Liver and Depot Lipids in Children on Normal and High Carbohydrate Diets

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TO BETTER understand the role of dietary carbohydrate in lipid metabolism, a detailed examination of lipid from the liver and adipose tissue was carried out in children from tropical countries in whom the main cause of death was a state of malnutrition associated with a high carbohydrate-low protein diet. Children with this condition have fatty livers and an abundance of adipose tissue. The findings in these children were compared with those in children living in the same environment in whom death had not been primarily due to malnutrition and with findings in normal children living in the British Isles. Previous work in animals,[1,2] has shown that on a low protein intake the composition of both the liver and depot lipids is related not only to the amount, but also to the type of carbohydrate consumed. Although it was not possible to assess with any exactitude the composition of the diet of the subjects in this series prior to death, it was thought that comparison of lipid changes in children with high carbohydrate intakes with those found in animals in which dietary carbohydrate intake could be measured might also be useful.

METHODS

Approximately 100 gm. fresh liver and 20 gm. perirenal adipose tissue were removed postmortem and frozen. The specimens were sealed in plastic bags and transported by air in carbon dioxide snow in a vacuum flask. The samples were obtained from the Departments of Pathology at the University Colleges of the West Indies (fourteen samples) and Western Nigeria (thirteen samples). Six specimens were obtained from children of the same age group in London who suffered a sudden accidental death. All samples came from children one to four years old, and the diagnosis was made postmortem by the pathologist. Since kwashiorkor can change imperceptibly into the low carbohydrate-low protein intake type of malnutrition (marasmus) making separation of the two types difficult, all specimens from children with kwashiorkor and marasmus have been put into one group and are referred to as being from the "malnourished" group. In fact, it may be that the proportion of liver lipid is as accurate a measure as any of the extent of the relative carbohydrate excess in the diet. The "control" group consisted of children from tropical countries whose death was not primarily due to malnutrition, and they are, therefore, control subjects rather than normal subjects. The pathologist's report stated that none of the control subjects was malnourished, and the reported cause of death for each is seen in Table I.

TABLE I
Cause of Death in the Control Group

Cause	No.
Gastroenteritis	3
Virus infection	3
Septicemia	2
Road accident	2

Aliquots from the specimens were weighed and dried over sulfuric acid *in vacuo*. The liver lipid was extracted with petroleum ether (boiling point 40° to 60°C.) in a Soxhlet's apparatus for twenty-four hours. Thus the proportion and amount (knowing the liver weight) of liver lipid could be obtained. The adipose tissue lipid was extracted by gently warming the tissue on a watch glass until the lipid had melted and could be poured off. Estimation of total depot lipid was not possible.

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The liver lipid then was fractionated on a silicic acid column[3] into four groups: sterol esters (fraction I), triglycerides and free fatty acids (fraction II), diglycerides and monoglycerides and free sterols (fraction III), and phospholipids (fraction IV).

Portions of the total (unfractionated) liver lipid of each fraction of this lipid and of the total depot lipid were methylated and the fatty acids separated and measured on a gas chromatograph. Cholesterol estimations[4] were carried out on the total liver and depot lipids and on silicic column fractions I and III.

The investigations carried out are summarized in Figure 1.

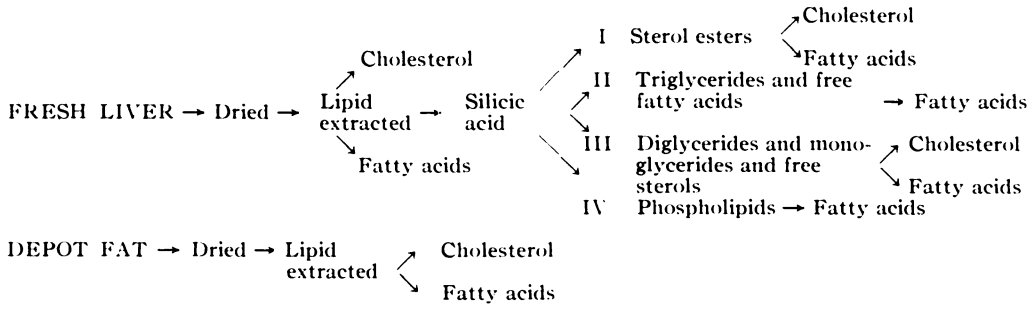

FIG. 1. Scheme of investigations carried out on the liver and depot fat.

RESULTS

Comparisons were made between the malnourished, control and normal groups. Significance has been assessed statistically when $P<0.05$.

Liver

Silicic Acid Fractions. Sterol esters (fraction I): The re isno relation between the amount of liver lipid and the amount of sterol esters. The mean amount of sterol esters is similar in each group (malnourished = 2.1 gm., S.D. = ±2.3; control = 1.4 gm., S.D. = ±1.2; normal = 1.2 gm., S.D. = ±0.5). These results suggest that the amount of sterol ester fraction of liver lipid in the children examined is independent of the amount of total liver lipid and matches the finding that the proportion of the nonsaponifiable fraction of liver lipid in children in South Africa with a similar malnutritional state decreases as liver lipid increases.[5]

Triglycerides and free fatty acids (fraction II): There is an almost perfect straight line relationship between the total amount of liver lipid and total amount of this fraction in the malnourished and control groups. The main increase in liver lipids is in this fraction which consists almost entirely of triglycerides.

Diglycerides and monoglycerides and free sterol (fraction III): With an increase in the amount of liver lipid there is an increase in the amount of this fraction in the malnourished group but not in the control group.

Phospholipids (fraction IV): In the malnourished group the amount of phospholipids increases as liver lipid increases but at a slower rate than the triglycerides or the diglycerides and monoglycerides, whereas in the control group the amount remains constant.

Cholesterol. Total liver lipid: There is no change in the amount of cholesterol in the liver as the liver lipid increases. The mean amount in the malnourished group (3.1 gm., S.D. = ±2.4) is not significantly different from the mean amount in the control group (2.4 gm., S.D. = ±1.1).

Diglyceride and monoglyceride and free sterol (fraction III): Any increase in the amount of this fraction is due to the glycerides and not the cholesterol as there is no correlation between the amount of this fraction and the amount of cholesterol it contains in either the malnourished or control groups.

Fatty Acids. As fraction I did not increase as liver lipid increased, the amount of this fraction available for fatty acid analysis after silicic acid chromatography was too small with the fatty livers and, therefore, it has not been possible to follow fatty acid trends in this fraction.

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The results in each lipid fraction are expressed as *amounts* of fatty acid, when possible. However, in the total liver lipid, phospholipid fraction and depot fat it has not been possible to do this, and each fatty acid is expressed as a proportion of all the fatty acids in that sample as determined directly by gas chromatography. The amounts or proportion in every case are correlated against total amount of lipid present in the liver. As before, any change is reported only when the probability of correlation statistically determined (student's t test) is less than 1 in 20.

Total liver lipid: Table II shows that the fatty acid pattern alters as the amount of liver lipid increases in the malnourished group but not in the control group.

TABLE II
Total Liver Lipid

Group	Fatty Acids (%)					
	C14:0	16:0	16:1	18:0	18:1	18:2
Malnourished...	0	0	+	0	0	−
Control...	0	0	0	0	0	0

NOTE: + = significant rise; − = significant fall; 0 = no change.

Triglycerides and free fatty acids (fraction II): As the free fatty acids are in a relatively small proportion in this fraction and the relative difference in the molecular weight between glycerol and acid radicals under these circumstances is insignificant, it was thought justifiable to convert the proportions of methyl fatty esters into amounts (Table III).

TABLE III
Triglycerides and Free Fatty Acids (Fraction II)

Group	Fatty Acid					
	C14:0	16:0	16:1	18:0	18:1	18:2
Malnourished...	+	+	+	0	+	+
Control...	+	+	+	0	+	+

NOTE: + = significant rise; 0 = no change.

Although this table shows that all fatty acids except stearic acid increase in amount, the rate of increase is not uniform. For instance, the amount of C14:0 acid increases more rapidly and the C18:2 fatty acid increases less rapidly in the malnourished group than in the control group.

Diglycerides and monoglycerides and free sterol (fraction III): After subtracting the amount of cholesterol in this fraction, the amounts of the various fatty acids have been calculated. This is subject to an error, because the amounts of diglyceride and monoglyceride are not known, but it is thought that the error is small and would not materially alter the results.

The rate of increase of the amount of C16:0 and C16:1 acids is less in the malnourished group than in the control groups (Table IV).

TABLE IV
Diglycerides and Monoglycerides and Free Sterol (Fraction III)

Group	Fatty Acid					
	C14:0	16:0	16:1	18:0	18:1	18:2
Malnourished...	0	+	+	+	+	0
Control...	0	+	+	0	+	0

NOTE: + = significant rise; 0 = no change.

Phospholipids (fraction IV): With the wide range of phospholipids, it was not possible to express the proportional findings of the fatty acids in terms of amount (Table V).

TABLE V
Phospholipids (Fraction IV)

Group	Fatty Acid					
	C14:0	16:0	16:1	18:0	18:1	18:2
Malnourished...	0	−	0	0	0	0
Control...	0	0	0	0	−	0

NOTE: − = significant fall; 0 = no change.

Depot Lipid

This lipid was not fractionated on the silicic acid columns (Table VI).

The percentage of linoleic acid in the depot lipid in the malnourished group showed a higher degree of correlation when plotted against the logarithm of the amount of liver lipid.

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TABLE VI
Depot Lipid

Group	Fatty Acid					
	C14:0	16:0	16:1	18:0	18:1	18:2
Malnourished...	0	0	+	0	0	−
Control...	0	−	−	0	+	0

NOTE: + = significant rise; − = significant fall; 0 = no change.

The percentage of linoleic acid in the depot fat in the normal group (from the United Kingdom) is about half that in the comparable control group, a statistically significant difference.

Cholesterol. No correlation is present between cholesterol in the depot fat and the amount of lipid in the liver.

COMMENTS

Dietary fat could only have contributed a small part of the amount of fat in the liver and depots of the malnourished children who were examined. It is unlikely that much of the protein, which was in short supply, would be utilized for the production of fat and, therefore, the major contribution to the liver and depot fat in the malnourished group must be from the dietary carbohydrate. Differences in the lipid composition between the malnourished and control group, therefore, would throw light upon the influence of carbohydrate.

Since it was not possible to record the carbohydrate intake in any of the children, the quantitative characteristics of lipid composition were studied using amount of liver lipid as the reference point. The justification for this procedure lies in the finding that in experimental animals on a diet comparable to that of the malnourished group there is a close correlation between the amount of liver lipid and the mean daily intake of carbohydrate, but no correlation between the amount of liver lipid and the mean daily protein intake.[1] The amount of the liver lipid in the malnourished group, therefore, may be an approximate measure of the extent carbohydrate contributed to the diet.

Dietary carbohydrate may influence the lipids somewhat even in the control group and this, it is thought, would mask differences when similarities are found and would fail to exaggerate any differences noted. As it stands, it is obvious that lipid metabolism is markedly different in the two groups.

Even the fundamental fractions of the liver lipid are different between the two groups. In the malnourished group the high values in liver lipid are derived from the triglycerides, diglycerides and monoglycerides and phospholipids in the liver, but in the control group the high values of liver lipid are confined to the triglyceride fraction. These findings compare favorably with those seen in rabbits on constant protein intakes accompanied by varying levels of sucrose when it was found that an increase in sucrose intake was accompanied by an increase in the amount of the fractions mentioned previously, although in the rabbits there was also an increase in cholesterol esters.

The pattern of fatty acids in the liver shows an increase in the palmitoleic acid and a decrease in the linoleic acid proportions in the malnourished group as opposed to the control group, which shows no change in the unfractionated liver lipid. That the changes in palmitoleic and linoleic fatty acids are associ-

TABLE VII
Similarity Between Fatty Acid Patterns in Rabbits on High Carbohydrate Diets and Those in Children with High Carbohydrate-Low Protein Malnutrition

Lipid	Fatty Acid				
	C16:0	16:1	18:0	18:1	18:2
*Rabbits**					
Liver.....	0	+	..	0	−
Depot....	0	+	..	0	−
Malnourished Subjects†					
Liver.....	0	+	0	0	−
Depot....	0	+	0	0	−
Control Subjects†					
Liver.....	0	0	0	0	0
Depot....	−	−	0	+	0

NOTE: + = significant rise; − = significant fall; 0 = no change.

* Alterations in fatty acid pattern in rabbits as dietary carbohydrate intake increases.

† Alterations in fatty acid pattern in children as amount of lipid in the liver increases.

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ated with the dietary carbohydrate of the malnourished group receive support from the evidence that in rabbits an increase in palmitoleic acid and a decrease in linoleic acid proportions are seen when they are plotted against the daily carbohydrate intake (Table VII). It could be reasoned that the fall in the proportion of linoleic acid is due to a deficient intake of this "essential" fatty acid, but it has been shown in rabbits that this is not so,[1] and there is no reason to believe that the nonfatty liver of the child with marasmus on its relatively low carbohydrate diet has more of this fatty acid in its diet than the child with kwashiorkor.

The decrease in the proportion of linoleic acid in the liver and depot fat as the liver lipid increased warrants more attention since this is believed to be a fatty acid that cannot be synthesized from carbohydrate, and some of the clinical features of kwashiorkor have been thought to be due to "essential" fatty acid deficiency.[6] When the percentage of linoleic acid in the liver lipid is plotted against that in the depot fat for the malnourished group, a significant positive correlation is obtained (Fig. 2). On the other hand, in the control group a decrease in the percentage of linoleic acid in the depot lipid is accompanied by a significant increase in this acid in the liver lipid. These correlations are compatible with the view that excess carbohydrate causes a relative reduction in linoleic acid which would result from the formation of new lipid in the liver and depots containing little, if any, linoleic acid. In the control group the increase

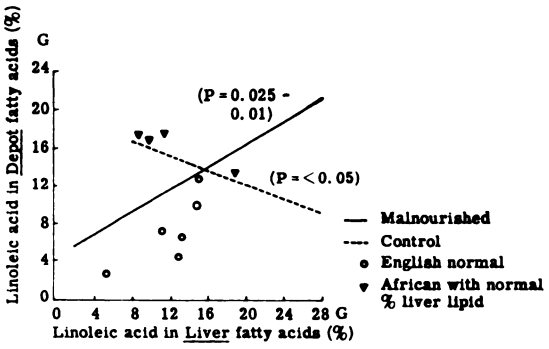

FIG. 2. Percentage of linoleic acid in liver fatty acids plotted against the percentage of linoleic acid in depot fatty acids for the malnourished and control groups.

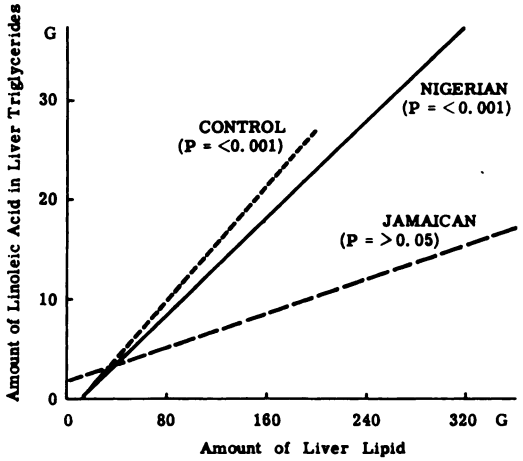

FIG. 3. Rate of increase of linoleic acid in the liver triglycerides of the malnourished groups from Nigeria and Jamaica and the control group. The difference between the slopes of the Nigerian and Jamaican groups is significant ($P = 0.001$).

in linoleic acid in the liver with a corresponding decrease in the depots is compatible with the classic view of fat transference from depots to liver in fatty livers.

The six normal values in children from the United Kingdom seem to follow the direction of the malnourished group (Fig. 2).

It has been shown that in the malnourished group the *proportion* of linoleic acid in various liver lipid fractions and in the depot fat decreases as the liver becomes more fatty, but that the *amount* of linoleic acid in the triglyceride fraction increases as amounts of liver lipid increase. However, if the Jamaican and Nigerian groups are considered separately it is found that there is no significant increase in linoleic acid in the triglyceride fraction in the livers of children from Jamaica but that there is a significant increase in the livers of children from Nigeria. Furthermore, there is no difference in the rate of increase of linoleic acid between the samples from Nigerian children and those of the control subjects (Fig. 3), 70 per cent of which come from Jamaica. The fact that the amount of linoleic acid does not increase in the triglyceride fraction, this fraction being largely responsible for the increase in liver lipid in the malnourished group from Jamaica, whereas it does increase in the group from Nigeria could mean that the

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diet leading to kwashiorkor in the latter group contains more of this fatty acid. However, it is also possible that the type of dietary carbohydrate in this diet may play a part for, as already stated, it has been shown in rabbits that dietary sucrose is associated with less linoleic acid in the liver than is starch. The diet of the Jamaican group before kwashiorkor developed contained a large amount of sugar,[7] whereas in the Nigerian group the principal carbohydrate in the diet was starch.[8]

SUMMARY

The alterations with increasing liver lipid seen in the liver and depot lipids of children who had calorie protein malnutrition are compared with those of the control subjects, that is children whose death was not primarily due to malnutrition. The differences are as follows: (1) there is an increase in the amount of all liver glycerides and phospholipid in the malnourished group, whereas in the control group there is only an increase in the triglycerides; (2) there is an increase in the proportion of palmitoleic acid and a decrease in the proportion of linoleic acid in liver fatty acids in the malnourished group, whereas no proportional changes are seen in any of the liver fatty acids in the control group; (3) similar changes are seen in the fatty acids of the depot lipid and the liver in the malnourished groups, whereas the palmitic and palmitoleic acids increased and the oleic acid increased in the depot lipid of the control group; and (4) in both groups the main accumulation of liver lipid was triglycerides.

The amount of linoleic acid in this fraction increases in samples from Nigeria but not in those from Jamaica. It is suggested that these findings may confirm experimental work in which dietary sucrose is associated with lower levels of linoleic acid than dietary starch.

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REFERENCES

1. MACDONALD, I. Some effects of carbohydrate in experimental low protein diets. *J. Physiol.*, 160: 306, 1962.
2. MACDONALD, I. Some influences of dietary carbohydrate on liver and depot lipids. *J. Physiol.*, 162: 334, 1962.
3. BARRON, E. J. and HANAHAN, D. J. Observations on the silicic acid chromatography of the neutral lipides of rat liver, beef liver and yeast. *J. Biol. Chem.*, 231: 493, 1958.
4. ZLATKIS, A., ZAK, B. and BOYLE, A. J. A new method for the determination of serum cholesterol. *J. Lab. & Clin. Med.*, 41: 486, 1953.
5. MACDONALD, I. Hepatic lipid of malnourished children. *Metabolism*, 9: 838, 1960.
6. BRONTE-STEWART, B. Dietary fats. In: Recent Advances in Human Nutrition, p. 182. Edited by Brock, J. F. London, 1961. J. & A. Churchill, Ltd.
7. RHODES, K. Some observations on the diet of Jamaican children with particular reference to liver disease. *Brit. J. Nutrition*, 6: 198, 1952.
8. NICOL, B. M. The nutrition of Nigerian children with particular reference to their energy requirements. *Brit. J. Nutrition*, 10: 181, 1956.

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