THE AMERICAN JOURNAL OF Clinical Nutrition

JULY 1963 VOLUME 13, NUMBER 1

Original Communications

Fasting and Postprandial Serum Triglyceride Levels in Healthy Young Americans

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In the last three years increasing attention has been directed to the possibility that elevated serum triglyceride levels may play a role in the pathogenesis of ischemic heart disease.[1] Triglycerides form the largest portion of our dietary fat, and it has been shown that the prevalence of ischemic heart disease in different racial groups correlates well with the percentage of calories each group derives from dietary fat.[2] Antonis and Bersohn[3] have demonstrated significantly higher triglyceride levels in South African European subjects than in Bantu in whom ischemic heart disease is rare. Albrink et al.[4,5] and others[3,6,7] have found high serum triglyceride levels in patients with ischemic heart disease. Likoff et al.,[8] Seller et al.[9] and Brown et al.[10] have all shown that postprandial lipemia in patients with ischemic heart disease is abnormal in degree and duration. More recently Stutman et al.[11] have demonstrated that this abnormality exists in relatively young persons with ischemic heart disease. In the majority of these studies, however, triglyceride levels have not been measured directly but have either been based on serum optical density measurements or obtained by calculating differences between total fatty acid and the combined cholesterol ester and phospholipid fatty acid. The results of the latter method will reflect any error present in these three determinations. Added to this paucity of more directly determined triglyceride values in ischemic heart disease is an almost complete lack of information on values in healthy young subjects. In view of the prevalence of atherosclerosis in middle-aged men it is likely that any large group of such persons, although apparently healthy, will contain many with coronary atherosclerosis and latent or preclinical ischemic heart disease. In a group of 1,843 clinically normal men examined in 1954,[12] ninety-six exhibited overt evidence of ischemic heart disease six years later. If elevated triglyceride levels bear a significant correlation to coronary atherosclerosis and not merely to overt clinical manifestation of the disease, it may be expected that in a large group of healthy middle-aged men a considerable

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This work was carried out under a grant-in-aid from the American Heart Association and supported in part by the Orange County (New York) Heart Association.

number would exhibit high triglyceride levels. In a recent study,[13] we found that over 40 per cent of 1,500 men aged forty-eight to sixty-four had triglyceride levels in excess of what, on the basis of present findings, we consider normal. It follows that a group of healthy middle-aged men cannot be considered an acceptable group for the establishment of normal triglyceride levels in adult men, nor should the triglyceride levels in this group be considered suitable control values for comparison with those obtained in patients with ischemic heart disease. We are in complete agreement with Albrink et al.[5] that normal triglyceride levels should be established by measurement in a population in which manifest ischemic heart disease is uncommon or absent. For this reason the subjects studied and described herein were all less than thirty years of age.

It has been demonstrated indirectly that a significant increase in fasting triglyceride values occurs after the ingestion of a 70 gm. fat meal.[10] No good estimate of the amount of dietary fat necessary to alter significantly the fasting triglyceride level is available. This information was sought. Were it demonstrated that significant increases in triglyceride values occurred only after a large fat meal (e.g., greater than 50 gm. of fat) it would eliminate the need for collecting fasting blood samples and thereby facilitate any large scale study of triglycerides.

MATERIALS AND METHODS

Blood was obtained from the umbilical cord of twenty-four infants at birth and from sixty-five children aged one month to fourteen years. Although most of the samples were obtained from children in the fasting state, some postprandial samples were probably included as the children on the pediatric ward had unlimited access to food. None of these children was acutely ill. No diabetic patients or any subjects with other disorders likely to affect lipid metabolism were included.

Thirty-two female student nurses aged eighteen to twenty provided blood after being on duty all night and prior to their morning meal. Some admitted to taking a light snack during the night.

Samples were obtained at 8:00 A.M. from thirty-six male medical students aged twenty-three to twenty-nine who had eaten nothing since 10:00 P.M. the previous evening. These students were divided into four groups (two of ten, one of nine and one of seven). Fat meals of different amounts were given to each group to study postprandial triglyceride levels. The meal administered to each group is shown in Table I. The fat content of the meals was 70 gm. for group I, 50 gm. for group II, 30 gm. for group III and 10 gm. for group IV. Immediately before ingesting any meal each student drank 100 μc. of I^{131} triolein* (5 ml. triolein) homogenized in water[11] with pluronic F^{68}† and phosphatide.‡ They were allowed water but no food for nine hours after the meal. Postprandial blood samples were obtained at three, five, seven and nine hours for measurement of triglyceride, cholesterol and lipid I^{131} content.

TABLE I
Content of Meals

Content	Group			
	I	II	III	IV
Fat (gm.)	70	50	30	10
Carbohydrate (gm.)	58	57	54	54
Protein (gm.)	25	21	18	11
Calories	960	770	600	360
Items				
Apricots (cups)	1/2	1/2	1/2	1/2
Bacon (strips)	3	1	0	0
Toast (slices)	2	2	2	2
Butter (gm.)	22	12	12	4
Scrambled eggs (no.)	2	2	2	1
Heavy cream (ml.)	30	15	0	0
Light cream (ml.)	40	40	40	0
Black coffee	*ad libitum*	*ad libitum*	*ad libitum*	*ad libitum*

An additional group of eighteen women and 143 men aged nineteen to thirty who attended as blood donors was studied. Blood donors at this hospital are requested to eat nothing for three hours prior to their attendance. As this and previous studies, however, have shown that maximum postprandial lipemia may occur at three hours and more commonly at five hours, these instructions would seem futile if the objective is to exclude lipemic plasma. Although the serum triglyceride levels in this group cannot, therefore, be considered fasting, most sub-

* E. R. Squibb & Sons, New York, New York.

† Kindly supplied by Wyandotte Chemical Corporation, Wyandotte, Michigan.

‡ Kindly supplied by Central Soya, Chicago, Illinois.

jects stated that they had consumed only light meals in the preceding five hours.

Serum triglycerides were measured on the basis of glycerol content using the method of Van Handel and Zilversmidt[14] and expressed as milliequivalents per liter of a pure triolein standard. Cholesterol was measured by the method of Abell et al.[15] and lipid I^{131} by the method of Beres et al.[16] Translucency of fasting and postprandial serum samples from male students was measured at 620 mμ in a Coleman Junior Spectrophotometer.

RESULTS

There was no difference by sex in the serum triglyceride levels of blood obtained from the umbilical cords of ten male and thirteen female infants. Values for all twenty-three infants ranged from 1.5 to 3.0 mEq. per L. with a mean of 2.6 ± 0.8 mEq. per L. Serum samples obtained from three bottle-fed infants aged one, two and five months demonstrated triglyceride levels of 4.8, 7.7 and 5.3 mEq. per L., respectively. In view of the frequency of feedings at this age, these samples must be considered postprandial.

The sixty-two children studied ranged from one to fourteen years of age with 50 per cent under nine years. Forty-two were boys and twenty girls. Although the number of children studied was insufficient to assess changes in triglyceride values from year to year, a comparison of five-year age groups revealed no significant age trend in average triglyceride levels. Values for the entire group ranged from 1.8 to 8.1 mEq. per L. with a mean and standard deviation of 3.6 ± 1.9 mEq. per L.

Triglyceride values from blood bank samples ranged from 1.6 to 9.9 mEq. per L. with a mean and a standard deviation of 5.0 ± 2.0 mEq. per L. The mean values of 143 men and eighteen women who comprised this group were 5.1 ± 1.9 and 4.6 ± 2.3 mEq. per L., respectively, and were not significantly different.

In thirty-two nurses aged eighteen to twenty, levels ranged from 2.5 to 5.6 mEq. per L. with a mean and a standard deviation of 4.0 ± 0.8 mEq. per L.

The lowest average triglyceride level, with the exception of that for blood from the umbilical cord, was found in the fasting male medical students. The mean and standard deviation in this group were 3.4 ± 1.0 mEq. per L. Ninety-five per cent of this group of students had a triglyceride level of 5.2 mEq. per L. or less. As this group was considered reliable with regard to fasting prior to blood withdrawal, it is thought that this value of 5.2 mEq. per L. is an accurate upper limit of normal for a fasting triglyceride level. The postprandial triglyceride levels and lipid I^{131} levels in these students are listed in Tables II through V. Although the maximum mean triglyceride level in each group was observed five hours after any meal, some subjects in these groups reached a maximum at three hours. In all students, with the exception of student R. G. in group IV, the seven hour triglyceride level was lower than the five hour value, and the nine hour value was equal to or lower than the fasting value. Student R. G. differed significantly from all others in having a highly abnormal fasting level of 13.7 mEq. per L. as compared to 2.7 mEq. per L. for the remainder of his group. Whereas the remainder of the group had a maximum mean increase in the triglyceride value of 0.8 mEq. per L. above the fasting value, R. G.'s value increased 2.2 mEq. per L. after the same 10 gm. fat meal. Of additional interest were the significantly higher radioactive lipid levels, which were 0.2, 4.3, 9.8 and 3.9 per cent of the ingested dose at three, five, seven and nine hours, respectively. Several fasting blood samples subsequently obtained from this student showed triglyceride levels in excess of 10.0 mEq. per L. His lipid values were excluded in calculating the mean level for his group.

Serum translucency values in the four groups are shown in the tables. In groups I and II in which significant increases in serum turbidity occurred after the meals, the levels continued to exceed the fasting value at nine hours in six of the seventeen students.

Serum cholesterol levels were determined in the fasting and postprandial samples of students who took the 50, 30 and 10 gm. fat meals. Insufficient serum was available for carrying out this analysis in the group ingesting the 70 gm. fat meal.

Results are included in Tables III, IV and V. Serum cholesterol level was significantly higher

TABLE II
Serum Optical Density and Fasting and Postprandial Lipid Levels After Ingestion of 70 gm. Fat Meal

Level	Triglyceride (mEq./L.)	Lipid I^{131} (% of dose)	Serum Optical Density
Fasting.....	3.2 ± 0.9	...	0.090
After 3 hours..	4.7 ± 2.1	2.9 ± 1.6	0.375
After 5 hours..	5.0 ± 1.4	3.0 ± 1.2	0.325
After 7 hours..	3.7 ± 1.2	2.2 ± 0.7	0.147
After 9 hours..	2.9 ± 0.7	1.3 ± 0.4	0.089

TABLE III
Serum Optical Density and Fasting and Postprandial Lipid Levels After Ingestion of 50 gm. Fat Meal

Level	Triglyceride (mEq./L.)	Lipid I^{131} (% of dose)	Serum Optical Density	Cholesterol (mg. %)
Fasting.....	4.7 ± 0.7	...	0.070	220 ± 41
After 3 hours..	6.3 ± 1.6	2.6 ± 2.2	0.141	223 ± 34
After 5 hours..	6.8 ± 1.2	3.5 ± 1.6	0.220	208 ± 21
After 7 hours..	5.7 ± 1.0	3.5 ± 1.3	0.150	243 ± 21
After 9 hours..	4.3 ± 1.0	1.9 ± 1.0	0.081	242 ± 38

at seven and at nine hours after the 50 gm. meal and at nine hours after the 30 gm. meal. No significant increase occurred at any time after ingestion of the 10 gm. meal. The mean cholesterol value for this last group excludes student R. G. whose fasting serum cholesterol was 267 mg. per cent. As in other members of his group, there was no increase in his fasting cholesterol level after the 10 gm. meal.

COMMENTS

If serum triglyceride levels bear a relationship to overt or preclinical ischemic heart disease it is essential that normal levels for this lipid be defined before the magnitude of any abnormality can be recognized. Some information on normal levels has been published by Albrink et al.,[5] who found a level of less than 5.4 mEq. per L. in 95 per cent of seventy-three healthy men twenty to twenty-nine years of age. Although this level was obtained by a different method, it is close to the level of 5.2 mEq. per L. (ninety-fifth percentile) obtained in the same age group in this study. In 248 healthy men aged forty to sixty-nine Albrink et al. found the mean level higher than in the younger group. Forty per cent of this older group had a value in excess of 5.4 mEq. per L. They speculated that these high levels may be predictive of future vascular disease.

Normal values for South African Europeans have been established by Antonis and Bersohn utilizing the method of Van Handel and Zilversmidt used in this study. Conversion of their results from mg. per cent to mEq. per L. of triolein yields a value of 3.0 ± 0.9 mEq. per L. for men under thirty, a value again close to that obtained in this study and to that obtained by Albrink et al. It is of considerable interest that in fifteen healthy men aged forty-one to sixty these investigators also found the considerably higher mean level of 5.9 mEq. per L. These two previous studies thus afford examples of abnormal triglyceride levels in middle-aged men and are in keeping with our experience in the same age group.[13] On the basis of levels found in young men in this study, it is believed that a fasting triglyceride level in excess of 5.2 mEq. per L. when determined by the method of Van Handel and Zilversmidt is probably abnormal. In view of the fact that the maximum triglyceride level observed after a 70 gm. fat meal in an admittedly small group

TABLE IV
Serum Optical Density and Fasting and Postprandial Lipid Levels After Ingestion of 30 gm. Fat Meal

Level	Triglyceride (mEq./L.)	Lipid I^{131} (% of dose)	Serum Optical Density	Cholesterol (mg. %)
Fasting.....	3.5 ± 1.0	...	0.079	201 ± 34
After 3 hours..	4.5 ± 2.0	2.1 ± 1.3	0.138	203 ± 34
After 5 hours..	5.4 ± 2.1	4.0 ± 1.6	0.346	206 ± 35
After 7 hours..	4.4 ± 2.2	3.3 ± 1.3	0.184	206 ± 43
After 9 hours..	3.2 ± 0.9	1.5 ± 0.7	0.076	217 ± 36

TABLE V
Serum Optical Density and Fasting and Postprandial Lipid Levels After Ingestion of 10 gm. Fat Meal

Level	Triglyceride (mEq./L.)	Lipid I^{131} (% of dose)	Serum Optical Density	Cholesterol (mg. %)
Fasting.....	2.7 ± 0.3	...	0.085	195 ± 32
After 3 hours..	3.1 ± 0.6	1.9 ± 1.2	0.089	185 ± 35
After 5 hours..	3.6 ± 0.8	3.5 ± 1.5	0.122	192 ± 32
After 7 hours..	2.8 ± 0.9	2.6 ± 1.7	0.098	185 ± 32
After 9 hours..	2.4 ± 0.3	1.1 ± 0.6	0.065	184 ± 32

of ten subjects was 9.0 mEq. per L., it would, nevertheless, seem appropriate to consider any nonfasting triglyceride level exceeding 9.0 mEq. per L. as probably abnormal. If a large number of postprandial blood samples were analyzed for triglyceride content, it is possible that normal levels in excess of 9.0 mEq. per L. may occur after consumption of meals containing more than 70 gm. of fat. As the serum triglyceride level at any time, and particularly in the first five hours after ingestion, is dependent both on absorption into and removal from blood, it is possible that the rate of the former process may limit the effect of any large fat meal on increasing the serum level. This may account for the fact that the magnitude of lipemia was approximately equal after the 30, 50 and 70 gm. meals. The fact that some subjects had a small increase in the triglyceride value after a 70 gm. meal would also suggest that the process of fat removal may be rapid. The concept of a normal maximum postprandial level of triglyceride may be useful when applied as part of a screening procedure. When triglyceride levels in large populations are to be determined, obtaining fasting blood samples may be impossible.

In a previous study[10] it was demonstrated that radioactive lipid levels tended to parallel triglyceride and optical density measurements when I^{131} triolein was fed with a 70 gm. fat meal in the form of heavy cream. In this study a constant amount of I^{131} triolein (100 μc.) was fed with varying amounts of fat, with a view to observing the influence of meal size on radioactive lipid levels. More physiologic meals were substituted here for the heavy cream used previously. Whereas it was anticipated that small amounts of ingested fat would be followed by rapid removal from the blood stream and would be associated with lower radioactive lipid levels, a comparison of the lipid I^{131} levels in the four groups revealed no difference at the corresponding times, despite the wide difference in meal size (Tables II–V). Lipid I^{131} levels differing significantly from all others were noted in student R. G. It is believed that the abnormally high levels observed at five to nine hours in this student indicated a delayed rate of removal of this fat. The higher absolute increase of triglyceride level in this student after a 10 gm. fat meal, *viz.* 2.2 mEq. per L. compared with 0.8 mEq. per L. in the rest of the group, is thought also to bear out the findings revealed by the radioactive fat. It is believed that this student suffers from idiopathic hyperlipemia and that his persistently elevated fasting triglyceride levels are attributable to delayed removal of fat ingested the previous day. It would appear from the studies of Hirschhorn et al.[17] that idiopathic hyperlipemia is not an uncommon disorder. Brown[18] has discussed the similarity of the lipid defect in this disorder with that found in ischemic heart disease.

In a recent study of postprandial lipemia by Stutman et al.[11] emphasis was placed on the value of nine hour postprandial serum translucency measurements as a means of separating healthy persons from those with ischemic heart disease. The simplicity of this measurement certainly has much to recommend it. In a previous study by this group[10] significant differences between mean nine hour serum translucency measurements were noted for healthy subjects and those with ischemic heart disease. We found this measurement did not, however, serve as a good index for separating persons with and without disease. Whereas this was, as Stutman suggests, possibly related to the age of our healthy control subjects, who may have had unsuspected ischemic heart disease, we have found that six of seventeen young subjects in this study who received 50 or 70 gm. of fat had more turbid serum samples at nine hours than in the fasting state. It is possible that a comparison of the magnitude of this fasting and nine hour difference with that in patients with ischemic heart disease fed the same type of meal may still prove this measurement to have value. If the index of normality is, however, a return to a fasting optical density measurement, the current data do not suggest it is a useful index.

SUMMARY AND CONCLUSION

The paucity of information on triglyceride levels in young subjects prompted this study of serum triglycerides in males and females from birth to thirty years of age. A mean level of 2.6 ± 0.8 mEq. per L. at birth increased within

a few months but did not increase further during the next thirty years. No sex difference in triglyceride levels was noted at any age. In thirty-two twenty year old nurses and thirty-six male medical students in their twenties, mean levels of 4.0 ± 0.8 and 3.4 ± 1.0 mEq. per L., respectively, were obtained. Ninety-five per cent of the latter had values of less than 5.2 mEq. per L. Blood from 161 blood donors, some of whom were not fasting, yielded a mean value of 5.0 ± 2.0 mEq. per L. Ingestion of 10, 30, 50 and 70 gm. fat meals by the medical students indicated that a significant increase in serum triglyceride values followed meals containing 30 gm. or more of fat. Feeding a constant amount of I^{131}-labeled triolein together with the aforementioned fat meals produced radioactive serum lipid levels which did not differ significantly in the four groups. The maximum individual triglyceride level noted after a 50 or a 70 gm. fat meal was 9.0 mEq. per L., and the maximum mean level after these meals was 6.8 ± 1.2 mEq. per L.

It is believed that either a fasting triglyceride level exceeding 5.2 mEq. per L. or a postprandial level exceeding 9.0 mEq. per L. is probably abnormal. Previous studies have indicated that abnormally high triglyceride levels are commonly found in patients with ischemic heart disease. It has also been shown that higher triglyceride levels are found in middle-aged men than in persons under thirty years of age. It is believed that a normal value established in this study will serve as a dividing line for separating middle-aged men into two groups. Speculation as to whether or not high triglyceride levels in healthy middle-aged men are associated with a high incidence of ischemic heart disease will be resolved by our continued study of these two groups. If a correlation between elevated triglyceride levels and future ischemic heart disease is established, these normal values may serve as a guide to the institution of such prophylactic therapy as will hopefully be available in the near future.

ACKNOWLEDGMENT

We wish to express our gratitude to the participants who donated their blood for this study, to Dr. William Peterson for providing us with pediatric blood samples and to Mrs. L. Cerri for the blood bank samples.

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