decile. Conversely, whereas the greatest degree of tracking was observed for C-HDL in children initially in the highest decile for C-HDL, C-HDL was very markedly dispersed in children initially in the lowest decile, with only 13% of children retaining C-HDL levels in the lowest decile after four years of follow-up. Despite evidence of considerable dispersion from both the lowest and highest deciles of the distribution, a majority of children having lipid-lipoprotein levels initially in the lowest and highest deciles remained in the lowest 30% and highest 30% of the distribution after four years, indicating relatively persistent rank position over time (Table 2). Moreover, the close correlation of initial and subsequent total plasma cholesterol levels ($r = 0.68$), indicates a substantive tracking of cholesterol for the entire group of children (9).

Prospective studies in adults indicated that even single determinations of plasma total cholesterol or C-LDL are related to the risk of developing CHD (1,6,8). Such findings raise the speculative possibility that children who tend to track in the bottom and top deciles for total cholesterol and C-LDL, may mature to become adults respectively at reduced or increased CHD risk (1,2,4,5,15). Although much less marked than for C-LDL, the relative tracking stability of children in the bottom decile for C-HDL might provide a cohort at increased CHD risk, by virtue of low C-HDL as adults (1). Conversely, the relatively high degree of tracking for C-HDL in children ini-

tially having top decile C-HDL levels might provide a cohort at reduced CHD risk, by virtue of high C-HDL as adults (1).

The concept that identifiable CHD risk factors in childhood track into adulthood is supported by the finding of Schrott et al (15), of an increased propensity for development of CHD in families of children with persistently elevated plasma total cholesterol levels. Ellefson et al (4) and Voors et al (17) from the Rochester, Minnesota, and Bogalusa, Louisiana, SCOR studies have also provided detailed data on identifiable risk factors for CHD in childhood and the importance of their early recognition and longitudinal evaluation.

CONCLUSION

Prospective studies in adults reveal that CHD risk is continuously related to total and LDL cholesterol levels (positively) and to HDL cholesterol levels (inversely) throughout their entire distribution. Such findings raise the possibility that children who tend to track in the lowest and highest decile for total and LDL cholesterol may mature to become adults respectively at reduced and increased CHD risk. Children who tend to track in the lowest and highest deciles for HDL cholesterol may also mature to become adults respectively at increased and reduced CHD risk. Although the degree of tracking at either extreme of the distribution, and for the group as a whole, appears to have adequate cohesiveness to be useful for prediction of future lipid and lipoprotein levels, individual children at the extremes of the distributions will need to be followed over longer periods of time, to identify the continuity of childhood and adult values, and eventual CHD risk.

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Table 1

Percentage of Children, Initially Having Plasma Lipids and Lipoproteins Either in the Lowest or Highest Deciles, Remaining There after Four Years of Follow-up.

Percent of Children Remaining in Initial Decile Group

Initial Decile Group	Cholesterol	Triglyceride	C-LDL	C-HDL
Lowest	60%	33%	56%	13%
Highest	46%	27%	27%	45%

Table 2

Percentage of Children, Initially Having Plasma Lipids and Lipoproteins Either in the Lowest or Highest Deciles, Located in the Lowest or Highest Three Deciles (Respectively) After Four Years of Follow-up

Percent of Children Initially in the Lowest and Highest Deciles Remaining in the Lowest or Highest Three Deciles Respectively

Initial Decile Group	Cholesterol	Triglyceride	C-HDL	C-LDL
Lowest	70%	67%	63%	78%
Highest	77%	55%	73%	91%

Table 3

Mean ($\pm$ SD) Plasma Cholesterol, Triglyceride, C-HDL, and C-LDL (mg/dl), Children in The Lowest Decile (low group) and those in the Other 9 Deciles (others) at Visit 1 for Cholesterol and Triglyceride, and At Visit 2 for C-HDL and C-LDL. Comparison of Mean Levels of Lipids and Lipoproteins from These Specific Children at Subsequent Visits.

Visit Group	Cholesterol	p	Triglyceride	p	C-HDL	p	C-LDL	p
Other	168.6 $\pm$ 22.8		65.0 $\pm$ 27.8					
Lowest	123.8 $\pm$ 12.3 <.0001		29.8 $\pm$ 6.0 <.0001					
Difference (Δ) in Means	44.8		35.2					
Other	168.9 $\pm$ 23.1		65.6 $\pm$ 28.8		56.3 $\pm$ 10.4		101.2 $\pm$ 19.3	
Lowest	134.6 $\pm$ 18.6 <.0001		38.4 $\pm$ 18.7 <.0002		35.3 $\pm$ 3.8 <.0001		57.2 $\pm$ 13.6 <.0001	
Difference (Δ) in Means	34.5		27.2		21.0		44.0	
Other	165.8 $\pm$ 21.1		67.3 $\pm$ 31.9					
Lowest	129.6 $\pm$ 17.2 <.0001		39.8 $\pm$ 18.4 <.0002					
Difference (Δ) in Means	36.2		27.5					
Other	162.4 $\pm$ 23.8		69.4 $\pm$ 32.2		57.6 $\pm$ 11.1		91.3 $\pm$ 20.1	
Lowest	126.8 $\pm$ 21.4 <.0001		39.9 $\pm$ 26.3 <.0006		43.8 $\pm$ 10.6 <.0006		52.5 $\pm$ 21.2 <.0001	
Difference (Δ) in Means	35.6		29.5		13.8		38.8	
Other	160.3 $\pm$ 22.6		67.4 $\pm$ 31.2		55.1 $\pm$ 11.2		91.3 $\pm$ 18.5	
Lowest	125.3 $\pm$ 22.7 <.0001		47.4 $\pm$ 27.7 <.0006		43.6 $\pm$ 7.7 <.003		58.6 $\pm$ 24.5 <.0001	
Difference (Δ) in Means	35.1		20.0		11.5		32.7	

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Table 4

Expected and Observed Mean Levels of Cholesterol, Triglyceride C-HDL, and C-LDL (mg/dl), for Children in the Lowest Decile Group at Visit 1 for Cholesterol and Triglyceride, and at Visit 2 for C-HDL and C-LDL. Comparison of Expected (by calculation) and Observed Values for these Specific Children at the Initial and Subsequent Visits.

		CHOLESTEROL		TRIGLYCERIDE		C-HDL		C-LDL	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Visit 1		123.2	123.8	29.1	29.8				
Visit 2		129.2	134.6	39.3	38.4	33.7	35.3	60.4	57.2
Difference	V2-V1	6.0	10.8	10.2	8.6				
Visit 3		135.9	129.6	42.5	39.8				
Difference	V3-V1	12.7	5.8	13.4	10.0				
Visit 4		135.8	126.8	43.4	39.9	41.5	43.8	70.8	52.5
Difference	V4-V1	12.6	3.0	14.3	10.1	7.8	8.5	10.4	-4.7
Visit 5		133.6	125.3	43.8	47.4	43.1	43.6	72.0	58.6
Difference	V5-V1	10.4	1.5	14.7	17.6	9.4	8.3	11.6	1.4
Difference	V5-V2

Frequency Distributions (At First Sampling and Over Time), For

Those Children Initially in the Bottom Decile for Cholesterol,

Triglyceride, C-HDL, and C-LDL.

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