Lack of Correlation Between Fatty Acid Patterns in Adipose Tissue and Amount of Coronary Arteriosclerosis

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This study is one of a series[1–7] designed to explore the possible relationships between the anatomic manifestations of coronary arterial disease and lipid patterns of blood and tissue. This report deals specifically with a comparison between the amount of coronary arteriosclerosis (with or without myocardial infarcts) and fatty acid patterns of adipose tissue (as determined by vapor phase chromatography) in fifty-seven New Yorkers who were examined at autopsy. In subsequent reports similar comparisons will be made using persons from other geographic areas, including East Africa and Central America.

Considerable evidence has accumulated in recent years suggesting that various fatty acids have differing biologic effects.[8,9] Particular attention has been directed toward the effects that might be related to arteriosclerosis and its complications. In rats, when the diet contains substantial fat, the adipose tissue reflects the fatty acid composition of the ingested diet. For example, rats fed a diet including 40 per cent corn oil (which contains much linoleic acid) show a high level of linoleic acid in their adipose tissue within six weeks or less compared to that of rats fed a diet containing 40 per cent butter (which contains little linoleic acid).[10] It has been demonstrated that the adipose tissue in man similarly reflects, at least in a general way, fatty acids in the diet.[11] In the studies of man, however, changes in the fatty acid composition of adipose tissue have occurred relatively slowly, possibly because the diets used have been less drastic than those that have been given to rats.

Despite these findings, no direct evidence has been presented linking any specific fatty acid with the development of coronary arteriosclerosis in man in either a negative or positive way. The aims of this study, therefore, were to try to find out in autopsy material if any correlation could be shown to exist between fatty acid patterns and gross anatomic changes in the coronary arteries and myocardium.

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MATERIALS AND METHODS

Fifty-seven autopsy patients from the Albany Medical Center Hospital (a general hospital), ranging in age from sixteen to eighty-eight years, were included in this study. The series was consecutive except for seven patients from whom the hearts were used for roentgenographic studies of the coronary arterial circulation, three who showed active tuberculosis, and one who showed viral hepatitis; four hearts were opened incorrectly for measuring, and in seven autopsy samples the adipose tissue was either inadvertently not taken or lost. The age and sex distribution of this group is shown in Table I. The autopsies were performed by the resident staff of the pathology department but the hearts were removed intact for special study by members of the senior staff involved in this project.

The samples of adipose tissue, each approximately

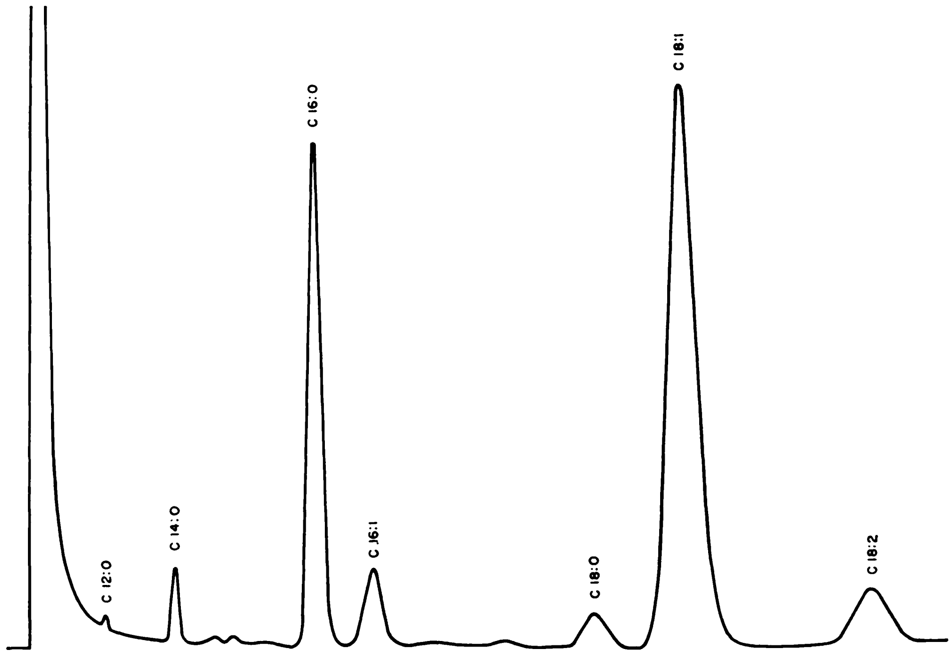

FIG. 1. Tracing of vapor phase chromatogram illustrating various fatty acid peaks in adipose tissue.

5 mm. in diameter, were all taken from the periumbilical region at the beginning of the autopsy (from one and a half to seventy-one hours after death). All included an ellipse of overlying skin to insure that the fat analyzed was at a constant depth below the skin. Each sample was placed in a glass tube, covered with methyl alcohol, and then sealed and stored at minus 29°c. To determine if the fatty acid patterns were affected by differing periods of time between death and the taking of the fat sample, we plotted all fatty acids against the time after death and found no correlation.

The fatty acids of the adipose tissue were methylated and extracted as follows: the adipose tissue was cut with scissors and placed (together with the methyl alcohol in which it had been stored) in a micro-interesterification tube with 10 ml. of a mixture of 2 per cent concentrated sulfuric acid and 98 per cent absolute methyl alcohol and refluxed for four hours at 98°c. Serial determinations at one, two and three hours showed no change in fatty acid ratios over this four hour period. The methyl esters of the fatty acids were then extracted twice with 5 ml. of hexane and the hexane washed twice with 10 ml. of distilled water. After the water layer was discarded the hexane was reduced under a stream of nitrogen to about 5 ml. and the mixture was analyzed in a vapor phase unit.

A model 10 Barber-Coleman vapor phase chromatography unit was used. Six foot glass columns with an internal diameter of 4 mm. were packed with 14 per cent diethylene-glycol succinate on 80 to 100 mesh chromosorb W.* The temperature of the column, cell bath and flash evaporator for all determinations were 159°c., 220°c. and 250°c., respectively. Argon was used as the carrier gas, with an inlet pressure of 14 pounds per square inch and an outlet flow of between 100 and 140 cc. per minute. The voltage was 650 with a buckout value of 80. The tracings were measured manually by triangulation. Only the peaks between 12:0 and 18:2 showing a deflection of over 3 per cent on the chromatogram record (and which could be measured accurately) were measured. The total of these acids was equated to 100 per cent so that the percentage figures used herein represent the percentage of the total of these acids, and not the percentage of all the fatty acids recorded on the chromatogram. Other fatty acids appeared on the tracing, but were in such low amount that no accurate measurements could be made (Fig. 1).

The hearts were examined carefully in the fresh state for infarcts or other abnormalities. Then, after fixation in 10 per cent formalin for six to seven days, the amount and severity of coronary arteriosclerosis was measured, using a method previously described by us in detail.[12] This method is based

* Obtained from Applied Science Laboratories, State College, Pennsylvania.

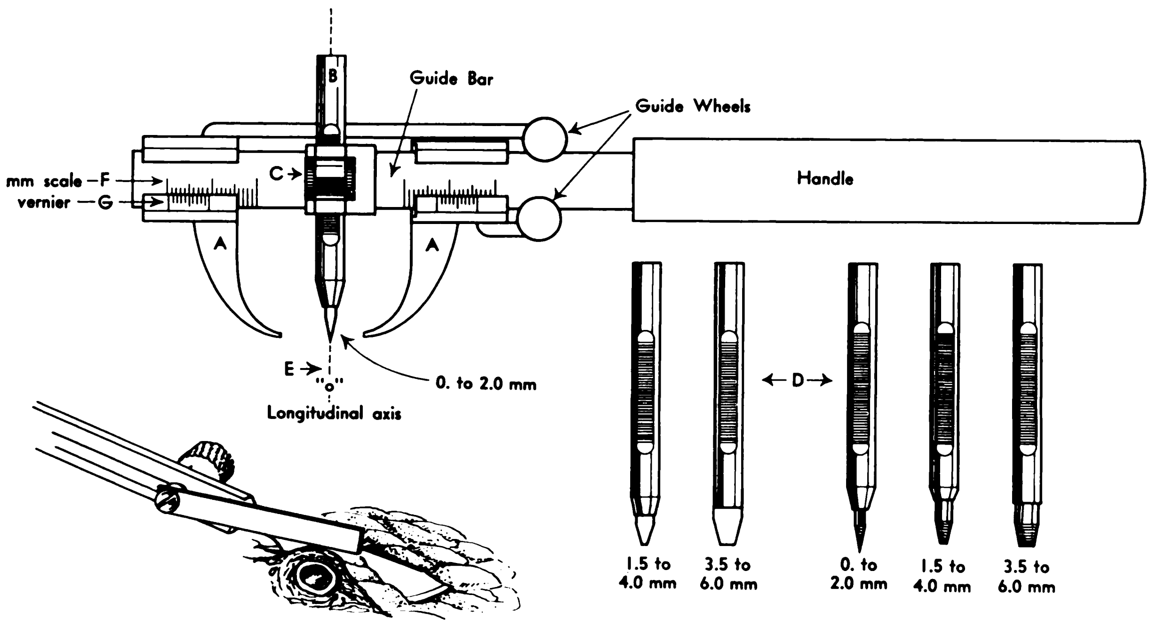

FIG. 2. Schematic drawing of specially designed calipers for the measurement of coronary wall thickness. Jaws (A), post in position (B), thumb screw for moving post (C), the longitudinal axis of the post representing the zero mark of the caliper scale (E), the millimeter scale (F) and the vernier (G). Shown separately are the interchangeable posts (D) of different sizes and shapes for various types of lumens, and the marking knife with its movable guide fixed 5.0 mm. from the blade.

on the premise that, in general, the wall of a coronary artery thickens when it is involved by arteriosclerosis and that, in general, the degree and extent of thickening provides a reasonably good estimate of the amount of arteriosclerosis.

In brief, the procedure for the examination of the coronary arteries consists of a series of cross sectional cuts along the course of the four main vessels (left, left descending, left circumflex and right coronary arteries). These right-angled cuts are made at accurately determined (with a special marking knife, Fig. 2) 5.0 mm. intervals until an external diameter of less than 1.0 mm. is reached. Alternate 5.0 mm. segments of vessel are then removed and the thickness of the wall is determined (with specially designed calipers) in two places on the proximal end of the segment on a plane parallel to the epicardial surface. After measurement, these segments and the segments remaining on the heart are cut in thirds and examined minutely for occlusions, atheromatous plaques or hemorrhage into the wall. The entire procedure is designed to insure thorough and consistent examination of the major portions of the coronary tree.

Description of Calipers

The chrome-plated steel calipers (Fig. 2) made specifically for this purpose, are composed of two separately movable jaws and a post held in position between the jaws. The post is removable but once in position can be moved only vertically, not horizontally. The longitudinal axis is at the 0 mark of the caliper scale. The guide bar on which the jaws travel is etched on each side of the post with a millimeter scale. Each jaw is equipped with a vernier scale, giving final readings to tenths of a millimeter.

Two sets of posts have been found necessary, with the tip of one set a flattened triangle for use in irregular lumens and with the other rounded to use in circular lumens. Each set contains three posts, with tapered tips accurately calibrated at 0 to 2.0, 1.5 to 4.0 and 3.5 to 6 mm. in diameter.

Use of Calipers

The segment of vessel to be measured is pushed gently upward, away from the surrounding fat or myocardium, and with a minimum of blunt dissection a sufficient length is exposed. The cone or post is inserted in the lumen until it completely fills but does not distend the wall. The post is now adjusted vertically until the proximal cut surface of the artery is at the level of the caliper jaws. The jaws are then moved separately until

TABLE I

Relative Percentage of Seven Fatty Acids in Adipose Tissue of Fifty-seven (twenty-eight males, twenty-nine females) Autopsy Subjects Arranged in Order of Amount of Coronary Arteriosclerosis as Indicated by Mean Wall Thickness

Case No.	Thickness of Wall (mm.)	Age (yr.)	Acid							Fat	
			Linoleic 18:2	Oleic 18:1	Palmitoleic 16:1	Palmitic 16:0	Stearic 18:0	Myristic 14:0	Lauric 12:0	Total Unsaturated	Total Saturated
Males (Twenty-Eight)											
1	0.81	55	4.2	47.9	4.5	33.0	5.5	4.2	0.7	56.6	43.4
2	0.76*	88	8.3	50.3	5.4	27.4	3.9	4.1	0.6	64.0	36.0
3	0.74*	47	9.6	48.7	5.8	25.7	5.4	4.1	0.7	64.2	35.9
4	0.69	81	8.1	54.3	4.8	20.9	6.7	4.4	0.8	67.2	32.8
5	0.65*	64	11.2	49.7	5.9	25.4	3.6	3.8	0.4	66.8	33.2
6	0.61*	60	7.8	50.8	7.6	26.5	3.6	3.3	0.4	66.2	33.8
7	0.61*	70	9.8	50.8	7.3	24.2	1.2	5.8	0.9	67.9	32.1
8	0.61*	72	8.9	52.2	4.1	22.3	7.7	4.1	0.7	65.2	34.8
9	0.59*	80	7.9	52.1	8.1	22.4	4.6	4.3	0.6	68.1	31.9
10	0.59	59	9.1	52.0	6.5	21.7	5.5	4.4	0.8	67.6	32.4
11	0.59	71	7.7	52.9	7.1	24.6	3.6	3.6	0.5	67.7	32.3
12	0.58*	61	11.6	47.3	7.1	26.0	3.8	3.7	0.5	66.0	34.0
13	0.58*	60	14.2	36.8	7.2	34.0	2.9	4.4	0.5	58.2	41.8
14	0.57	70	8.2	50.8	6.6	26.9	3.6	3.6	0.3	65.6	34.4
15	0.56	28	11.4	44.1	7.8	26.8	3.8	5.3	0.8	63.3	36.7
16	0.55	77	16.6	50.2	1.8	18.0	9.9	3.3	0.2	68.6	31.4
17	0.54	60	9.7	42.3	7.3	30.9	5.2	4.1	0.5	59.3	40.7
18	0.50	69	9.1	53.1	8.5	23.3	2.6	3.1	0.3	70.7	29.3
19	0.50	62	13.1	54.2	9.5	16.7	1.1	4.4	1.0	76.8	23.2
20	0.48*	73	10.9	46.0	5.3	27.0	5.2	4.7	0.9	62.2	37.8
21	0.48	62	12.3	50.3	5.2	23.0	4.6	3.9	0.7	67.8	32.2
22	0.47	85	10.0	51.5	6.8	24.7	3.0	3.6	0.4	68.3	31.7
23	0.45	46	3.7	54.4	11.9	23.8	2.0	3.8	0.4	70.0	30.0
24	0.44*	70	10.1	49.7	5.0	25.8	4.2	4.7	0.5	64.8	35.2
25	0.40	33	8.3	52.5	1.5	28.6	3.7	4.7	0.7	62.3	37.7
26	0.38	58	6.7	56.8	7.4	22.9	3.0	2.9	0.3	70.9	29.1
27	0.37	74	5.6	55.6	4.2	23.8	5.8	4.4	0.6	65.4	34.6
28	0.30	50	10.9	47.8	7.2	24.9	4.0	4.5	0.7	65.9	34.1
Females (Twenty-Nine)											
1	0.70*	74	8.2	54.8	5.5	20.5	6.1	4.4	0.5	68.5	31.5
2	0.65*	85	12.1	55.0	5.8	20.5	3.6	2.5	0.5	72.9	27.1
3	0.60	70	10.8	52.2	6.7	22.9	3.4	3.4	0.6	69.7	30.3
4	0.60	69	9.9	55.0	7.7	21.0	2.9	3.0	0.5	72.6	27.4
5	0.59*	73	10.8	52.0	7.8	23.4	2.5	3.1	0.4	70.6	29.4
6	0.59*	70	8.6	53.6	6.0	20.9	5.2	4.8	0.9	68.2	31.8
7	0.57	51	8.1	53.9	7.7	24.1	2.1	3.6	0.5	69.7	30.3
8	0.54*	72	9.0	55.7	5.8	20.7	4.6	3.8	0.4	70.5	29.5
9	0.50	70	6.7	54.8	9.8	18.8	4.0	5.0	0.9	71.3	28.7
10	0.49	72	12.8	53.2	6.7	21.8	2.7	2.5	0.3	72.7	27.3
11	0.48	70	10.3	49.0	7.8	23.8	3.7	4.7	0.7	67.1	32.9
12	0.48	65	7.4	58.4	5.3	23.5	3.1	2.1	0.2	71.1	28.9
13	0.48	79	6.5	52.4	8.1	26.4	3.1	3.2	0.3	67.0	33.0
14	0.47	66	9.4	56.4	4.9	23.9	2.8	2.4	0.2	70.7	29.3
15	0.46	80	10.3	59.0	7.1	17.0	3.7	2.5	0.4	76.4	23.6
16	0.46	37	9.4	51.8	6.9	22.9	3.6	4.5	0.9	68.1	31.9
17	0.46	67	7.8	51.2	9.6	24.1	2.1	4.7	0.5	68.6	31.4
18	0.44	30	12.0	47.8	5.6	26.8	3.9	3.5	0.4	65.4	34.6
19	0.40	35	10.6	54.0	8.4	21.7	2.3	2.7	0.3	73.0	27.0
20	0.40	76	7.3	59.0	5.6	21.2	3.7	2.9	0.3	71.9	28.1
21	0.38*	65	11.8	48.2	5.5	25.6	4.7	3.7	0.5	65.5	34.5
22	0.37	51	5.6	49.1	10.5	29.1	1.8	3.4	0.5	65.2	34.8
23	0.36	49	11.1	50.4	5.0	25.8	3.8	3.4	0.5	66.5	33.5
24	0.36	80	8.9	48.0	4.4	26.6	6.7	4.7	0.7	61.3	38.7
25	0.35	53	7.3	48.4	3.6	29.5	6.1	4.5	0.6	59.3	40.7
26	0.32	62	8.2	49.7	8.2	26.0	3.0	4.4	0.5	66.1	33.9
27	0.32	57	11.1	57.2	5.6	21.6	2.4	2.1	0.0	73.9	26.1
28	0.28	49	8.7	57.7	6.9	20.6	2.9	2.8	0.4	73.3	26.7
29	0.27	16	13.6	50.2	4.7	22.7	4.9	3.3	0.6	68.5	31.5

* Those with infarcts.

TABLE II

Summary of Comparisons of Fatty Acid Patterns of Adipose Tissue in Various Selected Groups from Table I (number of individuals indicated in parentheses)

Groups	Thickness of Wall (mean)	Mean Age (yr.)	Fatty Acids							Fat	
			18:2	18:1	16:1	16:0	18:0	14:0	12:0	Total Unsaturated	Total Saturated
Males (28)	0.55	63.8	9.5	50.2	6.3	25.0	4.3	4.1	0.6	66.0	34.0
Females (29)	0.46	61.8	9.5	53.0	6.7	23.2	3.6	3.5	0.5	69.2	30.8
Infarct* (18)	0.58	69.0	9.9	50.6	6.3	24.5	4.2	4.0	0.6	66.7	33.3
Without infarct* (39)	0.47	59.9	9.3	52.1	6.6	23.9	3.8	3.7	0.5	68.0	32.0
Most arteriosclerosis† (28)	0.60	66.7	9.6	50.9	6.6	23.9	4.2	3.9	0.6	67.2	32.7
Least arteriosclerosis† (29)	0.40	58.9	9.2	52.2	6.3	24.3	3.6	3.6	0.5	67.9	32.0
Most arteriosclerosis† (10)	0.68	69.6	8.8	51.5	5.7	24.6	4.7	4.1	0.6	65.9	34.0
Least arteriosclerosis† (10)	0.33	54.1	9.1	51.4	6.0	25.1	4.1	3.8	0.5	66.5	33.5
Youngest (29)	0.47	51.4	9.6	50.6	6.6	25.3	3.6	3.7	0.5	66.8	33.2
Oldest (28)	0.54	74.5	9.3	52.7	6.4	22.9	4.3	3.9	0.5	68.4	31.6

* Myocardial infarcts only.
† As judged by mean thickness of coronary wall. See text.

their tips just touch the external surface of the vessel wall.

Reading the Caliper Measurements

While the post is still in the lumen, the external diameter is read by noting on each side the distance traversed by the jaws from mark 0. The sum of these two readings, each of which measures the distance from one adventitial surface to the center of the post, is the external diameter of the vessel.

To measure the internal diameter, the calipers are removed from the artery, taking care not to disturb the position of the post or the jaws. The jaws are then moved until their tips touch the side of the post and the sum of the distances of the jaws from their respective 0 marks is the internal diameter.

We have found that the thickness of the wall is the most useful index of the degree of coronary arteriosclerosis. Other indices, such as wall volume, wall:lumen ratio and wall area added no further information and indeed were found to vary considerably with the weight and size of the heart, independent of the amount of coronary atherosclerosis.

One major advantage of this procedure is the fact that it is highly objective and can be performed by adequately trained technicians. If the method is to have value, however, it must be, in addition, accurate and reproducible. We have found that in duplicate measurements of forty-eight hearts, the average interobserver "error" is only 7 per cent.[12]

RESULTS

The results are summarized in the Tables I and II and Figure 3. In this series of fifty-seven patients, no significant correlation was demonstrated between fatty acid patterns of adipose tissue and anatomic alterations in the heart resulting from arteriosclerosis.

None of the fatty acid patterns appear to correlate with any of the anatomic variables that were studied. In Table I the findings are arranged according to sex of the patient and in descending order according to the mean thickness of the wall of the coronary arteries. The values for all the fatty acids appear to fall at random, and evaluation by statistical methods failed to show any significant correlations. In addition to arranging the findings by mean thickness of the wall of the coronary arteries, we also arranged them in descending order by coronary wall volume and also by wall:lumen ratio; in both instances the fatty acid values again appeared to be arranged at random.

In Table II we have paired selected anatomic variables and compared their mean fatty acid values. The most striking observation that can be made is that no correlation can be detected between anatomic extremes and fatty acids. Of particular interest is the closeness

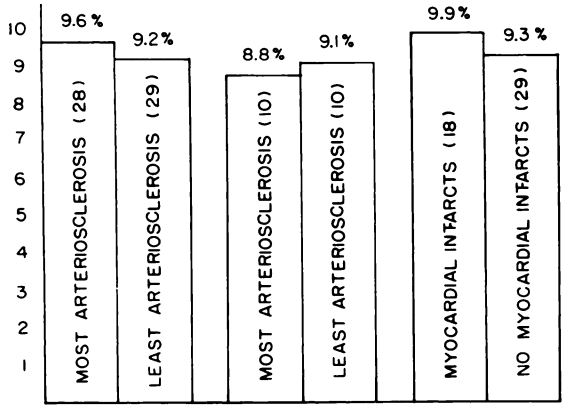

FIG. 3. Relative percentage of adipose tissue linoleic acid in autopsy patients showing the most and least degree of coronary arteriosclerosis as judged by mean thickness of coronary wall and patients with and without myocardial infarcts. It is apparent that no correlation exists between linoleic levels and these anatomic differences.

of the values for linoleic acid in patients with myocardial infarcts versus those without infarcts. The value for linoleic acid is actually slightly greater for those with myocardial infarcts than for those without, but the difference is not statistically significant.

COMMENTS

This study was designed primarily to explore possible correlations between certain fatty acids in the adipose tissue and certain anatomic changes in the heart. The most significant observation that we have made is the lack of demonstrable correlation between any fatty acid studied and the severity of coronary arteriosclerosis. Also, the fatty acid patterns in the range studied in patients with and without myocardial infarcts showed no significant differences.

It is possible that differences could be demonstrated if larger numbers were used. However, it seems likely that if a really significant trend were present, it would be apparent even in fifty-seven patients. It seems important to make a similar study of patients from different countries in which the amount of arteriosclerosis and the dietary backgrounds are different. Such a study, comparing patients from East Africa, Guatemala, and the United States, is in progress.

It may be that the fatty acids that are important in relation to coronary arteriosclerosis are not those in the 12:0 (lauric) to 18:2 (linoleic) range that were studied here. However, in a recent study of human adipose tissue,[11] using vapor phase columns, which allowed measurement of short chain fatty acids it was estimated that the seven fatty acids measured in this study comprise approximately 90 per cent of the fatty acids usually present in the adipose tissue of Americans. However, it is possible that some fatty acid or acids present in relatively small amounts are of importance in relation to arteriosclerosis. It is known that biologic substances, notably vitamins and minerals, are present normally in small amounts but are nonetheless of enormous significance.

Another factor to consider is that this study does not take into account the relative quantities of fats consumed. It is possible that a person who eats 300 calories of fat each day might have the same fatty acid composition in his adipose tissue as a person who eats 1,500 calories of fat per day. Studies similar to this one utilizing subjects from other geographic areas where less total fat is eaten may provide information on this point. It seems obvious that when the total amount of fat consumed is small, the fatty acids in the adipose tissue would be in part those synthesized by the individual.

The lack of correlation of fatty acids and coronary arteriosclerosis in this study might be because the population examined (fifty-seven autopsy patients) was too homogeneous as far as dietary habits were concerned. For example, the range of linoleic acid in the tissues of the fifty-seven patients was from only 3 to 16 per cent, whereas in experimental animals it is possible to produce a range from virtually zero to at least 50 per cent. If we had studied patients with relative linoleic acid levels ranging from 0 to 50 per cent, correlations with the degree of coronary arteriosclerosis might have become apparent. In another study now in progress we are attempting to find subjects with unusually high or low linoleic acid levels in their tissues, and the anatomic features of these persons will be compared with those of the fifty-seven patients presented in the current study.

SUMMARY

We have compared fatty acid patterns of adipose tissue as determined by vapor phase chromatography, with the amount of coronary arteriosclerosis and presence or absence of myocardial infarcts in fifty-seven Americans who have undergone autopsy.

Despite evidence in both man and animals that adipose tissue reflects, in a general way, the fatty acid content of the diet, and that various fatty acids have differing biologic effects, this study uncovered no correlation or relationship between the fatty acid composition of adipose tissue and the quantity of coronary arteriosclerosis or the presence or absence of myocardial infarcts.

Insufficient size of group, homogeneity of the population studied and possible importance of fatty acids outside the range for which we analyzed, are considered in relation to the negative results.

It is clearly important to examine in a like manner groups from other geographic areas, particularly where dietary fats and the degree of coronary arteriosclerosis are different from those found in New York.

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