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Metabolism of Octanoate and Its Effect on Glucose and Palmitate Utilization by Isolated Fat Cells (38593)

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The intramucosal metabolism and the physiochemical properties of medium chain fatty acids (1) lead to their transport through the portal vein rather than the lymph, the primary mechanism of transport for long chain fatty acids (2). In the liver, medium chain fatty acids are rapidly oxidized to CO_2 via β-oxidation. However, if the liver is bypassed by means of a postcaval shunt, adipose tissue can be enriched by ingested medium chain fatty acids (4).

Using intact rat adipose tissue, Knittle and Hirsch (5) have shown that tracer amounts of [^{14}C] octanoate are incorporated into tissue triglycerides.

In view of the clinical use of medium chain triglycerides in several malabsorptive disorders and the observed elevation of plasma octanoate under these conditions (1), we studied in some detail the metabolic interrelationships of octanoate, glucose and palmitate metabolism by isolated fat cells.

Experimental procedures. Isolated fat cells from rat epididymal fat pads were prepared essentially according to Rodbell's procedure (6), using Krebs-Henseleit buffer, pH 7.4, containing 4% albumin. In addition to the cells, all assay samples contained (unless otherwise indicated) 10 munits/ml insulin, 10 μmoles/ml glucose and 1 μCi of radioactive substrate, plus the indicated additions, in a total volume of 2.5 ml. After 20 sec aeration with 95%O_2/5%CO_2 the siliconized 25 ml flasks were stoppered with rubber caps fitted with hanging plastic wells (Kontes). After terminating the 2 hr incubation at 37° by the addition of HCl at 0°, NaOH was introduced into the wells to trap CO_2 and the samples were stored overnight. In the experiments in which [1-^{14}C] octanoate was used as the radioactive precursor, absorption of the moderately volatile labeled octanoate along with the $^{14}CO_2$ was minimized by repeating the absorption of CO_2 twice in the presence of about 90 mg of unlabeled octanoic acid

Triglycerides were extracted by the Dole procedure (7) and washed with base to remove fatty acids; an aliquot of the heptane extract was counted. Total triglycerides were determined by the procedure of Rapport and Alonzo (8). The contribution of different lipid classes to the radioactivity of the extracted lipids was determined after separation on a Florisil column (9). Over 98% of the final extract was in the form of triglycerides. Fatty acids recovered after hydrolysis of the triglycerides with alcoholic KOH were separated on a Celite column (10) after mixing with authentic palmitic or octanoic acid.

Sprague–Dawley rats weighing 200–250 g were obtained from Charles River Laboratories and maintained on a regular diet and water *ad libitum*. Collagenase was a product of Worthington Corporation and radiochemicals were obtained from New England Nuclear, Boston, Massachusetts.

Results and Discussion. When isolated adipocytes were incubated in 10 m*M* glucose to which was added 0.5 m*M* of labeled [1-^{14}C] octanoate, [U-^{14}C] alanine, [U-^{14}C] pyruvate, [U-^{14}C] lactate, or [1-^{14}C] acetate, it was found that the rate of triglyceride synthesis from the [1-^{14}C] octanoate was much higher than that from the other substrates, while the rate of CO_2 formation from octanoate was low and comparable to that from [1-^{14}C] acetate. The relative rates of triglyceride synthesis and CO_2 formation from equimolar concentrations of octanoate and glucose (2.5 m*M* each) were then investigated, and the results are shown in Fig. 1. At all times the incorporation of label from [1-^{14}C] octanoate into triglycerides was much higher, and into CO_2 much lower, than from [U-^{14}C] glucose.

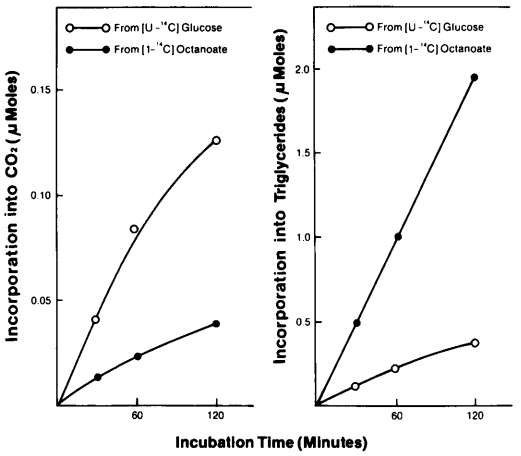

FIG. 1. Incorporation of [U-^{14}C] glucose or [1-^{14}C] octanoate into triglycerides and CO_2 by isolated fat cells. Each flask contained both substrates at concentrations of 2.5 m*M*, with either [U-^{14}C] glucose (○——○) or [1-^{14}C] octanoate (●——●) as the labeled precursor, and cells corresponding to 157 mg of cell triglyceride. Values are expressed as μmoles of labeled substrate converted to product, and are the means of duplicate experiments.

The relative rates of CO_2 and triglyceride formation from [1-^{14}C]-octanoate as compared to that from other substrates and [U-^{14}C] glucose (Table I) suggested that there was extensive direct esterification of octanoate, without prior β-oxidation to acetyl CoA. This was shown by separation of the products formed from [1-^{14}C] octanoate into lipid classes using Florisil columns, and after alkaline hydrolysis of the triglyceride fraction, by cochromatography with authentic fatty acids on reverse-phase Celite columns.

The diet of the donor animal has a pronounced effect on the capacity of fat cells for lipogenesis from glucose (11), but dietary changes such as fasting for 24 hr or feeding a high-fat diet had little effect on the incorporation of [1-^{14}C] octanoate.

The extensive incorporation of [1-^{14}C] octanoate or [1-^{14}C]-palmitate into cell triglycerides was greatly depressed (90%) when glucose was omitted from the incubation medium. Glycerol could not substitute for

TABLE I. RELATIVE CONTRIBUTIONS OF GLUCOSE, OCTANOATE, AND PALMITATE IN THE FORMATION OF CO_2, GLYCERIDE GLYCEROL (GG), AND ESTERIFIED FATTY ACIDS (FA).

Substrate	Radioactive precursor: [U-^{14}C] glucose CO_2	GG	FA	[1-^{14}C] octanoate CO_2	GG	FA	[U-^{14}C] palmitate CO_2	GG	FA
	μmoles[a]								
Glucose, 10 m*M* + octanoate, 0 m*M*	172	168	200	—	—	—	—	—	—
Glucose, 10 m*M* + octanoate, 0.25 m*M*	225	195	229	0	6	323	—	—	—
Glucose, 10 m*M* + octanoate, 0.50 m*M*	204	208	213	0	16	675	—	—	—
Glucose, 10 m*M* + octanoate, 1.0 m*M*	126	227	40	0	10	942	—	—	—
Glucose, 10 m*M* + octanoate, 2.5 m*M*	67	147	4	9	10	847	—	—	—
Glucose, 10 m*M* + octanoate, 5.0 m*M*	73	188	4	17	22	1250	—	—	—
Glucose, 10 m*M* + palmitate, 1.0 m*M*	260	493	110	—	—	—	2	3	2583
Glucose, 10 m*M* + palmitate, 2.0 m*M*	299	563	51	—	—	—	2	4	2773

[a]Fat cells corresponding to 93 mg of cell triglyceride were present in each flask. Other conditions were as outlined under "Experimental Procedures". Results are averages of duplicate experiments and are expressed as μmoles of substrate converted to product.

glucose, and this is in line with the low activity of glycerol kinase in adipose tissue (12).

Octanoate strongly inhibits pyruvate oxidation in isolated mitochondria (13). It was of interest to see whether this effect was demonstrable in intact fat cells and to assess the contribution of such an inhibitory influence on the relative rates of glucose and octanoate metabolism. Table I shows that both esterified fatty acids and CO_2 formation from [U-^{14}C] glucose were drastically reduced when more than 0.5 m*M* octanoate was present in the medium.

At any octanoate concentration, total triglyceride synthesis from glucose plus octanoate was much higher than that obtained from glucose alone. Concentrations of octanoate below 0.5 m*M* caused an increase in the conversion of glucose to triglycerides and to esterified fatty acids. The same effect was observed by Felber *et al.* (14) with the addition of long-chain fatty acids to adipose tissue incubation medium. This facilitation of fatty acid synthesis was attributed to the fact that free fatty acids taken up and esterified by the tissue require ATP for activation and NADH for the synthesis of glycerol phosphate. Platt (15) has suggested that both the generation of reducing equivalents (NADH) in the cytoplasm, in excess of that required for fatty acid synthesis, and the obligatory generation of ATP in adipose tissue associated with the conversion of carbohydrate to fat, provide a regulatory control for adipose tissue lipogenesis. The increase in glucose utilization in the presence of exogenous octanoate could explain the hypoglycemia induced by the infusion of medium-chain triglycerides to intact dogs, independently of the increased insulin secretion observed by Bach *et al.* (16).

When the effects of octanoate on glucose metabolism were compared with those of palmitate, marked differences as well as similarities were observed. Both caused a marked depression of fatty acid synthesis when present at millimolar concentrations, but the effect was much more pronounced with octanoate than with comparable concentrations of palmitate. This is consistent with the idea of an inhibitory effect on pyruvate dehydrogenase by octanoate, which is much more marked than the effect of long-chain fatty acids on this enzyme (13).

The formation of glyceride glycerol from [U-^{14}C] glucose was stimulated by the presence of palmitate and to a lesser extent by levels of octanoate up to 1.0 m*M*. The presence of 2.0 m*M* palmitate caused a 3.5-fold increase in net glyceride glycerol synthesis from the labeled glucose; maximum stimulation with octanoate (obtained with 1.0 m*M*) was about 1.3-fold. The increase in the formation of glyceride glycerol was far below the amounts required for the esterification of the [^{14}C] palmitate or [^{14}C] octanoate found in the triglyceride fraction. When 2 m*M* palmitate was present, there was a 13-fold increase in the total esterified fatty acids synthesized over the synthesis obtained with glucose alone.

Since the net glyceride glycerol formed from the labeled glucose in the presence of palmitate or octanoate cannot account for all of the newly synthesized glyceride fatty acids, and since the adipose tissue has a limited capacity for the reactivation of glycerol formed from hydrolysis of triglyceride to glycerol phosphate, it must be concluded that in fat cells the esterification of the added fatty acids must occur mainly via the monoglyceride pathway (17). This pathway has recently been demonstrated to exist in adipose tissue (18) and it probably contributes to the large amounts of glyceride fatty acids constantly turned over by adipose tissue. This can also explain the small contribution of glucose to the newly synthesized glycerides in adipose tissue (19).

The results in Table I suggest that the rate of incorporation of palmitate is higher than that of octanoate. It was of interest to learn whether the simultaneous presence of the two fatty acids would reveal any mutual interactions with respect to the rates of esterification. Table II summarizes the results of experiments showing that the presence of increasing amounts of palmitate in the incubation medium greatly reduced the rate of octanoate esterification. At a ratio of palmitate to octanoate of 1:4, the esterification of octanoate was depressed to 7% of the level obtained in the absence of palmitate. Under similar conditions, the addition of

TABLE II. COMPETITION BETWEEN PALMITATE AND OCTANOATE FOR INCORPORATION INTO TRIGLYCERIDE (TG).[a]

Substrates	TG	Substrates	TG
[U-^{14}C] Palmitate, 0.5 m*M*		[1-^{14}C] Octanoate, 0.5 m*M*	
+ Octanoate, 0 m*M*	1197	+ Palmitate, 0 m*M*	707
0.25 m*M*	1173	0.25 m*M*	641
0.5 m*M*	1185	0.5 m*M*	522
1.0 m*M*	1176	1.0 m*M*	270
2.0 m*M*	1043	2.0 m*M*	51
[U-^{14}C] Palmitate, 1.0 m*M*		[1-^{14}C] Octanoate, 1.0 m*M*	
+ Octanoate, 0 m*M*	2372	+ Palmitate, 0 m*M*	1502
0.25 m*M*	2485	0.25 m*M*	1436
0.5 m*M*	2329	0.5 m*M*	1385
1.0 m*M*	2328	1.0 m*M*	675
2.0 m*M*	2268	2.0 m*M*	216

[a] All samples contained glucose 10 m*M* in addition to the substrates indicated, and cells corresponding to 130 mg of cell triglyceride. Results are averages of duplicate experiments, expressed as μmoles of the labeled substrate converted to product.

octanoate had very little effect on the esterification of palmitate (Table II). Therefore, there must be a competition between palmitate and octanoate for the available esterifying capacity of the fat cell, favoring palmitate. These experiments cannot distinguish whether this competition occurs at the activation stage, i.e., in the formation of the CoA esters in the cytoplasm, or at the esterification process itself. One could also suppose there might be preferential esterification of octanoate over palmitate due to its weaker binding to the albumin in the medium resulting in a fatty acid–albumin bond more readily broken on contact with the cell membrane. However, the experiments described exclude this possibility.

Separation of the neutral lipids formed from [1-^{14}C] octanoate plus glucose on a Florisil column showed that ^{14}C-activity was largely (84.5%) recovered in the triglyceride fraction and that there was negligible contamination from labeled free fatty acids. When the triglyceride fractions isolated from such experiments were hydrolyzed and the extracts containing the fatty acid residues separated on Celite columns, the radioactivity was quantitatively recovered with the octanoate carrier peak, with no evidence of conversion to other long or medium-chain fatty acids. Thus incorporation of labeled octanoate into cell triglycerides is taking place by activation and direct esterification. Moreover, the labeling patterns of fatty acids isolated from the triglycerides derived from [1-^{14}C] octanoate, obtained by Schmidt degradation, show that 80–90% of the label is in the carboxyl group.

In summary, all the data obtained from the present study show that adipose tissue cells have a tremendous capacity to incorporate octanoate into triglycerides; under normal conditions, however, the physiological implications of this process are limited because of the competition with the more readily available long-chain fatty acids.

Summary. Octanoate is avidly incorporated into triglycerides by isolated rat adipocytes in the presence of glucose via direct esterification without prior β-oxidation to acetyl CoA. This was shown by separation of the products formed from [1-^{14}C] octanoate into lipid classes using Florisil columns, and after alkaline hydrolysis of the triglyceride fraction, by cochromatography with authentic fatty acids on reverse-phase Celite columns.

The relative contribution of [U-^{14}C] glucose and [1-^{14}C] octanoate to triglyceride synthesis and CO_2 formation were studied under a variety of conditions. Concentrations of octanoate below 0.5 m*M* have a stimulatory effect on the conversion of [U-^{14}C] glucose to CO_2, triglycerides and esterified

fatty acids. However, a marked depression of fatty acid synthesis from [U-^{14}C] glucose was observed in the presence of millimolar concentrations of octanoate.

Octanoate had no effect on the esterification of palmitate, but palmitate strongly depressed the ability of rat adipocytes to esterify octanoate.

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