Successive Trials of Induced Alimentary Lipemia

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THE OCCURRENCE of alimentary lipemia following the ingestion of various test meals has been used to test an individual's ability to handle a large fat load. A number of reports suggest that this mechanism is impaired in patients with ischemic heart disease as compared with normal subjects.[1-3] Until a few years ago, the measurements made during alimentary lipemia were mostly of serum turbidity or serum total lipids. Cholesterol levels usually were little changed by an acute fat load while phospholipid levels tended to rise to varying degrees.[4,5] Optical density of serum increased predictably with peak readings from three to six hours.[6-8] Total fatty acids increased comparably with peaks at three to six hours (more recently, measurements of serum triglycerides have revealed the same pattern).[7-10] Free fatty acids have generally increased also, although not consistently.[7,11,12] When the fat load was given intravenously as a fat emulsion, the increase in free fatty acids was more consistent and greater than that which occurred following intragastric administration.[13] Recent evidence indicates that the carbohydrate and protein content of meals of equal fat content influence the pattern of lipemia[14,15]—a high carbohydrate meal tending to decrease lipemia and high protein meal tending to increase and prolong it. A curious finding in these studies was that elevated fasting triglyceride levels actually decreased following the fat load, either with high or low carbohydrate content.

The present study was designed to test the replicability of alimentary lipemia on repeated trials, to describe the separate measurements of lipemia following a specific type of meal, to determine the relationship between certain of these measurements and to obtain a preliminary idea of the comparative variability of fasting serum triglyceride and cholesterol levels.

METHODS

Twenty-four trials were run in twelve subjects, the second trial following the first by an interval of one week. All subjects were men in apparent good health. Two were in the third decade of life, four each in the fourth and fifth decade and two in the sixth decade. After fasting for twelve to fourteen hours, the subjects were given a test meal in the early morning consisting of 455 gm. of vanilla ice cream which contained 100 gm. fat (butterfat), 90 gm. carbohydrate and 14 gm. protein with a total caloric value of 1,450. The feeding took about twenty minutes, following which the subjects were kept at rest and prohibited from smoking for the next six hours.

Prior to feeding, a blood sample was obtained for the following measurements: (1) optical density—serum was read against a water blank in a Coleman Junior spectrophotometer at 660 mμ, using 10 by 75 mm. cuvettes. (2) Total cholesterol—serum values were determined by alcohol extraction, production of the Liebermann-Burchard reaction with cholesterol acetate as the standard.[16] (3) Triglycerides—serum levels were measured directly.[17] (4) Free fatty acids—plasma levels were measured by a modification of the Dole technic.[18,19] Determinations of optical density and free fatty acids were repeated one, two, three, four and six hours after feeding was complete; triglyceride levels were determined at two, four and six hours and total cholesterol levels at six hours.

RESULTS

The test meal of vanilla ice cream evoked definite alimentary lipemia in all trials. The

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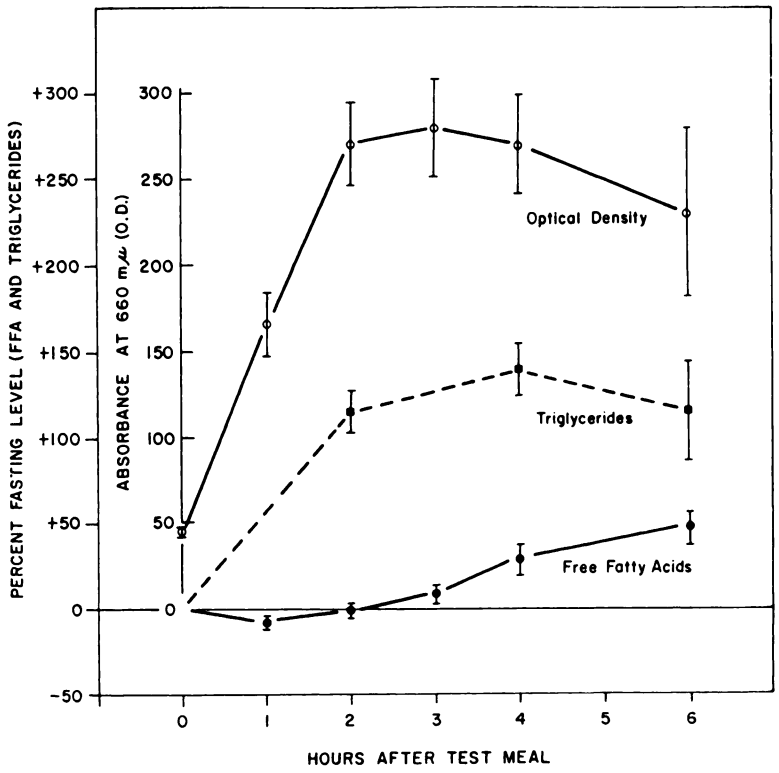

FIG. 1. Effects of alimentary lipemia on optical density (O.D.) of serum triglycerides and plasma free fatty acids (FFA) in twenty-four subjects. Means and standard error of means.

pattern of the separate measurements of lipemia is shown in Figure 1. Optical density of serum increased sharply during the first two hours following the test meal, remained relatively constant during the next two hours, and declined slightly during the last two hours. The curve of response of serum triglycerides was similar, rising to 115 per cent over fasting levels after two hours with a peak of 139 per cent at four hours. Free fatty acids deviated from this pattern, with levels declining slightly in the first two hours and rising somewhat slowly to a peak of 48 per cent over fasting levels by the sixth hour.

Serum triglyceride and cholesterol levels at various time periods following the meal are shown in Table I. As indicated, fasting serum triglyceride levels varied somewhat more from one trial to another than serum cholesterol levels, the former tending to be lower on the second trial, the latter remaining relatively constant. Correlation between fasting levels in the first and second trial was high, being 0.75 for triglycerides and 0.78 for cholesterol, both highly significant coefficients ($p < 0.01$).

Although variation among subjects is to be expected, the degree of replicability of alimentary lipemia in the same subject is important if this test is to be used for evaluating therapy. To correct for variations in initial serum triglyceride levels, the percentage change from fasting levels at two, four and six hours was used for comparing the results of the first and second trials. While there is a tendency for the general pattern of lipemic response to be repeated from one trial to another, one cannot be sure that this will occur in the early phases. Correlation coefficients for changes in triglyceride levels from the first to second trial were 0.12 at two hours, 0.55 at four hours and 0.95 at six hours. Only the triglyceride increase at six hours was significantly correlated ($p < 0.01$) between the two trials. A nonparametric analysis of ranks of change in-

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TABLE I
Serum Triglyceride and Cholesterol Levels Following Induced Alimentary Lipemia

Subject	Trial	Serum Triglycerides (mg./100 ml.) Hours After Feeding				Serum Cholesterol (mg./100 ml.) Hours After Feeding	
		0	2	4	6	0	6
E. C.	1	161	223	260	191	295	281
	2	89	301	247	79	236	218
M. G.	1	169	316	223	127	182	173
	2	159	317	322	155	201	201
F. G.	1	61	111	213	103	172	172
	2	63	165	170	96	172	167
C. M.	1	127	264	244	273	188	191
	2	82	292	338	236	218	198
M. S.	1	83	121	172	145	139	128
	2	58	100	141	139	122	124
J. B.	1	103	169	112	101	140	144
	2	51	92	63	66	188	135
L. R.	1	87	186	136	122	225	217
	2	57	117	162	113	206	209
G. I.	1	61	149	146	102	206	219
	2	45	91	86	59	164	172
L. H.	1	115	121	213	206	187	174
	2	92	96	127	132	181	201
W. P.	1	185	455	453	504	282	264
	2	115	288	410	869	305	289
J. P.	1	168	569	602	843	223	248
	2	219	385	646	838	212	226
C. B.	1	76	197	170	134	173	173
	2	77	217	226	206	192	211

creased the correlations at two and four hours, the latter becoming significant ($p = 0.05$).

Optical density values were correlated with serum triglyceride levels to assess the accuracy of the former as an indicator of triglyceride levels. Fasting and two-hour values of triglycerides showed only small correlations ($r = 0.20$ and 0.51, respectively) with optical density readings; by four and six hours the correlations were rather high ($r = 0.76$ and 0.80, respectively, both highly significant). Although the units of measure are completely different, the direct reading of optical density at four and six hours gives a reasonable approximation of the triglyceride level in milligrams per 100 milliliters in many cases. Nonparametric analysis by rank order of each measurement yielded comparable results.

Confirming previous results, there was no change in serum cholesterol levels at six hours after the meal as compared with fasting levels. (Mean value was 200.38 before the meal and 197.21 mg. per 100 ml. six hours after the meal.) On the other hand, serum triglyceride levels were significantly elevated at six hours after the meal as compared with fasting levels (mean of 90.8 mg. per 100 ml. before and 139.25 after). This change held true despite the elimination of triglyceride data from four trials in two subjects with aberrant responses to the fat load, both of whom had marked and prolonged lipemia. These subject were the two oldest in the study; one had a mild myocardial infarct three months after participating in these tests.

Also of interest is the fact that the initial serum triglyceride levels correlated quite well with the peak levels obtained ($r = 0.70$, $p < 0.0005$) following the meal. This was true even when the data from the four aberrant trials were omitted. Including these, and using a nonparametric analysis by rank order, the correlation was even better ($r = 0.78$). None of our subjects, regardless of their initial serum triglyceride level, showed any decrease in these levels following the test meal. In only seven of the twenty-four trials were the initial triglyceride levels over 120 mg. per 100 ml. (only two were over 180 mg. per 100 ml.).

The fact that the subjects underwent two separate trials one week apart allowed for some comparison of the variability of repeated measurements of serum cholesterol and triglycerides. The total variance (trial 1 minus trial 2) for serum cholesterol was 786.91 (S.D. 28.05) in twelve subjects, and for triglycerides 1,429.91 (S.D. 37.81). Since the scale of normal values for triglycerides (80 to 120 mg. per 100 ml.) is half that for cholesterol (160 to 240 mg. per 100 ml.), the actual increased variability of the serum triglyceride level was about threefold.

Levels of free fatty acids at various times following the test meal are shown in Table II. Fasting levels of plasma free fatty acids varied considerably from one trial to another in the same subject ($r = 0.36$, not significant). Such variation was expected, being due perhaps to

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differing adherence to the fasting schedule, states of excitement, or amount of physical exercise prior to each trial. I have also found this variation in other repeated trials in which fasting levels of free fatty acid were measured. Correlation of percentage change from fasting levels of free fatty acids between trials was rather high. At one hour, the coefficient was 0.57, just missing significance at the 0.05 level; two, three, four and six hours after the meal, coefficients were 0.85, 0.82, 0.97 and 0.91, respectively, all highly significant ($p < 0.001$).

COMMENTS

Alimentary lipemia induced by ingestion of a test meal has its limitations as a test of therapeutic agents because of considerable variability in the results of successive trials in the same subject. Greatest variability occurs early in lipemia. On the other hand, the reproducibility of six-hour levels of lipemia, and the fairly high correlation at this time between optical density readings and serum triglyceride levels, suggest that a simple measurement of optical density is adequate for the clinical detection of aberrant responses.

Following the test meal given in this study, free fatty acid levels fell slightly initially, probably due to the fairly high content of carbohydrate inhibiting endogenous mobilization of fatty acids. The later rise was probably due to lipolysis of the exogenous fats. No subject, regardless of his initial serum triglyceride level, showed a fall in this fraction following ingestion of the meal. Indeed, the fasting serum triglyceride level was a good predictor of the peak level attained following the meal.

With the increasing popularity of serum triglyceride measurements as predictors of ischemic heart disease or as gauges of lipopenic therapy, it is well to point out that on repeated trials the results of this measurement are even more variable than those for serum cholesterol. The few data from this study indicate a threefold increase in variability, rather similar to what we have also observed in sample data from patients during various therapeutic trials.

SUMMARY

Two successive trials of induced alimentary lipemia were made one week apart in twelve normal subjects. The test meal consisted of ice cream containing 100 gm. fat, 90 gm. carbohydrate and 14 gm. protein.

As judged by serum triglyceride levels, responses to successive trials of alimentary lipemia were poorly replicated at two and four hours, but rather consistent at six hours. This early variability limits the usefulness of this procedure for evaluating therapy. All subjects showed varying increases in serum triglyceride levels with a peak mean response of 139 per cent over fasting levels at four hours. Fasting serum triglyceride levels were good predictors of peak levels, being significantly correlated. Optical density rose in a similar fashion, being rather highly correlated with serum triglyceride levels at four and six hours.

TABLE II
Free Fatty Acid Levels Following Induced Alimentary Lipemia

Subject	Trial	Free Fatty Acids (μM/L.) Hours After Feeding 0	1	2	3	4	6
E. G.	1	423	385	473	460	585	625
	2	360	440	493	520	493	493
M. G.	1	335	480	610	755	843	570
	2	373	268	528	653	963	845
F. G.	1	435	410	553	603	720	...
	2	558	485	503	558	948	855
C. M.	1	298	248	235	273	485	610
	2	348	155	318	435	603	795
M. S.	1	708	410	398	423	540	708
	2	855	380	335	415	508	768
J. B.	1	498	473	435	435	510	585
	2	520	478	480	573	628	735
L. R.	1	498	598	585	728	640	598
	2	498	448	680	695	913	858
G. I.	1	448	410	573	548	640	598
	2	640	708	853	708	858	783
L. H.	1	343	343	330	305	343	585
	2	523	435	370	343	410	753
W. P.	1	615	428	403	403	335	403
	2	298	124	162	236	236	323
J. P.	1	410	162	162	261	347	670
	2	378	273	298	298	465	910
C. B.	1	318	423	423	398	448	523
	2	668	468	418	468	405	468

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Mean levels of free fatty acids tended to fall initially, then rose slowly to an increase of 48 per cent over fasting levels by the sixth hour. The variability of successive measurements of fasting serum triglycerides is about three times that of serum cholesterol, a point of practical importance when this measurement is used as a predictor of ischemic heart disease or as a gauge of therapy.

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