Lipogenesis in Monkey Liver and Adipose Tissue[1] (36213)

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(Introduced by M. B. Yatvin)

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The carbon pathway and regulation of lipogenesis varies in different tissues, as well as species of animals (1, 2). Studies with human adipose tissue have shown a lack of adaptation of lipogenic enzymes (3), as well as a poor capacity to synthesize fatty acids *de novo* (4). This is in marked contrast to the dramatic changes in enzyme activity and lipogenesis following various metabolic alterations in rat liver and epididymal fat (5). Examples of human liver enzymes have also been described, which show considerable differences from those in rat liver (6). Because of inherent difficulties, studies with human liver cannot be carried out under rigid experimental conditions. The rhesus monkey was, therefore, considered a representative example for metabolic studies in primate liver. In the present communication, adaptive enzyme changes were determined in the liver after fasting and refeeding, a known stimulus for lipogenesis in rat liver. In addition, similar studies were carried out in monkey adipose tissue. The results suggest that the potential for, and control of, lipogenesis in rat and primate liver may be similar, while differences in adaptive properties exist in adipose tissue.

Materials and Methods. Rhesus monkeys, weighing between 8 and 17 lb, were housed in individual cages and fed standard laboratory chow, with additional supplements of fresh fruit and vegetables. Each animal acted as its own control; and tissue samples were obtained prior to, after 3 days fasting, and again after 3 days of refeeding. Liver and omental fat were obtained by open biopsy from animals heavily sedated with phencyclidine HCl (Sernylan) and anesthetized with light ether.

Freshly washed tissues, immediately weighed and homogenized in 0.25 *M* sucrose, were diluted to 10% for liver and 40% for fat. After an initial low speed spin to remove cell debris and the overlying fat cake from adipose tissue, the extracts were centrifuged at 105,000*g* for 1 hr in a Spinco model L-2 ultracentrifuge to obtain the high speed supernatant fraction for experimental determinations. All assays were carried out under standard procedures previously described (4); and results represent maximum velocities, which are linear with respect to both protein concentration and time. The reaction was stopped with 5 vol of a chloroform methanol solution (2:1); and, after saponification and acidification, the fatty acids were extracted with petroleum ether. Aliquots added to 20 ml of Scintisol solution were counted in a Packard liquid scintillation spectrometer. Results are expressed as micromoles of substrate incorporated per hour per gram of wet weight or milligram of protein.

Reaction mixtures contained the following components in 1.0 ml: Experiment with radioactive acetate: 50 μmoles of glycylglycine (pH 7.5); 10 μmoles of ATP; 0.1 μmole of CoA; 0.3 μmole of TPNH; 25 μmoles of $NaHCO_3$; 10 μmoles of $MgCl_2$; 5 μmoles of dithiothreitol; 10 μmoles of citrate; 10 μmoles acetate-1-^{14}C(0.2 μCi/μmole); and enzymes. The experiment with citrate-1,5-^{14}C(0.1 μCi/μmole) was similar, except that acetate was omitted. The experiment with radioactive acetyl CoA was similar, except that reagents for synthesis of acetyl CoA were omitted and 0.2 μmole of acetyl CoA-1-^{14}C (0.1 μCi) was

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[1] This investigation was supported by U.S. Public Health Service Grant AM-09187.

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TABLE I. Comparison of Rates of Lipogenesis in Monkey Liver and Adipose Tissue.

	(nmoles/hr/g of wet wt)	
Substrate	Liver	Adipose tissue
Acetate-1-^{14}C	530 ± 120[a]	160 ± 50
Citrate-1,5-^{14}C	80 ± 20	280 ± 30
Acetyl CoA-1-^{14}C	560 ± 200	310 ± 100
Acetyl CoA-1-^{14}C + malonyl CoA (fatty- acid synthetase)	1480 ± 580	870 ± 330

[a] Standard error; av of 5 animals.

included. The experiment with acetyl CoA-1-^{14}C (0.1 μCi), 0.1 μmole; and malonyl CoA, 0.2 μmole; also included 50 μmoles of potassium phosphate (pH 7.0) and 0.3 μmole of TPNH.

Radioactive substrates were obtained from New England Nuclear, Boston, MA; malonyl CoA and nucleotides (P-L Laboratories, Chicago IL; Scintisol (Isolabs, Inc., Akron, OH; and Sernylan (Parke, Davis, Detroit, MI. All other reagents were of the highest purity commercially available and were prepared in distilled deionized water prior to use.

Results. Lipogenic activity was measured by the incorporation of acetate-1-^{14}C, citrate-1,5-^{14}C, and acetyl CoA-1-^{14}C into fatty acids. The first two radioactive substrates measure the entire sequence of lipogenic enzymes, beginning with either acetyl CoA synthetase, or citrate cleavage enzyme. Radioactive acetyl CoA eliminates these initial reactions and begins the sequence with acetyl CoA carboxylase. The conversion of acetyl CoA to malonyl CoA was also assayed directly using $H_2{}^{14}CO_3$ and the fatty acid synthetase complex with acetyl CoA-1-^{14}C plus malonyl CoA. These determinations represent the entire series of enzymes for the *de novo* synthesis of fatty acids and are a good reflection of overall lipogenic activities in the animal.

Table I gives values for lipogenic enzyme activities in liver and adipose tissue of the rhesus monkey under normal conditions. There appears to be poor incorporation of citrate-1,5-^{14}C into fatty acids, relative to that of other substrates in monkey liver, which would suggest low citrate cleavage enzyme activity. This low rate of citrate incorporation may not be representative of endogenous lipogenesis since the animal, though not fasting, was deprived of food overnight prior to surgical biopsy. The marked increase in citrate cleavage enzyme activity after refeeding (Table II) indicates the functional importance of the citrate pathway during active lipogenesis.

In rat liver and adipose tissue, lipogenic enzyme activities show marked adaptive properties to fasting and refeeding (5, 7, 8). As shown in Table II, monkey liver also demonstrates this capacity. There is a 10-fold and 100-fold overall change from fasting to refeeding conditions when radioactive acetate and citrate, respectively, are used as substrates for fatty acid synthesis. The 20-fold change in incorporation of acetyl CoA-1-^{14}C is undoubtedly the result of adaptive properties of both the acetyl CoA carboxylase and fatty acid synthetase enzymes. Rather small differences in acetyl CoA carboxylase activity between fasting and refeeding conditions were measured directly by the $^{14}CO_2$ fixation essay. The results, which are not included, may not represent the true concentration of the enzyme, since, in a crude system, other products be-

TABLE II. Adaptive Changes in Lipogenesis in Livers of Fasted and Refed Monkeys.

	Fasted (nmoles/hr per:		Refed (nmoles/hr per:	
Substrate	mg of P)	g of wet wt)	mg of P)	g of wet wt)
Acetate-1-^{14}C	2.14 ± 1.40[a]	203 ± 85	25.5 ± 10.8	2038 ± 526
Citrate-1,5-^{14}C	0.03 ± 0.01	3.0 ± 1	3.9 ± 1.0	312 ± 86
Acetyl CoA-1-^{14}C	1.41 ± 0.70	134 ± 53	33.5 ± 14.5	2681 ± 732
Acetyle CoA-1-^{14}C + malonyl CoA	7.82 ± 2.86	743 ± 230	43.7 ± 14.9	3495 ± 985

[a] Standard error; av of 5 animals.

TABLE III. Adaptive Changes in Lipogenesis in Adipose Tissue of Fasted and Refed Monkeys.

Substrate	Fasted (nmoles/hr per: mg of P)	Fasted (nmoles/hr per: g of wet wt)	Refed (nmoles/hr per: mg of P)	Refed (nmoles/hr per: g of wet wt)
Acetate-1-^{14}C	16.8 ± 4.3[a]	168 ± 38	21.5 ± 7.2	225 ± 59
Citrate-1,5-^{14}C	7.2 ± 2.9	114 ± 23	13.1 ± 3.5	260 ± 27
Acetyl CoA-1-^{14}C	24.8 ± 14.1	265 ± 89	34.9 ± 16.3	335 ± 150
Acetyl CoA-1-^{14}C + malonyl CoA	65.7 ± 21.4	660 ± 210	110.2 ± 30.7	1180 ± 300

[a] Standard error; av of 5 animals.

side malonyl CoA could be formed. Further studies will be necessary, however, to determine the rate-limiting potential of acetyl CoA carboxylase for fatty acid synthesis in monkey liver.

In contrast to liver, there is a lack of any adaptive lipogenic enzyme change in monkey adipose tissue (Table III). In this respect, monkey adipose tissue resembles the human rather than the rat. Citrate is, however, a relatively good precursor for fatty acids. The present experiments do not preclude the possibility that, under more prolonged periods of fasting and refeeding or in younger animals, more marked adaptive changes might occur.

Discussion. Induction and repression of enzymes play an important regulatory role in the growth and metabolism of microorganisms. In higher animals, such as the rat, responsive tissues show similar adaptive properties. The present study illustrates that primate liver responds to nutritional changes with a marked fluctuation in lipogenic enzyme activity. Similar results have been published recently for urea-cycle enzymes in monkey liver (9). Isolated studies with human liver have shown an induction of tryptophan pyrrolase with cortisone administration (10) and derepression of *δ*-aminolevulinic acid synthetase in porphyria (11).

It is less certain that induction and repression of enzymes plays as important a regulatory role in mammalian metabolism as it does in microorganisms. For the most part, adaptive changes in rate-limiting enzymes occur far less rapidly than the overall change in macromolecular synthesis. The response of rat liver citrate cleavage enzyme to fasting and refeeding falls behind the overall change in fatty acid synthesis, indicating that it could not act as a rate-limiting step (12). Because of the complexity in mammalian metabolism, it is likely that minute-to-minute control is due to changes in the concentration of substrates and cofactors, as well as activation and inactivation of enzymes by allosteric modifiers. Adaptive changes, as reported here and elsewhere (5, 7, 8), are important, however, in that they contribute to the long-term regulation of biosynthetic and catabolic pathways. It is possible that certain clinical hyperlipidemic states particularly those produced by high carbohydrate feedings might, in some part, be due to increased enzyme activity controlling rate-limiting steps in the biosynthesis of fatty acids and/or glycerol. In fact, certain hypolipidemic drugs of clinical interest have been found to inhibit acetyl CoA carboxylase and reduce lipogenesis in cultured cells from lactating rate mammary gland (13).

The lack of adaptive lipogenic enzyme response in adipose tissue is similar in the monkey and human species and in marked contrast to rat adipose tissue. While it is possible that longer periods of fasting and refeeding or use of younger animals would bring out an adaptive property of monkey adipose tissue, these factors were not required for the adaptive response in liver. In the rat, liver and epipidymal fat respond in identical fashion within the same time period. The significance of the poor adaptive response in monkey adipose tissue is not readily apparent. While it may suggest a poor capacity for the synthesis *de novo* of fatty acids, the required enzymes are present in significant concentrations. However, these studies do suggest that, in most animals, the control of lipogenesis is more similar in liver than adipose tissue.

Summary. Monkey liver has been used as a model system to study adaptive lipogenesis in primates. Fasting and refeeding produced marked fluctuation in overall fatty acid synthesis and related lipogenic enzyme activity. Adaptive enzyme formation does not appear to play a regulatory role in lipogenesis from monkey adipose tissue. Rates of incorporation of citrate-1,5-^{14}C relative to other substrates appears to be sufficiently high in both monkey liver and adipose tissue to account for the citrate pathway in fatty acid biosynthesis.

The authors thank Mr. Terry Spennetta for his excellent technical assistance.

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Received Sept. 1, 1971 P.S.E.B.M., 1972, Vol. 139.