

# Lipid composition of the vascular system during infancy, childhood, and young adulthood

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**ABSTRACT** The object of this study was to determine the changes in lipid composition that occur in blood vessels from infancy to young adulthood. Analyses included levels of total cholesterol, total triglyceride, phospholipid, and cholesteryl ester fatty acids, and the distribution of saturated and unsaturated fatty acids.

Triglyceride, total and monoenoic fatty acids, and linoleic acid were lower in the ascending, thoracic, and abdominal aorta than in the pulmonary artery and inferior vena cava. Phospholipids and arachidonic acid were higher in aortic segments than in the other two vessels.

Aortic lipids showed significant changes with increasing age: total cholesterol and total fatty acids decreased from <1 wk to 5 yr, then increased to 22 yr of age. Triglycerides decreased whereas cholesteryl esters increased from 10 to 22 yr of age. Saturated fatty acids decreased from 1 wk to 10 yr, then remained relatively constant. Linoleic acid (3.7–9.8% of total fatty acids) and arachidonic acid (15.8–21.7%) both increased with age; the increase in cholesteryl linoleate was highly significant. After 10 yr of age, total cholesterol and total fatty acids were significantly higher in abdominal than in ascending and thoracic segments of aorta.

**KEY WORDS** infants · children · adolescents · coronary vessels · aorta · lipid composition · fatty acids · cholesteryl esters

**T**HERE IS CONSIDERABLE EVIDENCE from gross and histologic examination of heart vessels that fatty streaks and early atheromatous lesions occur in coronary arteries and in aortas of infants and children (1–6). Since the publication of the early histologic reports describing various degrees of sudanophilic deposits in aortas of young patients, physicians have been concerned about the amount and kind of fat deposited in aortic tissue.

Most lipid analyses have been on adult subjects and

scant attention has been given to characterization of fatty acids present in aorta during the growth of the cardiovascular system. If one accepts the hypothesis that fatty streaks seen in aortas of children may be the beginning of atherosclerotic lesions that become evident clinically later in life, it seems pertinent to know the sequence of changes that takes place in the histologic appearance and chemical composition of heart vessels from infancy to young adulthood. Hence, a study was designed to establish possible relationships between histologic and lipid changes during the growth and development of the cardiovascular system.

This report presents data obtained from analyses of five vessel segments for major lipid components, including distribution of saturated and unsaturated fatty acids, during the first 10 yr of life, and of aortic segments from patients <1 to 22 yr of age. Histologic features of these same vessels will be presented in a subsequent report.

## MATERIALS AND METHODS

### *Autopsy Material*

Vessel segments were obtained from infants, children, and young adults without selection according to cause of death. The time interval between death and autopsy was <4 hr in 11.5%, 4–24 hr in 70%, and 24–52 hr in 18.5% of the patients.

Each vessel segment was washed in saline to remove adhering blood and freed from all removable adventitial tissue. No adventitial tissue was histologically evident on the segments. In the first phase of the work, five vessel segments were taken from patients <1 to 10 yr of age. The segment from just above the aortic valve to the ductus arteriosus or ligamentum arteriosum was taken

as the ascending aorta, that from the ductus to just above the celiac axis as the thoracic aorta, and the remainder to the bifurcation as the abdominal aorta. For comparison with segments of aorta, the pulmonary artery and inferior vena cava were examined in about 75% of these cases. A sample was taken from the posterior wall of each vessel segment for histologic examination and placed in an aqueous solution containing 10% formalin and 1% calcium acetate. The remainder of the specimen was placed in a tightly stoppered vial, frozen immediately, and stored at  $-10^{\circ}\text{C}$  until analyzed for lipid components. For 89% of the segments analyses were completed within 1–9 months after autopsy and the remaining 11% were stored (frozen) for 9–12 months. The mean storage time for all specimens was 6 months.

In the second phase of the work, lipid analyses were confined to the ascending, thoracic, and abdominal segments of aorta from patients <1 to 22 yr of age.

#### *Methods of Analysis*

Extraction of total lipids and all subsequent analyses were carried out on individual vessel segments and not on pools. All analyses were performed on the full thickness of each vessel segment. Intima and media of aortas were not separated. Each specimen was cut into small pieces, weighed, and ground in a porcelain mortar with washed, ignited sea sand.

Total lipids were extracted from the ground specimen with a hot 3:1 mixture of redistilled, aldehyde-free ethanol and peroxide-free diethyl ether. Aliquots of the extract were taken for determination of total fatty acids (7) and for silicic acid chromatography (8). The extract used for silicic acid chromatography was evaporated to dryness under reduced pressure in a stream of nitrogen. 2 ml of water was added to the residue and the total lipids were redissolved by repeated extraction with redistilled petroleum ether.

A 2 ml concentrate containing 1.5–5 mg of total lipid in petroleum ether (bp  $20-40^{\circ}\text{C}$ ) was added to the silicic acid column, which was prepared and packed with 1 g of silicic acid<sup>1</sup> according to Lis, Tinoco, and Okey (8). Four individual fractions were collected in 15 ml portions of the following four eluents: fraction I, 1% diethyl ether in petroleum ether (cholesteryl esters); II, 4% diethyl ether in petroleum ether (triglycerides); III, 50% diethyl ether in petroleum ether (free cholesterol, free fatty acids, mono- and diglycerides); IV, absolute methanol (phospholipids). Recovery of fatty acids from these four fractions was 95–98% in preliminary studies on mixed standards of tripalmitin, trilinolein, stearic acid (1% mono- and diglycerides),

free cholesterol, and lecithin. Recovery of cholesterol as measured by the Liebermann-Burchard reagent (9) was 97–99%. In extracts of vessel segments, cholesterol was found only in the unsaponifiable portion of fractions I and III and agreed with the amount of total cholesterol determined in the original extract and of cholesteryl esters determined after precipitation with digitonin.

The original total lipid extract and the four silicic acid fractions were saponified with alcoholic KOH on a water bath for 30 min, during which time the soaps were evaporated nearly to dryness. Details of the precipitation of fatty acid, removal of the unsaponifiable fraction, and drying and weighing of the fatty acids have been described previously (7). Oxidation of unsaturated fatty acids was prevented by addition of hydroquinone during saponification and by evaporation of all petroleum ether extracts in a stream of nitrogen.

The efficiency of extraction of the total lipids in petroleum ether was determined by the total fatty acid content of the original alcohol-ether extract and the amount of fatty acid found after saponification of the silicic acid fractions. For 123 separate vessel segments, mean recovery of fatty acids was 97.8%.

Fatty acids were methylated in 2% sulfuric acid in redistilled methanol under reflux for 1.5 hr at  $70^{\circ}\text{C}$ . The mixtures were cooled and the esters were washed with water and extracted with petroleum ether. The methyl esters in hexane were chromatographed in a Beckman GC-2A instrument on 183-cm columns containing Chromosorb W coated with 15% diethylene glycol succinate polyester<sup>2</sup> at  $220^{\circ}\text{C}$ . Helium was used as the carrier gas. Peaks were identified by comparison with retention times of known methyl esters.<sup>2</sup> Peak areas were measured by triangulation. Correction factors were applied to compensate for differences in detector response to individual fatty acid esters. Detector response was linear within a given instrument sensitivity setting; injected sample sizes were chosen to allow use of a single sensitivity setting. Results obtained with National Heart Institute fatty acid standard F (10) agreed exactly with those of the stated composition data for fatty acids composing <10% of the mixture and with a relative error of less than 1.0% for fatty acids composing >10% of the mixture.

#### *Statistical Comparisons*

Within each group, i.e. vessel, lipid fraction, age, or diagnosis, equality of variance for cholesterol and fatty acids (mg/100 mg dry weight) and for distribution of saturated and unsaturated fatty acids (per cent of fatty acid fraction) was determined by Bartlett's test (11). Equality of means within each of the same groups was

<sup>1</sup> The silicic acid used was specially prepared for the chromatography of lipids by Bio-Rad Laboratories, Richmond, Calif.

<sup>2</sup> Applied Science Laboratories Inc., State College, Pa.

determined by Student's "t" test (11). Although some of the data do not appear to have a normal distribution, the fact that the variances are equal justifies the use of the *t* test, especially since the sample sizes are not too small (12).

## RESULTS

### *Lipid Components in Five Vessel Segments from Patients <1 to 10 Yr of Age*

Total cholesterol, total fatty acids, and distribution of the saturated and unsaturated fatty acids were determined in each of 230 vessel segments from 60 patients. In 124 of the segments, fatty acids were also determined in the triglyceride, phospholipid, and cholesteryl ester fractions. A summary of these data is presented in Fig. 1.

The five vessel segments from infants and children 1 day to 10 yr of age were low in total cholesterol and total fatty acids, with means of 0.46–0.68% and 1.59–3.17% of dry weight respectively. There was considerable variation in total lipid content among the vessel segments, but total cholesterol was significantly higher in the inferior vena cava than in pulmonary artery or in any segment of aorta ( $P < 0.05$ ). Both pulmonary artery and inferior vena cava gave significantly higher values than the three segments of aorta for total, monoenoic, and linoleic acids ( $P < 0.05$ ), but were significantly lower than aorta in arachidonic acid ( $P < 0.05$ ). Palmitic acid

was the predominant saturated fatty acid and oleic acid the predominant monoenoic fatty acid in all segments. The relatively low level of linoleic acid (5% of total fatty acids), and the relatively high level of arachidonic acid (20% of total fatty acids) in aortas from these young patients is the reverse of the values reported in serum of children (13).

Triglyceride fatty acids comprised the largest fraction of total fatty acids in the pulmonary artery and inferior vena cava (58–68%) and were significantly higher than in segments of aorta (29–43%). Phospholipid fatty acids were significantly higher in segments of aorta (33–43% of total fatty acids) than in pulmonary artery and inferior vena cava (12–26% of total fatty acids). The fraction composed of free fatty acids, monoglycerides, and diglycerides was more abundant than the cholesteryl esters in all vessels, particularly in the aortic segments. The thoracic aorta contained significantly more of this fraction than did the pulmonary artery and inferior vena cava ( $P < 0.05$ ).

### *Lipid Components in Three Segments of Aorta with Respect to Age*

According to an analysis of variance test for distribution of fatty acids in aortic segments from young children, linoleic acid increased significantly from 1 month to 10 yr of age. Hence, in the second phase of the study, lipid

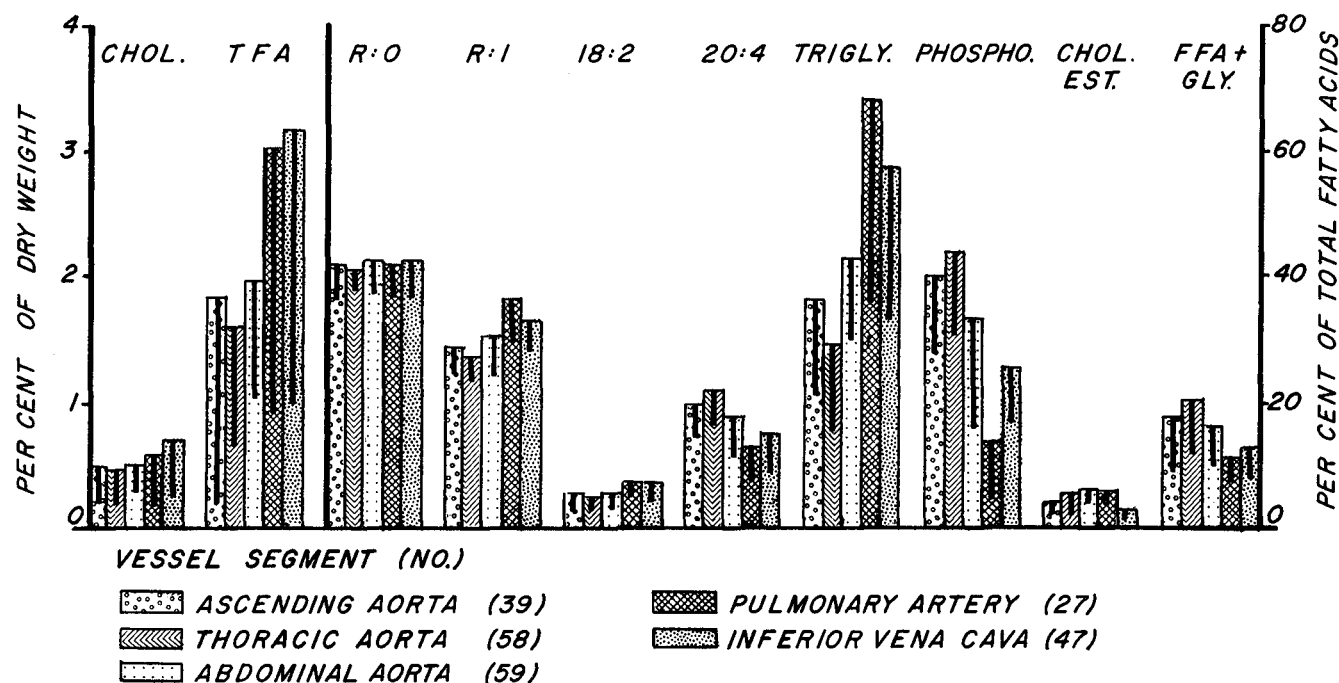


Fig. 1. Major lipid components in segments of aorta, pulmonary artery, and inferior vena cava. Mean values are indicated by height of each bar; one sd is indicated by the vertical line in each bar. TRIGLY., PHOSPHO., CHOL. EST., and FFA + GLY. represent fatty acids in triglycerides, phospholipids, cholesteryl esters, and the fraction containing free fatty acids and partial glycerides, respectively, expressed as percentages of the total fatty acids derived from the tissue. CHOL., cholesterol; TFA, total fatty acids. Fatty acids are designated by number of carbon atoms : number of double bonds.

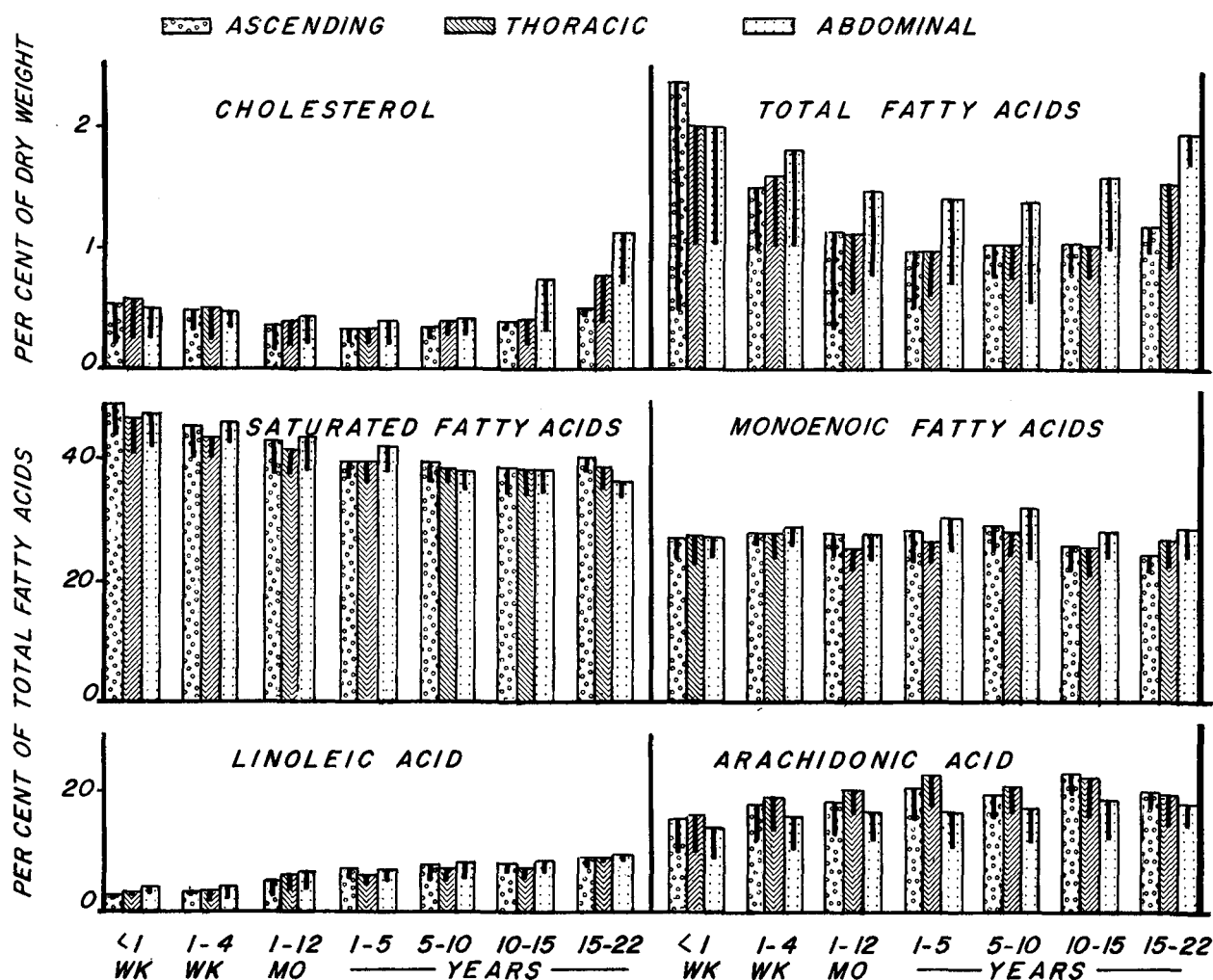


Fig. 2. Lipid components in three segments of aorta in relation to age. Mean values with one SD are represented by bars and vertical lines, respectively.

components were determined in the ascending, thoracic, and abdominal segments of aorta from patients <1 to 22 yr of age. The data for 587 aortic segments from 215 patients were examined with respect to the following age groups: <1 wk, 1–4 wk, 1–12 months, and 1–5, 5–10, 10–15, and 15–22 yr. Fig. 2 gives the mean value with one SD for total cholesterol, total, saturated, and monoenoic fatty acids, and linoleic and arachidonic acids in the three segments of aorta according to these age groups.

Total cholesterol decreased from <1 wk to 5 yr of age in each aortic segment ( $P < 0.01$ ) but increased from 5 to 22 yr ( $P < 0.01$ ). After 10 yr of age, total cholesterol was higher in the abdominal segment than in the ascending and thoracic segments ( $P < 0.05$ ).

Total fatty acids decreased from <1 wk to 5 yr of age in the ascending and thoracic segments ( $P < 0.01$ ), then increased from 5 to 22 yr of age ( $P < 0.01$ ). In the abdominal segment, total fatty acids decreased from <1 wk to 10 yr of age ( $P < 0.01$ ), then increased from 10 to

22 yr ( $P < 0.01$ ). After 5 yr of age, total fatty acids in the abdominal segment were significantly higher than for the ascending and thoracic segments ( $P < 0.05$ ).

Saturated fatty acids decreased from <1 wk to 10 yr of age in the aortic segments ( $P < 0.01$ ). The abdominal segment showed the greatest decrease up to 22 yr of age. There was no significant trend in the level of monoenoic fatty acids from infancy to 22 yr of age. Linoleic acid increased from <1 to 10 yr in the ascending and thoracic segments and from <1 wk to 22 yr in the abdominal segment ( $P < 0.01$ ). Arachidonic acid increased from <1 wk to 5 yr of age in the ascending and thoracic aorta and from <1 to 15 yr in the abdominal aorta ( $P < 0.01$ ). The arachidonic acid level remained considerably higher than the linoleic acid level in the three aortic segments in all age groups.

Trends with age for major lipid components in aorta are clearly demonstrated in Fig. 3 by the mean values for the three segments of aorta.



### Aortic Lipids and Sex of Children

Total cholesterol and total saturated, monoenoic, linoleic, and arachidonic acids in aortic segments showed no consistent differences between male and female children in any age group.

### Fatty Acids of Aortic Lipid Fractions

Lipid extracts of individual segments of ascending, thoracic, and abdominal aorta were separated into four fractions by silicic acid chromatography. Since the amount of material available for these separations in each aortic segment was relatively small in some age groups, the following data represent averaged values from individual analyses of the three aortic segments from 95 patients. Fig. 4 illustrates the relative amount of each lipid fraction with respect to five age groups.

Expressed as a percentage of the total fatty acids, the fatty acids derived from the triglyceride and cholesteryl ester fractions varied widely, but in spite of this mean values for the triglyceride fatty acids showed a progressive increase from <1 to 10 yr of age. This increase was not significant, but the progressive decrease in triglycerides from 10 to 22 yr was ( $P < 0.01$ ). Phospholipids did not change significantly from <1 to 22 yr. The progressive decrease in cholesteryl ester fatty acids from <1 to 10 yr of age was not significant but the increase from 10 to 22 yr was ( $P < 0.01$ ). Except in the

15-22 yr age group the free fatty acids, monoglycerides, and diglycerides exceeded the cholesteryl esters in amount. However, the former fraction decreased significantly from 10 to 22 yr ( $P < 0.01$ ) whereas the latter fraction increased significantly during this period.

Fig. 5 shows the distribution of saturated and unsaturated fatty acids in each fraction with respect to age. The triglycerides consisted mostly of saturated and monoenoic fatty acids, predominantly palmitic and oleic acids. There were no significant trends with age for any of the fatty acids in this fraction.

Phospholipids contained a high percentage of saturated fatty acids (45-50%), which generally consisted of approximately equal amounts of palmitic and stearic acids. There was no trend with age. Monoenoic fatty acids (mainly oleic) were less abundant than the saturated fatty acids and their percentage decreased slightly after 10 yr of age. Linoleic acid levels increased from 3.5 to 5.5% from <1 to 10 yr, and then remained stable to 22 yr of age. Arachidonic acid levels varied considerably within each group, with mean values of 18.3-21.3%. Except for the 10-15 yr group, mean values indicated a slight trend for decreasing levels from 1 to 22 yr of age.

There was insufficient material for determination of fatty acid distributions in the cholesteryl ester fractions from infants <1 yr of age. However, saturated fatty acids decreased significantly from 10 to 22 yr ( $P < 0.001$ ).

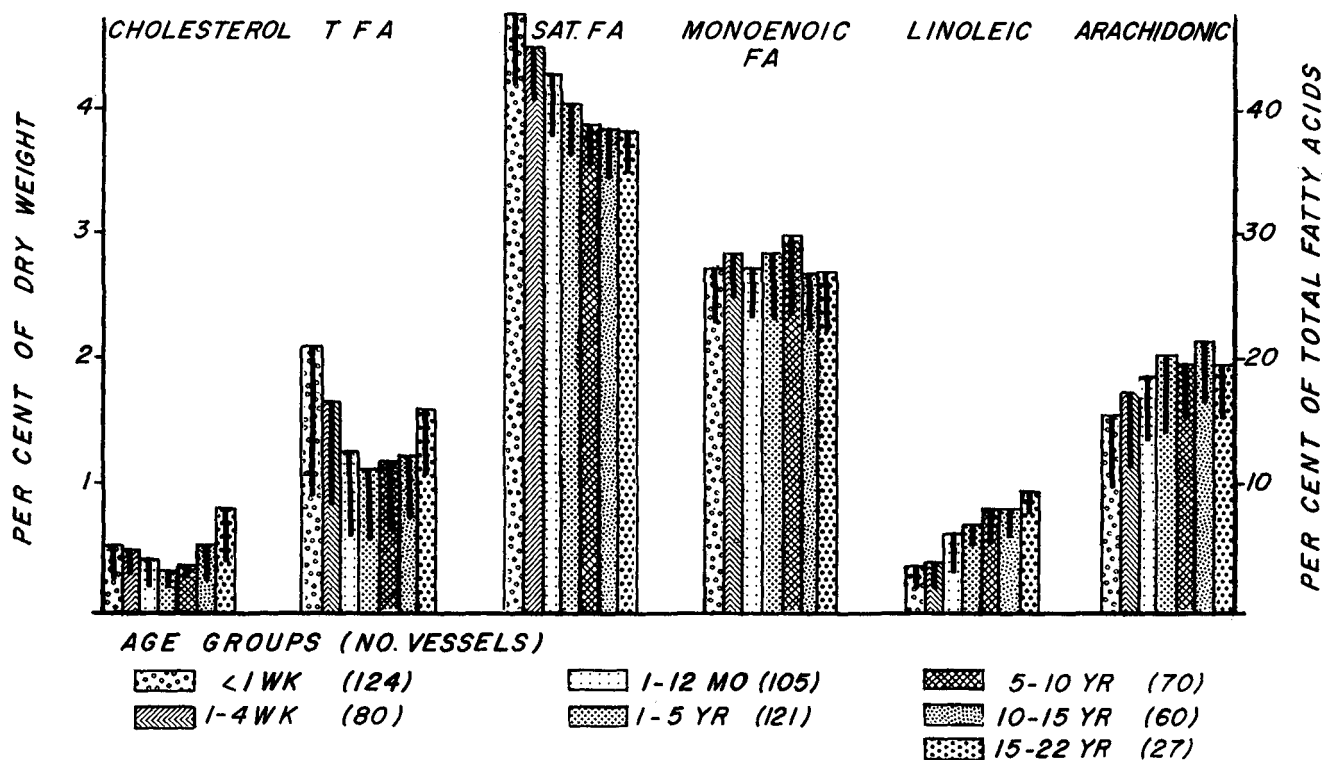


FIG. 3. Mean values with one SD for cholesterol and major fatty acids in aorta in relation to age. TFA, total fatty acids; SAT. FA, saturated fatty acids.

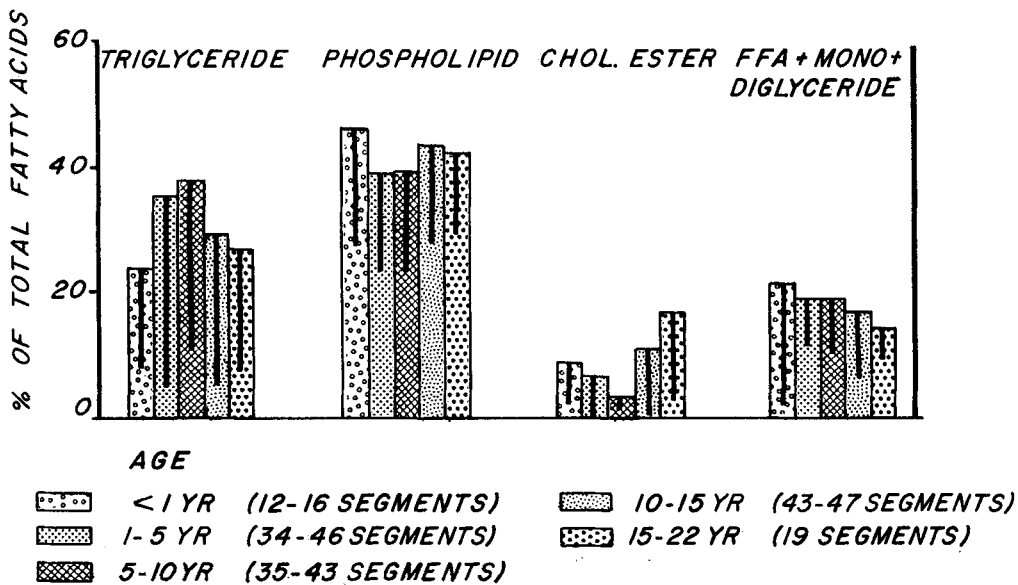


Fig. 4. Fatty acids in aortic lipid fractions from silicic acid column in relation to age. See Fig. 1 for abbreviations.

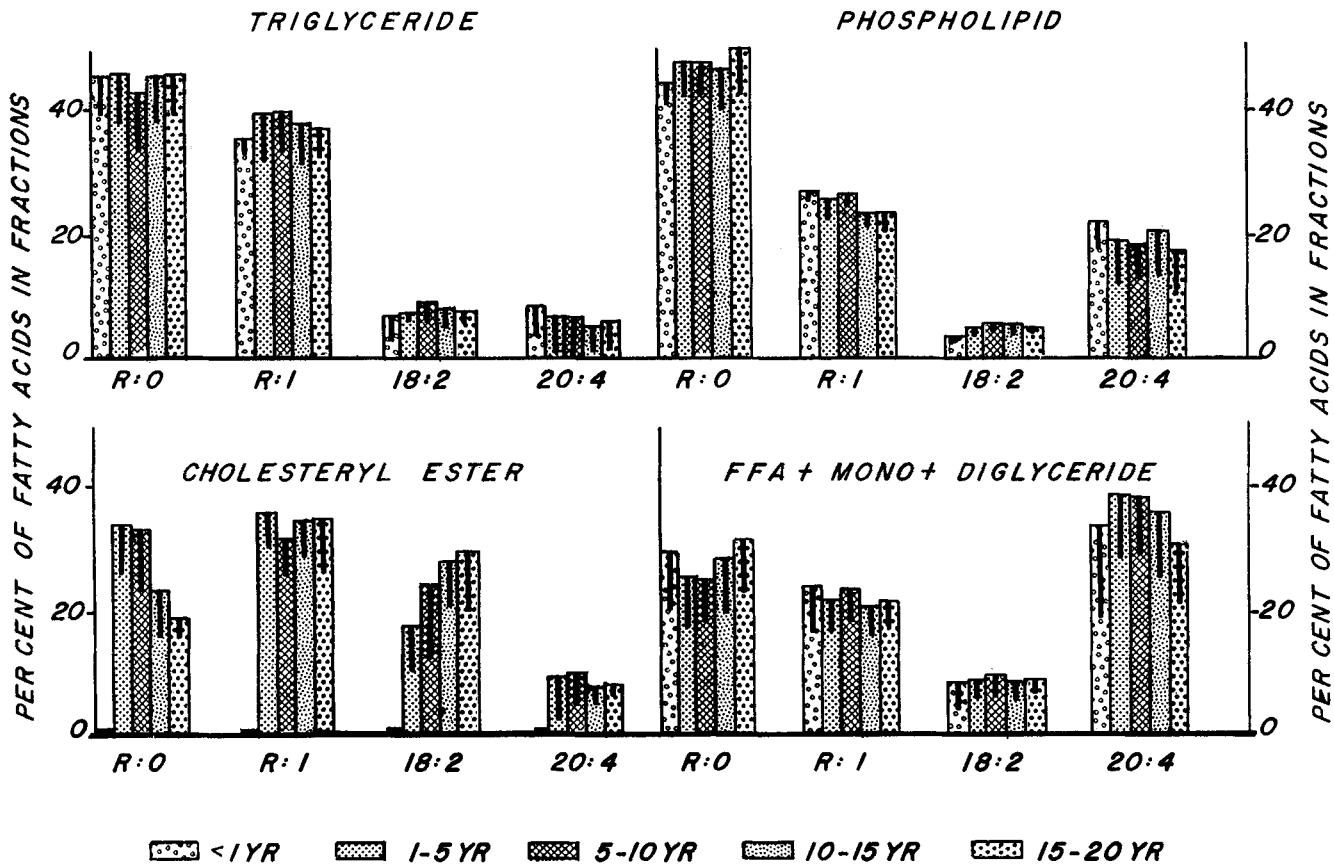


Fig. 5. Saturated and unsaturated fatty acids in four aortic lipid fractions in relation to age. See Fig. 1 for abbreviations.

Monoenoic fatty acids did not differ significantly with age. Linoleic acid increased from 1 to 22 yr ( $P < 0.001$ ) whereas arachidonic acid was significantly lower from 10 to 22 yr than from 1 to 10 yr ( $P < 0.05$ ).

Saturated fatty acids in the fraction composed of free fatty acids, monoglycerides, and diglycerides increased significantly from 10 to 22 yr of age ( $P < 0.01$ ). Monoenoic and linoleic acids comprised approximately

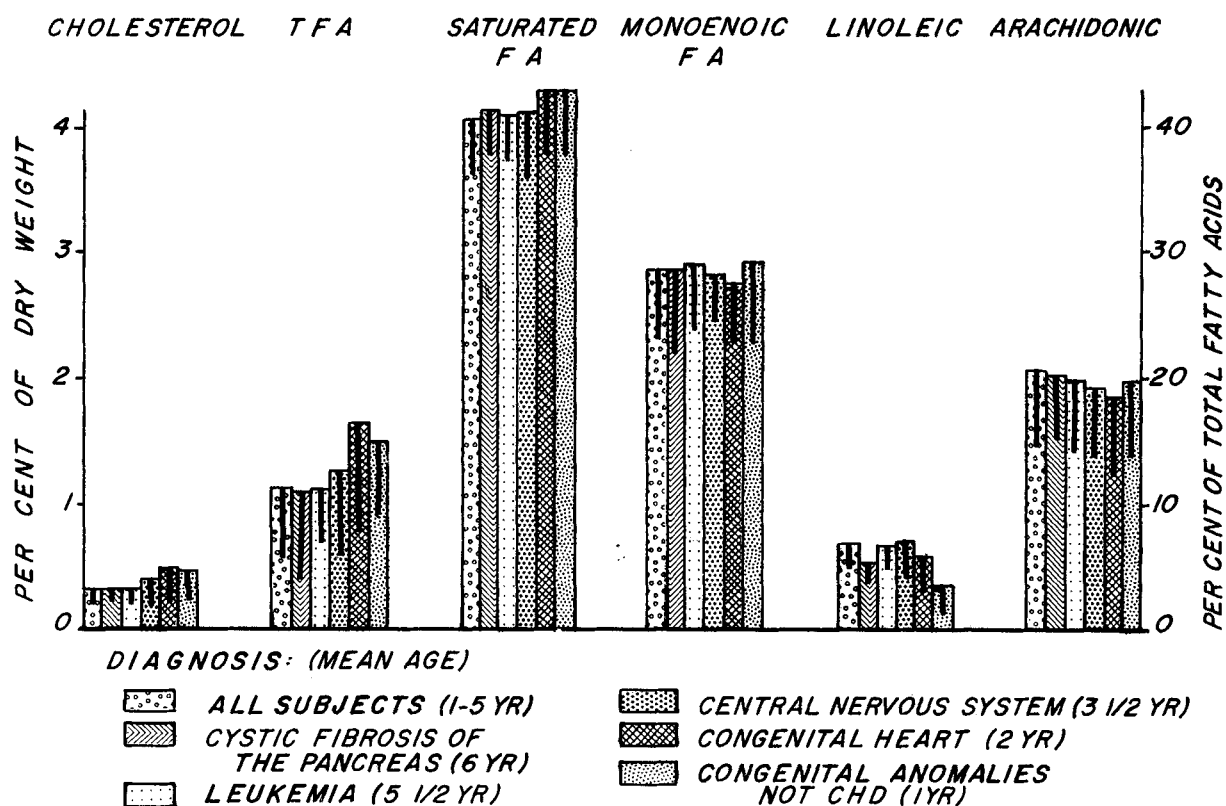


FIG. 6. Mean values with one SD for aortic lipids in relation to diagnosis. CHD, congenital heart disease; see Fig. 3 for other abbreviations

20 and 10% of the fatty acids in this fraction, respectively, and remained quite constant from 1 to 22 yr of age. The progressive decrease in arachidonic acid from 5 to 22 yr was not significant; mean levels of this acid ranging from 38.5 to 31.0% were higher than in the triglyceride, phospholipid, or cholesteryl ester fractions.

#### *Aortic Lipids in Relation to Five Diagnostic Categories*

Fig. 6 shows mean values of lipids in aortas from children in five diagnostic groups. There were 27 segments of aorta from patients with cystic fibrosis of the pancreas, 53 with leukemia, 103 with central nervous system disease, 114 with congenital heart disease, and 25 with congenital anomalies other than heart disease. Since the mean ages for patients in these categories were between 1 and 6 yr of age, distributions of the fatty acids were compared with those for 122 segments from all children 1-5 yr of age. The data were not analyzed statistically because of the small number of patients in some diagnostic categories, but certain trends were evident from the mean values. Aortas from children with cystic fibrosis of the pancreas were noticeably low in linoleic acid content. Lipids in aortas from children with congenital heart disease or anomalies other than congenital heart disease were higher in cholesterol and in total and saturated fatty acids but lower in linoleic and

arachidonic acid than those from all children 1-5 yr of age. The lipid composition of aortas from children with leukemia and those with central nervous disease did not differ from that of the total group.

#### DISCUSSION

Although large variations were observed in lipid composition of the heart vessels analyzed in this study, significant differences and trends were evident. For example, the data for 60 children <1-10 yr of age show that total cholesterol, total fatty acids, and triglycerides were lower in the aorta than in the pulmonary artery and inferior vena cava. These data confirm the results of Meyer, Meyer, Pepler, and Theron for 51 children <1-9 yr of age (14). The concentrations of phospholipids in our series were higher in aorta than in pulmonary artery and inferior vena cava. The present data for patients from <1 to 22 yr of age showed a trend after 5 yr toward increasing levels of cholesterol and total fatty acids in aortic segments, particularly the abdominal segment. Moreover, from 10 to 22 yr, cholesteryl esters and the linoleic acid content of these esters increased significantly. The significant decrease in the fraction containing free fatty acids, monoglycerides, and diglycerides may conceivably represent a decrease in turnover

rate of fatty acids in the aorta associated with the accumulation of cholesteryl esters.

The ratio of cholesterol to phospholipids in aortas has been suggested as an indication of atherosclerotic involvement (15). In a study of aortas from 80 children <1-14 yr of age, Giersten has reported an increase in this ratio after 5 yr of age (16). In the present work, the ratio rose sharply from 10 to 22 yr.

Although Böttcher and coworkers (17, 18) found sphingomyelin in aortic tissues from six children 3-12 yr of age, sphingomyelin has not been reported in aortic tissue of young patients <22 yr of age by other workers (14, 16, 19). It was not possible in our study to fractionate the phospholipids in the original ethanol-ether extract or in the methanol eluates from the silicic acid columns. Hence, no data are available regarding the presence or absence of sphingomyelin in the aortas of our young patients. However, total phospholipid fatty acids expressed as a percentage of the total fatty acids in aorta agree with the data of other workers for the phospholipid content of aortic tissue from patients <22 yr of age (14, 16, 19).

In an evaluation of the data obtained in a study such as ours, consideration must be given to various factors which may influence the lipid composition of the heart vessels, i.e., the genetic background, the dietary history, and the clinical condition of the patients, as well as the methods used during analysis of the tissues. It was not possible in the present study to obtain direct information regarding the genetic and dietary factors of the patients. However, data are available (13) for dietary intakes and blood serum lipid patterns for 324 infants and children from a population group similar in age and diagnostic categories to most of the patients described in the present paper. Although Wiese, Bennett, Braun, Yamanaka and Coon found total cholesterol and total fatty acids in serum to be relatively high in some children with a family history of heart disease, there were no statistical differences for mean blood serum levels of cholesterol, total fatty acids, saturated, monoenoic, linoleic, or arachidonic acids between children from families with a history of heart disease in the parents or grandparents and children with a negative family history for heart disease. In two diagnostic categories, i.e., congenital heart disease and cystic fibrosis of the pancreas, saturated fatty acid and linoleic acid levels in serum and in aortas showed similar differences from other diagnostic groups of the same age. Trends with age from these same fatty acids were also similar in blood serum and in aortas.

An examination of the data for segments of aorta procured from 37 patients 24-52 hr after death did not indicate uniform changes that could be attributed either to the time interval between death and autopsy or to the length of time the frozen specimens were stored. In two

instances (2/37), linoleic acid in the aortic segment was less than the mean  $\pm$  SD for the particular age group.

The results presented here, which show a significant increase from 10 to 22 yr of age only in the cholesteryl ester content of aortic tissue and only in the linoleic acid content of the cholesteryl esters, appear particularly noteworthy in view of data demonstrating a high content of cholesteryl esters and linoleic acid in aortas of adult subjects with proven atherosclerosis (15, 17-21). Morphologic studies to be published elsewhere show a good correlation between increase in intimal thickness with age and the increase in cholesterol and cholesteryl ester content of the whole aorta.

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