Odd-Carbon Fatty Acid-Enriched Rat: Model for Study of Depot Fatty Acid Turnover[1] (36844)

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Previous studies of fatty-acid turnover in adipose tissue have involved naturally occurring long chain, even-numbered fatty acids. The approach has been to label the adipose tissue by administration of fatty acids, their radioisotopes, or their isotopically labeled precursors. As a result of such studies, summarized by Stein and Stein (1), different estimations of half-life of fatty acids in adipose tissue have been made, ranging from 3 to 187 days in the rat, and up to 750 days in man. A variety of factors has contributed to this wide range of fatty acid mobilization, including differences in species, sex, age, length of experimental feeding periods, proportions of nutrients in the diet and methods of analysis. Also, the adipose tissue label may be influenced by recycling of the labeled fatty acid from other organs as well as reincorporation of the label after breakdown of the original acid.

In the present study, odd-carbon fatty acid incorporation and turnover in adipose tissue were studied in animals fed triundecanoin, a C11 triglyceride. By administration of a medium chain odd-carbon triglyceride, the adipose tissue is readily enriched with undecanoate. This fatty acid is not synthesized *de novo* by the animal and, once released from depot fat as the free acid, does not reappear to any appreciable extent in circulating lipid esters. Thus, adipose tissue enriched with undecanoate may provide a model that permits more valid estimation of fatty acid turnover in the intact animal.

Materials and Methods. Seventy weanling male Sprague Dawley rats approximately 4 wk of age (mean wt 78.4 ± 1.0 g) were fed a nutritionally complete diet and weighed weekly. The fat in the diet consisted of a 7:3 mixture of triundecanoin (Drew Chemical) and corn oil, and provided 30% of total calories. All animals were fed the triundecanoin-rich diet for a period of 4 wk. In order to determine the rate of enrichment of adipose tissue with odd-carbon fatty acids, groups of animals (six each) were killed prior to and at weekly intervals during triundecanoin administration for a period of 4 wk. At these times perirenal fat samples were obtained for analysis. Following enrichment with odd-carbon fatty acids for 4 wk, 10 animals were subjected to total calorie starvation for a period of 7 days. Perirenal fat samples were obtained at 4 and 7 days of starvation. In the remainder of the animals triundecanoin feeding was discontinued and the animals were switched to an identical diet except that corn oil was now the sole source of fat. They were maintained on this diet for an additional period of 4 wk. Prior to and at weekly intervals during corn oil feeding, the animals were killed in groups of six, and samples of perirenal fat were obtained.

The lipids of all fat samples were extracted by a modification of the Folch, Lees and Sloane Stanley procedure (2, 3). The solvent (chloroform–methanol; 2:1) was evaporated under nitrogen at room temperature. Thin layer chromatography (3) of adipose tissue extracts revealed that over 99% of the lipids consisted of triglycerides. The lipids in each extract, weighing approximately 100 mg, were

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transmethylated with 5 ml of 0.5 *N* sodium methoxide in methanol in a Teflon-capped tube and allowed to stand in a water bath (55°) for 1 hr. The tube then was cooled in an ice bath. The solution was neutralized with HCl, and 4 ml of spectrograde *n*-heptane were added, shaken, and allowed to separate into two layers overnight in closed tubes. A 10 μl aliquot of the heptane layer containing the fatty acid methyl esters was injected into a Hewlett Packard 5750 gas–liquid chromatograph (equipped with temperature programming and a hydrogen flame detector), using a 6-ft column (3/16 in. O.D.) packed with 12% diethylene glycol succinate as the liquid phase on 80/100 mesh Gas-Chrom P, and a carrier gas flow rate of 50 ml/min. In order to insure detection of undecanoate, as well as the longer chain fatty acids, temperature programming was begun at 120° and was increased by 4°/min until a temperature of 175° was reached. Under these conditions undecanoate was detected clearly at 138°.

Results. The rate of weight gain (mean ± standard error) of the animals during triundecanoin feeding was 4.1 ± 0.2 g/day, or 5.2 ± 0.2%/day based on initial body weight at weaning. The rate of weight gain following discontinuance of triundecanoin and during corn oil feeding was 3.9 ± 0.3 g/day, or 1.9 ± 0.1%/day based on weight attained at the end of 4 wk of triundecanoin administration. The animals subjected to total calorie deprivation lost weight at the rate of 3.0 g/day, or 1.6%/day based on their body weight prior to starvation.

The fatty acid pattern of adipose tissue at weaning consisted entirely of even-numbered long chain fatty acids. Following triundecanoin administration, the pattern of deposition of undecanoate in adipose tissue revealed that a first order compartment in equilibrium, when exposed to a step-change, would alter its concentration according to an equation $Y = Y_{max}\ (1 - e^{-kt})$. Fitting the data to this model demonstrated that the proportion of undecanoate approached the asymptote (Y max) of 36.5%, while the rate of exchange was 8.0 ± 1.5%/day, and its $t_{1/2}$ was 8.7 days (Fig. 1).

In the group of animals that were subjected to prolonged starvation at the end of 4 wk of triundecanoin feeding, the proportions of undecanoate in adipose tissue at the beginning, on the 4th, and on the 7th day of the fast were, respectively: 32.9 ± 1.7; 32.7 ± 0.4; and 29.4 ± 2.6% of total fatty acids. Starvation up to 7 days did not change significantly the proportion of undecanoate in adipose tissue.

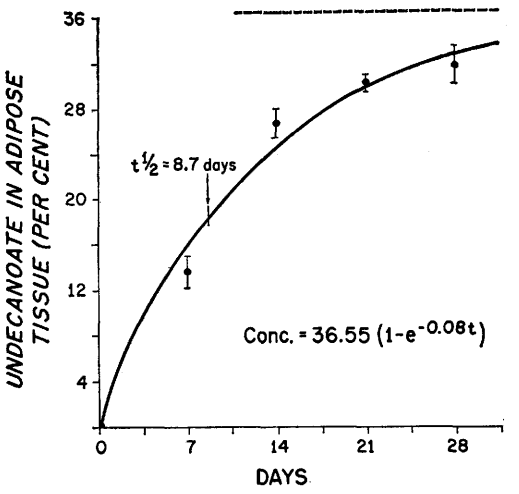

FIG. 1. Incorporation of undecanoate into perirenal fat of rats maintained on a diet containing triundecanoin as 70% of its fat source. The mean ± SE represent experimentally derived molar proportions of undecanoate in adipose tissue. The calculated maximum proportion of undecanoate is indicated by the horizontal interrupted line.

In the animals that were maintained on the corn oil diet following cessation of triundecanoin feeding and during continued growth, the proportion of undecanoate in adipose tissue diminished in an exponential manner. From the disappearance curve for undecanoate, as constructed on a semilog plot according to the equation $Y = Y_0\ e^{-kt}$, the calculated half-life of this fatty acid was 12.1 days, and its tunover rate (K) was 5.7 ± 0.6%/day, as determined by the slope of the disappearance curve (Fig. 2).

Discussion. The data indicate that it is feasible to utilize the odd-carbon fatty acid-enriched rat for study of fatty acid turnover in adipose tissue. The unique feature of this model is that adipose tissue turnover is determined only by the entry of dietary undecan-

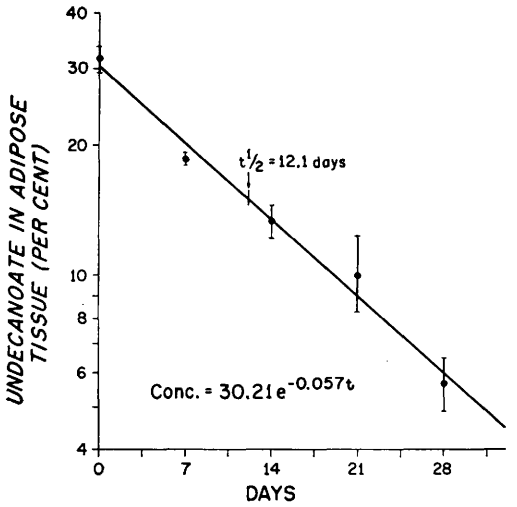

FIG. 2. Disappearance of undecanoate (C11) from perirenal fat of undecanoate enriched rats following cessation of dietary triundecanoin. Animals were maintained on a diet containing corn oil as the sole source of fat. The mean ± SE represent experimentally derived molar proportions of undecanoate in adipose tissue.

oate (in the form of chylomicrons) and its mobilization as free fatty acid, with minimal recirculation of the undecanoate in lipid ester form (4). Undecanoate is not synthesized in the body; yet, this fatty acid is capable of participating in physiologic mechanisms that influence adipose tissue (4). Continued administration of triundecanoin to rats at levels used in the present study for periods up to 12 wk did not result in appreciably greater proportions of undecanoate in adipose tissue (5). Despite the fact that undecanoate constituted 70% of dietary fatty acids, adipose tissue undecanoate did not exceed 36%. This can be explained by the fact that only 50% of orally administered undecanoate enters the systemic circulation as a part of chylomicron triglyceride, the remainder travels in the portal vein (6). Thus, the theoretical maximal incorporation of undecanoate into adipose tissue appears to have occurred under the conditions of the present study.

Since the proportion of undecanoate in adipose tissue did not change significantly during progressive starvation, there does not appear to have been preferential release of this fatty acid. This phenomenon has been observed in previous studies, showing relative stability of adipose tissue fatty acid composition in response to starvation (7–9). However, upon cessation of triundecanoin administration, undecanoate incorporation into adipose tissue necessarily ceases, while its mobilization continues. In contrast to the pattern of incorporation of undecanoate which followed a simple order kinetic, the disappearance of this fatty acid from adipose tissue was exponential. During this phase, fatty acid accretion to adipose tissue would consist only of even-numbered long chain fatty acids derived from dietary and endogenous sources (10, 11). Since this accretion would not induce an exponential decrease in undecanoate, the modifying influence of newly added even-numbered fatty acids should not be significant. A kinetic behavior for fatty acid disappearance from adipose tissue similar to that of undecanoate has been described for even-numbered long chain fatty acids, such as palmitate and linoleate (9). Yet, unlike the long chain fatty acids, undecanoate, once it leaves the adipose tissue as free fatty acid, does not reappear in circulating phospholipids or cholesterol esters; only a small proportion of undecanoate not exceeding 3% appears in circulating triglycerides (4, 5). The mechanism of the lack of appreciable recirculation of undecanoate may be related to the fact that medium chain fatty acids are rapidly and virtually completely oxidized (12). Thus, the half-life of 12 days for undecanoate in the rat may more accurately reflect fatty acid turnover in adipose tissue than previously reported values derived from studies with long chain even-numbered fatty acids (1, 9).

Summary. Administration of triundecanoin to young rats results in substantial enrichment of adipose tissue with undecanoate. This fatty acid is not synthesized *de novo* and does not recirculate in lipid ester form in significant quantity once it is released from adipose tissue. Incorporation of undecanoate into adipose tissue triglyceride proceeds linearly toward an asymptote and its disappearance follows in an exponential manner, thus providing a new model for the study of adipose tissue fatty acid kinetics.

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