Lipids and Vitamins

Effects of Vitamins on Lipid Metabolism in Diabetes Mellitus

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Brady¹⁾ and Numa²⁾ demonstrated decreased synthesis of long chain fatty acids from ^{14}C -acetate in the liver of diabetic rats. Using alloxan diabetes rats, we have already shown that expiration of $^{14}\text{CO}_2$ following administration of ^{14}C -acetate was normal in spite of diminished incorporation of ^{14}C into liver fatty acids. When ^{14}C -palmitate was used, expiratory $^{14}\text{CO}_2$ was decreased in the face of increased ^{14}C in liver fatty acids³⁾. These findings led us to an assumption that both synthesis and breakdown of fatty acids are decreased in diabetes mellitus. However, these results were not sufficient to fully explain the aberrant lipid metabolism in diabetes. The present investigation was, therefore, undertaken to study the aspect of mobilization or transfer of fatty acids and the influence of vitamins in this disease.

Experimental

1) Animal: Rats of the Wistar strain were made diabetic by an intraperitoneal injection of alloxan monohydrate, 18 mg. per 100 g. body weight, and those which had continuous glycosuria for at least two months were used. The body weight of these rats was approximately 150 g. The blood glucose levels were 238-490 mg.%, whereas those of the control averaged 67.8 ± 5.3 mg.%. Experiments were carried out after overnight fasting.

2) Method: Aqueous suspension of palmitic acid-1- ^{14}C with a specific activity of 2.0 mc/mM. was prepared with the aid of Tween 80, given to rats intravenously in the dose of $5.0 \mu\text{c}/100 \text{ g.}$, and the liver and blood taken at intervals were subjected to analyses.

A homogenate of 2 g. of liver and 5 ml. of plasma pooled from a group of 5 rats were immediately extracted with 50 ml. of ethanol-ether mixture (3:1) at 60°C for one hour. The residue was reextracted with 50 ml. of chloroform-methanol (2:1). Both extracts were combined, evaporated, and dissolved with 2.0 ml. of ether. To this were added 14 ml. of acetone and 6 drops of saturated MgCl_2 , the mixture was left in a cold room overnight and the precipitate was taken as the phospholipid fraction. The supernatant was evaporated, dissolved with 4.0 ml. of ethanol, and then 5 ml. of 1% digitonin and

5 ml. of ethanol were added to remove free cholesterol. The supernatant was evaporated again, dissolved with 10 ml. of hexane and subjected to florisil chromatography according to Carroll's method⁴⁾ to separate esterified cholesterol, triglycerides, mono- and di-glyceride, and free fatty acids.

A preliminary study using cholesterol acetate, tripalmitate and palmitic acid indicated that 80 ml. of 5% ether in hexane is adequate for the elution of esterified cholesterol, 120 ml. of 15% ether in hexane for triglycerides, and 80 ml. of 4% acetic acid in ether for free fatty acids. These lipid fractions thus separated were evaporated, dissolved quantitatively in ethanol, 0.1 ml. of which was mixed with 15 ml. of toluene-PPO and counted in a liquid scintillation counter. Cholesterol ester was chemically determined by Bloor's method⁵⁾, glyceride was determined as palmitate utilizing Hanahan's method⁶⁾ for glycerol, fatty acid was titrated with 0.1 N NaOH after washing thoroughly with water and expressed as palmitic acid, and phospholipid by Fiske's method⁷⁾. The value herein are expressed as specific activity for mg. of each component.

For the determination of CO_2 , the animal container was aerated with a CO_2 free air at a rate of 300 ml./min., CO_2 was trapped in $\text{Ba}(\text{OH})_2$, and precipitate of BaCO_3 was determined for radioactivity.

Results

1) Normal time relation: Palmitic acid-1- ^{14}C was injected intravenously to normal rats, the liver was removed 1/2, 1, 2, 4 and 6 hour later and incorporation of ^{14}C in each fraction was determined. The count of free fatty acid (FFA) was already markedly diminished at 30 minutes, but triglyceride fraction (TG) gave the most count at the same time. The peak of count for phospholipid (PL) was reached at 1 hour and the count stayed rather constant for about 6 hours. Cholesterol ester (Ch-E) showed a peak at 2 hours with a decline thereafter (Fig. 1).

2) Diabetic rats: Palmitic acid-1- ^{14}C was administered to 6 diabetic and 5 normal rats. The result showed that incorporation of ^{14}C into PL was markedly reduced in the diabetic with a tendency of more ^{14}C remaining as FFA. Similarly, in plasma, more of the radioactivity was found in FFA. No significant difference was observed in other fractions, but less CO_2 was expired by the diabetic (Fig. 2).

3) Effect of vitamins on liver lipids in diabetes: Groups of 5 diabetic rats received vitamins as Fig. 3, one dose in the evening of the previous day and a second dose in the morning, and radioactive palmitic acid was administered 2 hours later. The result suggested a tendency of vitamin B_6 enhancing incorporation of ^{14}C in PL fraction. Vitamin B_2 seemed to reduce the count in PL and FFA fractions. With pantothenic acid, count in FFA decreased

Fig. 1. Incorporation of ¹⁴C into lipid fraction of rat liver following palmitic acid-1-¹⁴C injection.

with a slight increase in expiratory CO₂. Nicotinamide reduced count in PL and FFA and slightly increased CO₂. Administration of linoleic acid had an apparent effect of increasing count in PL fraction, decreasing count in TG and FFA with a marked increase of CO₂. A tendency for reduced counts in TG and FFA and increased CO₂ was observed with tocopherol (Fig. 3).

4) Comparison of radioactive palmitic and linoleic acids: Both compounds labelled with ¹⁴C were administered in the same dose to normal and diabetic rats, 5 each. In the normal, incorporation of linoleic acid into liver PL was definitely small, and so was that into Ch-E and FFA; its incorporation in plasma lipid fractions was different, more being found in PL and less in Ch-E and TG. Expiration of CO₂ was greater with linoleic acid than palmitic acid. Similar results were obtained with diabetic rats, except that incorporation of linoleic acid into liver PL was almost normal in spite of a marked reduction in the case of palmitic acid (Fig. 4).

Fig. 2. ¹⁴C specific activities of rat liver and plasma lipid fraction, and expiratory CO₂ at 2 hours after palmitic acid-1-¹⁴C injection.

* B₆: Pyridoxal phosphate 1mg subc. inj.
 B₂: FAD 0.5mg subc. inj.
 PaA: Pantothenic Acid (Co) 2mg subc. inj.
 NAA: Nicotinamide 2mg. subc. inj.
 Linol: Linoleic Acid 10mg. per os
 E: α-Tocopherol 250γ subc. inj.

Fig. 3. Effect of vitamins on ¹⁴C incorporation into lipid fraction of diabetic rat liver.

Fig. 4. Incorporation of palmitic acid-1-¹⁴C and linoleic acid-¹⁴C (U) into lipid fraction of rat liver and plasma.

Discussion

Oxidation of fatty acids is now fairly well understood. It has been shown that a two carbon fragment is split off the molecule at a time and C₂ becomes acetyl-CoA, enters the TCA cycle and is finally broken down to H₂O and CO₂. It is assumed that phospholipids play an important role in the transfer of lipids. Fatty acid is converted to triglyceride through acyl-CoA, phosphatidic acid and diglyceride, and partially to lecithine. Being hydrophobic, lecithine enhances solubility of triglyceride and cholesterol and hence their mobility in blood. Therefore, inference may be warranted that, following administration of palmitic acid-1-¹⁴C its oxidation was accelerated if ¹⁴CO₂ in expiratory air was found to be increased; and mobility of fatty acids was enhanced if greater incorporation of ¹⁴C into phospholipid was demonstrated.

Following this logic, and in view of the lack of impairment in the pathway from acetate to CO₂ as shown in the previous study, the above data may be interpreted that in diabetes, steps in oxidation, particularly β -oxidation, are impaired, and mobility of fatty acids and synthesis of phospholipid are reduced. These alterations may account in part for the increase of lipids, mainly triglycerides, in blood plasma.

Our data demonstrated some effect of each of the tested vitamins on the abnormal lipid metabolism. Vitamin B₆ showed a trend to enhance incorpora-

tion of fatty acid into phospholipid, pantothenic acid, niacin and vitamin E increased oxidation of fatty acids, and linoleic acid had some effect of enhancing oxidation of fatty acids and synthesis of phospholipid. No information has as yet been available concerning direct effect of vitamins on fatty acid metabolism, except that CoA, FAD and DPN are known to be involved in β -oxidation. Thus, the effect of pantothenic acid and niacin to increase CO_2 was not unexpected, yet mechanisms for the demonstrated influences of other vitamins are not clear. It may be that these vitamins are involved in various reactions coupled directly or indirectly with those for fatty acid metabolism. In this connection, it is important to elucidate whether deficiencies of these vitamins occur in diabetes. Our earlier studies have indicated disturbed utilization of vitamins B_1 and B_2 and niacin in large percentages of the patients⁸⁻¹¹.

The experiments first with unlabelled and then with labelled linoleic acid in comparison with palmitate demonstrated against expectation, that incorporation of linoleic acid into liver phospholipid was smaller than the latter, but this relation was reversed in plasma phospholipids. Stein¹², using the same two compounds, demonstrated a smaller incorporation of linoleic acid into the total liver fat, and also found that its incorporation into phospholipid was greater in the early stage but incorporation of palmitic acid became greater after 2 hours. Orth et al.¹³ infused these compounds into dogs to study the incorporation in plasma lipids, and found more linoleic acid in phospholipid fraction and in free fatty acids. Considering these reports, the result of experiment 4 may be taken as indicating that smaller incorporation of linoleic acid into liver phospholipid was due not to decreased synthesis of phospholipids but rather due to a rapid synthesis resulting in its faster transfer to other tissues. Thus, the present study has demonstrated that unsaturated fatty acids enhance synthesis of phospholipids and influence indirectly tissue transfer and breakdown of lipids in general

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