Lipogenesis in rabbit adipose tissue

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Abstract Previous reports that rabbit adipose tissue does not synthesize fatty acids at significant rates led us to study in detail the pathways of lipogenesis and glyceroneogenesis in this tissue. We found that rabbit adipose tissue has a low capacity for de novo fatty acid synthesis from glucose but a high capacity for synthesis from pyruvate and acetate. The tissue can also convert pyruvate to glyceride-glycerol via the dicarboxylic acid shuttle and gluconeogenic pathways. Experiments with hydroxycitrate, a potent inhibitor of citrate cleavage enzyme, demonstrated that this is an obligatory enzyme in lipogenesis from pyruvate. The lipogenic system of rabbit adipose tissue resembles that of a ruminant in that it is adapted to utilize acetate rather than glucose. However, in contrast to ruminant tissues, the limited ability to convert glucose to fatty acid results not from a deficiency in the enzymes concerned with the transport of acetyl units out of the mitochondria but from a block prior to the level of pyruvate, most likely at the hexokinase and pyruvate kinase reactions.

Supplementary key words de novo fatty acid synthesis · transport of acetyl units · glyceroneogenesis · ruminant · nonruminant

In a study on glucose metabolism in adipose tissue of several species, Di Girolamo and Rudman (1) noted that epididymal and perirenal adipose tissue from rabbits metabolized glucose at a lower rate than did adipose tissue from rats. The very low capacity of rabbit adipose tissue to convert glucose to fatty acid was most striking, the rate being less than 10% of that observed with rat adipose tissue. Rudman and Di Girolamo (2) concluded that fatty acids are not formed de novo at a significant rate in rabbit adipose tissue.

Herbivores such as the rabbit have enlarged hindguts, in which dietary carbohydrate is broken down to volatile fatty acids through microbial action (3). There is considerable evidence to suggest that, in the rabbit, these volatile acids, mainly acetic acid, are absorbed by the colon (4). Thus, the colon of the rabbit functions in a manner similar to the rumen and omasum of ruminants. In the ruminant, the volatile acids acetic and butyric are probably the major precursors for lipogenesis (5–8).

We have therefore investigated whether the adipose tissue of the rabbit resembles that of a ruminant in that it is adapted to utilize acetate rather than glucose for de novo lipogenesis.

EXPERIMENTAL

Animals

Young male rabbits of the New Zealand White strain (1.5–2.5 kg) were used. The rabbits were fed Purina rabbit chow and water ad lib. and were killed by injection of 20 ml of air into the ear vein. Young male rats of the Long-Evans strain (250–300 g) were fed rat breeder chow (Feedstuffs Processing Co., San Francisco, Calif.) and water ad lib. and were killed by a blow on the neck. The rat diet contained 23.6% protein, 4.3% fat, 3.7% fiber, and 8.4% ash; the rabbit diet contained 15% protein, 2.4% fat, 14.6% fiber, and 7.3% ash, with carbohydrate accounting for most of the balance in both cases. All animals were maintained in a regular light–dark (5 a.m./5 p.m.) cycle and were killed between 8 and 10 a.m.

Tissue incubations

Perirenal adipose tissue was removed as rapidly as possible, rinsed at 37°C (9) in Krebs-Henseleit bicarbonate buffer (10), and gently blotted dry. Portions of tissue 1–2 mm thick were weighed (90–130 mg) and transferred to the incubation media, contained in 25-ml conical flasks with center wells. The incubation media contained the various substrates in 2 ml of Krebs-Henseleit bicarbonate buffer, and the atmosphere consisted of 95% oxygen and 5% carbon dioxide. Flasks were incubated for 3 hr at 37°C. Radiochemical yields of carbon dioxide were determined (11), and adipose tissue was rinsed in Krebs-Henseleit bicarbonate buffer and gently blotted dry. The tissue was then homogenized in 10 ml of chloroform–methanol 2:1 (v/v), and the lipid extract was washed by the procedure of Folch, Lees, and Sloane Stanley (12). The solvent

was evaporated under N_2 and the lipids were dissolved in 10 ml of hexane. The hexane extract was washed twice with 10 ml of water and evaporated to dryness under N_2. The lipids were saponified at 90°C overnight with 0.8 ml of 4 M KOH and 0.5 ml of ethanol in loosely stoppered tubes. The stoppers were removed and heating was continued for 2 hr to ensure removal of ethanol. In the initial experiments, extraction of nonsaponifiable material was carried out prior to acidification. However, the amount of radioactivity recovered was negligible, and this practice was discontinued. The reaction mixtures were acidified with 1.0 ml of 12 M HCl, and the fatty acids were extracted by shaking twice with 5 ml of hexane. A portion of the extract was evaporated to dryness and radioactivity was determined by liquid scintillation spectrometry. The radioactivity in the aqueous phase was assumed to represent glyceride-glycerol, as water-soluble material present prior to saponification was removed by the procedure of Folch et al. (12). On the average, about 88% of the radioactivity in the total lipid fraction was recovered in fatty acid plus glyceride-glycerol fractions.

In this paper all the results are expressed on a per gram wet weight basis. Since we used animals of the same sex and of approximately the same age and weight and fed them the same diet, differences in cell size for animals of the same species would be expected to be small. The protein content per wet weight of tissue was approximately the same in rat and rabbit, so expressing the results on a protein basis did not change the interpretation.

Preparation of subcellular fractions

Adipose tissue to be used for enzyme assays was rinsed in 0.25 M sucrose at 37°C and homogenized quickly (20–30 sec) in 0.25 M sucrose (2 vol/g) at 30–37°C using a Potter-Elvehjem homogenizer. The homogenate was cooled to 0°C, and nuclei and cell debris were removed by centrifugation at 1200 *g* for 10 min. The mitochondrial fraction was sedimented at 13,000 *g* for 20 min and the microsomal fraction at 100,000 *g* for 60 min. The final supernatant fraction is referred to as the cytosol. Particulate fractions were resuspended in the original volume of 0.25 M sucrose and recentrifuged at the appropriate speed to sediment the particles. The washed particulate fractions were gently homogenized in 0.25 M sucrose, approximately one-fifth of the original homogenate volume.

Gas–liquid chromatography

Fatty acids were methylated with diazomethane (13) and chromatographed isothermally at 190°C on a column (6 ft × 0.5 in) containing 15% diethylene glycol succinate on Chromosorb W (80–100 mesh). A column flow rate of 60 ml of argon/min was used. Methyl esters, determined with an ionization detector, were trapped either in scintillation fluid or in hexane as they emerged from the column. For analytical work, scintillation fluid was used to trap the esters, and the radioactivity was determined directly. For preparative work, the esters were trapped in hexane, and a portion was rechromatographed to verify homogeneity of the sample. The esters were then saponified, and the fatty acids were extracted and subjected to the chemical degradation procedure.

Decarboxylation of labeled fatty acids

Fatty acids synthesized by adipose tissue from [1-^{14}C]-acetate were decarboxylated by a modification of the Schmidt reaction (14). The fatty acid (less than 2 mg) was transferred to a conical flask fitted with a center well, and the hexane was evaporated. A mixture (1 ml) of fuming sulfuric acid–concentrated sulfuric acid 1:5 (v/v) was added to dissolve the fatty acid. A small vial containing 25 mg of NaN_3 was inserted into the flask, and a plastic capsule (Better Equipment for Electron Microscopy, Bronx, N.Y.) was placed in the center well. The flask was sealed with a rubber cap and the pressure was reduced by inserting for a few minutes a needle connected to a vacuum line. The flask was then gently shaken to tip the NaN_3 into the sulfuric acid. The flask was incubated for 2 hr at 70°C with occasional swirling, then cooled to 0°C. A solution (0.5 ml) of 0.5 M NaOH was injected into the center capsule, and the flasks were shaken gently for 2 hr at room temperature to allow absorption of the CO_2. Pressure was then equilized by gently inserting a needle through the rubber cap, and the center capsule was removed, wiped carefully, and transferred to a scintillation vial for determination of radioactivity. The recovery of radioactivity was checked by decarboxylating [1-^{14}C]palmitic acid and [2-^{14}C]palmitic acid, using a range of 1–25 mg of fatty acid. Recovery of $^{14}CO_2$ from [1-^{14}C]palmitate was 100%, and from [2-^{14}C]palmitate, 5%. The proportion of radioactivity found in the carboxyl carbon was used to estimate the percentage of acetyl equivalents anabolized by the de novo and elongation pathways according to the formula (15):

$$P = \frac{100n\left(m - \frac{T}{C}\right)}{\frac{T}{C}(m - n)}$$

where P = percentage of acetyl units anabolized de novo, n = number of acetyl units in the fatty acid, T/C = total radioactivity in the fatty acid/radioactivity in the carboxyl carbon, and m = number of acetyl units added by the elongation pathway, assumed to be 1 in these calculations.

Liquid scintillation spectrometry

Radioactivity of ^{14}C-labeled samples was determined using 10 ml of toluene–ethoxyethanol 2:1 (v/v) containing 50 mg of diphenyloxazole. Radioactivity of ^{3}H-labeled samples was determined using 10 ml of toluene–ethoxy-

ethanol 2:1 (v/v) containing 90 mg of Omnifluor (New England Nuclear). The radioactivity of samples that contained a large amount of water was determined in the same mixture with the addition of a further 5 ml of ethoxyethanol. The efficiency was determined by the internal standard, external standard, or channel ratio method.

Estimation of protein

Protein was determined by the method of Lowry et al. (16).

Materials

Radioactive compounds were obtained from New England Nuclear Corp., with the exception of [3-^{14}C]malate and [2-^{14}C]palmitate, which were gifts from Dr. S. Abraham. Cofactors and enzymes were obtained from Calbiochem, Sigma, or Boehringer Mannheim. Crystalline zinc insulin (25 U/mg) was a gift from Eli Lilly and Co.; it was stored at 0–5°C, pH 3, at a concentration of 1 mg/ml. Hydroxycitrate lactone, a gift from Dr. J. Watson, was converted to free hydroxycitrate prior to use. Radioactive pyruvate was stored frozen at −20°C in an equimolar solution of HCl, as recommended by Von Korff (17); nonradioactive pyruvate was made up fresh for each experiment.

RESULTS

Preliminary experiments confirmed the observations of Di Girolamo and Rudman (1) that, in contrast to rat adipose tissue, rabbit adipose tissue was unresponsive to insulin and had a low capacity for converting glucose to fatty acid. To investigate this latter point further, we compared the utilization of [6-^{14}C]glucose, [3-^{14}C]pyruvate, and [2-^{14}C]acetate. Each of these substrates would be expected to give rise to acetyl CoA labeled in the methyl position. For comparative purposes, identical experiments were performed with rat adipose tissue, as the lipogenic pathway is relatively well documented for this tissue. Substrate concentrations of 20 mM were found to be sufficient to saturate the lipogenic system. Although these concentrations may be unphysiologically high, in order to estimate the maximum lipogenic capacity from a particular substrate or metabolite, it is essential to ensure that the concentration of that compound is not rate limiting. The results are in **Table 1.** The rate of oxidation of [2-^{14}C]acetate was similar in rat and rabbit, but whereas the rabbit adipose tissue was able to convert [2-^{14}C]acetate, in the absence of glucose, to fatty acid at a considerable rate, the rate of conversion of [2-^{14}C]acetate to fatty acid was 30-fold lower in the rat adipose tissue. On the other hand, the ability of rat adipose tissue to convert [6-^{14}C]glucose to fatty acid was much greater (over 100 times) than that of the rabbit. Metabolism of [3-^{14}C]pyruvate by the two tissues was more comparable, conversion to CO_2 and fatty acid being slightly higher in the rat tissue. The incorporation of [2-^{14}C]acetate into fatty acid by adipose tissue from both species was stimulated by glucose, although the effect was much more marked in the rat (70-fold) than in the rabbit (approximately 3-fold), due probably to the high incorporation of acetate in the absence of glucose by the rabbit tissue. Additional experiments with [1-^{14}C]glucose and [6-^{14}C]glucose were performed to determine whether significant pentose phosphate cycle activity could be demonstrated in the rabbit. The values for the ratios of fatty acid from 1-^{14}C:6-^{14}C (2.4:1) and of CO_2 from 1-^{14}C:6-^{14}C (3:1) indicated that, of the glucose metabolized, a significant proportion flowed via the pentose phosphate cycle (18, 19).

Our results confirmed the observation of Di Girolamo and Rudman (1) that rabbit adipose tissue has a low capacity for fatty acid synthesis from glucose but revealed that the conclusion of these workers that fatty acids were not formed at a significant rate by this tissue was probably unjustified.

Estimation of contributions of de novo and elongation pathways to fatty acid synthesis

To determine whether fatty acid synthesis in rabbit adipose tissue occurred largely by the de novo pathway, portions of tissue were incubated with [1-^{14}C]acetate, and the

TABLE 1. Comparison of metabolism of glucose, pyruvate, and acetate by perirenal adipose tissue of rabbit and rat

		Substrate Converted to			
		CO_2		Fatty Acid	
Labeled Substrate	Unlabeled Substrate	Rabbit	Rat	Rabbit	Rat
		nmoles/hr/g wet wt			
[6-^{14}C] Glucose		152 ± 24 (4)	276 ± 27 (3)	119 ± 48 (4)	12,500 ± 3,200 (2)
[3-^{14}C] Pyruvate		1,105 ± 62 (5)	2,790 ± 260 (2)	3,820 ± 390 (4)	4,580 (1)
[3-^{14}C] Pyruvate	Glucose	616 ± 31 (5)	2,270 ± 80 (2)	7,070 ± 360 (4)	8,550 ± 1,100 (2)
[2-^{14}C] Acetate		1,065 ± 110 (5)	1,010 ± 350 (2)	1,803 ± 174 (3)	65 ± 36 (2)
[2-^{14}C] Acetate	Glucose	781 ± 92 (5)	590 ± 20 (2)	6,440 ± 880 (4)	4,700 ± 1,600 (2)

Incubations contained labeled substrates (20 mM) with or without unlabeled glucose (20 mM). Those containing glucose also contained 0.2 unit of insulin. Number of animals is in parentheses.

fatty acids were isolated and decarboxylated (**Table 2**). Palmitic acid was the major product, and the de novo pathway predominated for the synthesis of all fatty acids, 14:0, 16:0, 18:0, and 18:1. It can be calculated that 98.5% of the acetyl units incorporated into these fatty acids arrived by the de novo pathway. Thus, the elongation pathway described by Kanoh and Lindsay (20) for rat adipose tissue does not seem to play an important role in rabbit adipose tissue.

Glyceroneogenesis in rabbit adipose tissue

It has been demonstrated that, in rat adipose tissue, pyruvate carbon is incorporated into glyceride-glycerol by a pathway similar to the gluconeogenic route in liver and kidney (21). Functioning of this pathway is therefore dependent on the presence of both pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Our early experiments had indicated considerable ability of rabbit adipose tissue to convert pyruvate to glyceride-glycerol. To determine whether the incorporation of pyruvate into glyceride-glycerol did indeed proceed via the gluconeogenic pathway or whether it proceeded by some other route (perhaps after entry of pyruvate into the citric acid cycle and exit as malate or oxalacetate), we compared the metabolism of 1-^{14}C-, 2-^{14}C-, and 3-^{14}C-labeled pyruvate (**Table 3**).

As expected, the relative rates of oxidation of the isotopically labeled pyruvate carbons were 1-^{14}C > 2-^{14}C > 3-^{14}C. The incorporations of [2-^{14}C]- and [3-^{14}C]pyruvate into fatty acid were the same, as would be expected if pyruvate were converted directly to acetyl CoA prior to incorporation into fatty acid.[1] The incorporations of [2-^{14}C]- and [3-^{14}C]pyruvate into glyceride-glycerol were also equivalent, ruling out the possibility of pyruvate being converted to oxalacetate via the citric acid cycle. The incorporation of [1-^{14}C]pyruvate into glyceride-glycerol was half that of [2-^{14}C]- and [3-^{14}C]pyruvate, ruling out the possibility of a direct conversion of pyruvate to phosphoenolpyruvate by reversal of pyruvate kinase. Ballard, Hanson, and Leveille (22) have also shown that in rat adipose tissue twice as much [2-^{14}C]- as [1-^{14}C]pyruvate is incorporated into glyceride-glycerol. These authors suggested that the incorporation must proceed via the randomization of the label as a symmetrical four-carbon intermediate, probably fumarate (pyruvate → oxalacetate ↔ malate ↔ fumarate).

The activity of pyruvate carboxylase (180 nmoles/g of tissue/hr at 30°C) is just about sufficient to account for the observed rates of incorporation of [2-^{14}C]- and [3-^{14}C]pyruvate into glyceride-glycerol (100–300 nmoles/g of tissue/hr at 37°C). The activities of some other enzymes involved in glyceroneogenesis (enolase, 3-phosphoglycerate kinase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase) are adequate to account for the rate of pyruvate incorporation. The activity of phosphoenolpyruvate carboxykinase was considerably higher than that of pyruvate carboxylase, 3180 nmoles/g of tissue/hr at 30°C.

These results suggest that, in common with rat adipose tissue, incorporation of pyruvate into glyceride-glycerol in rabbit adipose tissue proceeds via the dicarboxylic acid shuttle and the gluconeogenic pathway.

Role of citrate cleavage pathway in fatty acid synthesis by adipose tissue of rat and rabbit

Our observation that rabbit adipose tissue had a high capacity for converting acetate to fatty acid but a low capacity for fatty acid synthesis from glucose is similar to observations made with ruminant tissues. In the case of ruminants, the block in fatty acid synthesis from glucose is thought to result from the low capacity for translocation of acetyl units from mitochondria to cytosol via the citrate cleavage pathway. However, the high capacity of rabbit adipose tissue for converting pyruvate to fatty acid indicated that

TABLE 2. Products synthesized by rabbit perirenal adipose tissue

Fatty Acid	% Total Incorporation	T/C[a]	% Acetyl Equivalents Incorporated de Novo
14:0	15.7 ± 1.3 (3)	6.82 ± 0.72 (2)	99.6
16:0	60.1 ± 4.6 (3)	7.92 ± 0.06 (4)	99.9
18:0	14.4 ± 2.9 (3)	6.45 ± 0.05 (2)	95.0
18:1	9.8 ± 0.5 (3)	5.70	92.8

Incubations contained 10 mM glucose, 20 mM (160 μCi) [1-^{14}C]-acetate, 0.25 unit of insulin, and 20 mg of rabbit perirenal adipose tissue. Individual fatty acids were isolated by preparative gas–liquid chromatography and decarboxylated as outlined in the Experimental section. The de novo pathway is estimated in terms of the percentage of acetyl equivalents incorporated by this pathway rather than by the percentage of the fatty acid synthesized de novo (Smith and Abraham [15]). See Experimental section for further details. Results are expressed as means ± SE; number of animals is in parentheses.

[a] T/C, total radioactivity in fatty acid/radioactivity in carboxyl carbon.

TABLE 3. Metabolism of isotopically labeled pyruvate by rabbit perirenal adipose tissue

Labeled Substrate	Substrate Converted to		
	CO_2	Fatty Acid	Glyceride-glycerol
	nmoles/hr/g wet wt		
[1-^{14}C] Pyruvate	2775 ± 128	30 ± 2	64 ± 3
[2-^{14}C] Pyruvate	792 ± 46	491 ± 66	126 ± 6
[3-^{14}C] Pyruvate	492 ± 34	535 ± 48	122 ± 8

Incubations, in triplicate, contained 20 mM pyruvate.

[1] We are unable to explain the incorporation of a small amount of [1-^{14}C]pyruvate into fatty acid. The washing procedure was sufficient to remove all water-soluble materials from the lipid extract. According to New England Nuclear Corp., the [1-^{14}C]pyruvate was prepared from cuprous [1-^{14}C]cyanide, via [1-^{14}C]pyruvonitrile, so contamination with [2-^{14}C]- or [3-^{14}C]pyruvate is extremely unlikely.

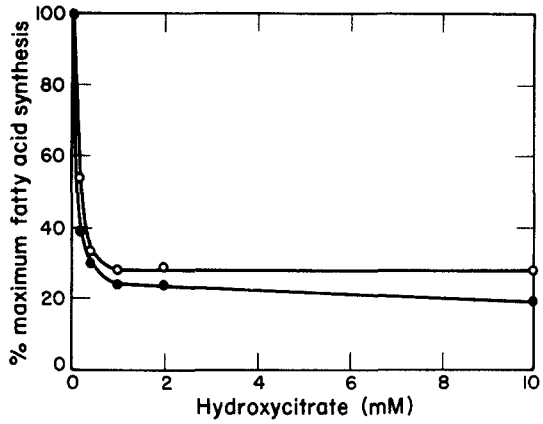

Fig. 1. Effect of hydroxycitrate on fatty acid synthesis by rabbit and rat perirenal adipose tissue. The substrate was [2-^{14}C]pyruvate. Details are given in the Experimental section; O, rat; ●, rabbit.

there was no lack of capacity for translocating acetyl CoA in this tissue. To determine whether pyruvate was incorporated into fatty acid via the citrate cleavage enzyme or by some alternative route, we made use of hydroxycitrate, a potent inhibitor of citrate cleavage enzyme. The inhibition of hepatic fatty acid synthesis by this compound has been shown to be a direct consequence of inhibition of the cleavage enzyme: neither citrate production and oxidation by mitochondria nor citrate transport from the mitochondria is affected by hydroxycitrate (23).

When hydroxycitrate was added to adipose tissue incubation systems, synthesis of fatty acids from [2-^{14}C]pyruvate was inhibited to similar extents with tissue from rats and rabbits (**Fig. 1**). These results provide strong evidence that citrate cleavage enzyme plays a comparable and major role in lipogenesis in both rat and rabbit adipose tissue.

Malic enzyme is also thought to play a role in lipogenesis via the citrate cleavage pathway. This enzyme converts malate to pyruvate, thus generating reducing equivalents for fatty acid biosynthesis. The pyruvate after reentering the mitochondria can be carboxylated to replenish the oxalacetate required for citrate synthesis. The importance of malic enzyme in fatty acid synthesis was assessed on a comparative basis by determining the relative extent of transfer of labeled isotope from [2-^{3}H]- and [3-^{14}C]malate to fatty acid in rat and rabbit adipose tissue. The rationale behind this approach has been discussed extensively by Lamdin et al. (24). In tissues with malic enzyme activity, the tritium at C-2 of malate can be transferred to NADP. The [^{3}H]NADPH formed can subsequently donate the ^{3}H for the reductive steps in the fatty acid synthetase reaction. The carbon at C-3 of malate becomes successively the methyl carbon of pyruvate and of acetyl CoA. Thus, through the functioning of the malic enzyme, both radioactive isotopes are incorporated into fatty acid. In the absence of malic enzyme activity, malate could conceivably be converted successively to oxalacetate, phosphoenolpyruvate, and pyruvate, so that the carbon at C-3 of malate would again become the methyl carbon of pyruvate. In this case, however, the tritium at C-2 would be transferred to NAD and thus would be unavailable for fatty acid synthesis. The net result of this sequence of reactions would be a relatively low ^{3}H:^{14}C ratio in the synthesized fatty acids. The situation is complicated, however, by the presence of the dicarboxylic acid shuttle in adipose tissue. Thus, equilibration of malate with fumarate would result in a shift of half of the tritium from C-2 to C-3 of malate. Only negligible amounts of this tritium would be incorporated into fatty acid because there is a loss of tritium in several ways: (*a*) Some tritium on the methyl group of acetyl CoA will be lost on conversion to citrate intramitochondrially; (*2*) some tritium on the methyl group of acetyl CoA will be lost on conversion to malonyl CoA extramitochondrially; (*3*) the tritium at the active methylene group of malonyl CoA is highly labile, and much of the label would be lost by exchange. Thus, the only route that would allow for appreciable incorporation of tritium label relative to carbon label from [2-^{3}H, 3-^{14}C]malate would be via the malic enzyme reaction. A comparison of the ^{3}H:^{14}C ratios in fatty acids synthesized by different tissues should therefore provide a qualitative indication of the relative involvement of malic enzyme in lipogenesis in the tissues. Lamdin et al. (24) found that the ^{3}H:^{14}C ratio in the liver fatty acids of mice injected with [2-^{3}H, 3-^{14}C]malate was 7:1 but it was only 0.2:1 in carcass fatty acids. These workers were able to conclude that, in the liver, malic enzyme is important for the delivery of reducing equivalents for fatty acid biosynthesis.

In our experiments with rat and rabbit adipose tissue, the incorporation of radioactive malate (1 mM) was found to be stimulated three- to fivefold by pyruvate (40 mM). The reaction proceeded as a linear function with time for 3 hr. The ratio of incorporation into fatty acid of 2-^{3}H:3-^{14}C was significantly lower in adipose tissue of the rabbit than in that of the rat (**Table 4**), indicating a relatively lower contribution of malic enzyme to lipogenesis in the rabbit.

DISCUSSION

It is believed that adipose tissue is an important site of lipogenesis in several species of mammals and that glucose is an important physiological precursor for de novo fatty acid synthesis (25). Consequently, Rudman and Di Girolamo (2) concluded from their observation that rabbit adipose tissue had a poor ability to convert glucose to fat that this tissue was not a major site for lipogenesis. Our experiments clearly demonstrate that rabbit adipose tissue has, in fact, a high capacity for lipogenesis if acetate rather than glucose is the substrate. Physiologically, acetate may be a major

precursor of fatty acids in the rabbit because large quantities of acetic acid are produced and absorbed in the colon of this species (4). Thus, if one considers only the data obtained using glucose and acetate as substrates, one would probably conclude that lipogenesis in rabbit adipose tissue follows a course very similar to that observed in ruminant tissues. However, the results obtained with pyruvate as substrate dispel this notion. The poor ability of ruminant tissues to convert glucose to fat, coupled with the low activities of the enzymes of the pyruvate cycle in these tissues, is interpreted to indicate that lipogenesis from glucose is limited largely because of the low capacity for transfer of mitochondrial acetyl CoA into the cytosol. Yang and Baldwin (26) have in fact shown with cow adipocytes that a large proportion of glucose carbon accumulates as pyruvate and lactate. Rabbit adipose tissue, however, is able to convert pyruvate to fat very rapidly, and because the de novo pathway is the only one operative (Table 2), the acetyl units formed intramitochondrially from pyruvate must be translocated to the cytosol for conversion to fatty acid. The experiments with hydroxycitrate indicate that citrate cleavage enzyme is involved in this process. However, the experiments with the isotopically labeled malates raised some doubt as to whether malic enzyme plays a significant role in fatty acid synthesis in rabbit adipose tissue. Measurement of the malic enzyme activity in rabbit (5.0 ± 1.6 nmoles/min/g wet weight) and rat (2030 ± 852 nmoles/min/g wet weight) adipose tissue also indicated a minor role for malic enzyme in rabbit adipose tissue.

Pyruvate carboxylase can play a dual role in lipogenic tissues: the enzyme is required for the generation of glyceride-glycerol from pyruvate and for the conversion of pyruvate, produced in the malic enzyme reaction, back to oxalacetate. The activity of pyruvate carboxylase in rabbit adipose tissue is sufficient to account for the observed rates of glyceroneogenesis from pyruvate but is probably too low to play a major role in the formation of the oxalacetate required for the production of citrate for fatty acid synthesis. Although the presence of the citrate cleavage enzyme is obligatory for the transport of acetyl equivalents into the cytosol, the requirement for malic enzyme and pyruvate carboxylase could be circumvented if malate (rather than pyruvate) were transported back into the mitochondria. Thus, oxalacetate produced in the cytosol by cleavage would be converted to malate by malic dehydrogenase. The malate would be translocated to the mitochondria and reconverted to oxalacetate by the mitochondrial malic dehydrogenase (27). In support of this idea, we have observed malic dehydrogenase activity in both mitochrondria (157 nmoles/min/mg of protein) and cytosol (587 nmoles/min/mg of protein). Operation of this pathway, however, deprives the cytosol of reducing equivalents that would have been generated in the conversion of malate to pyruvate. In nonruminants, activity of the malic enzyme and the dehydrogenases of the pentose phosphate cycle is believed to supply the reducing equivalents required for fatty acid synthesis. The latter dehydrogenases are present in rabbit adipose tissue at appreciable levels, and in fact a considerable proportion of the glucose metabolized by the tissue flows through this pathway, as evidenced by the experiments with [1-^{14}C]- and [6-^{14}C]glucose. It is possible that in rabbit adipose tissue additional reducing power could be generated if some of the citrate, instead of being cleaved in the cytosol, flowed forward to α-ketoglutarate via an abbreviated form of the citric acid cycle. Isocitrate dehydrogenase is present in high activity in rabbit adipose tissue (316 nmoles/min/g wet weight), and most of the activity is found in the cytosol. In fact, the specific activity of the cytosolic isocitrate dehydrogenase was higher than that of the mitochondrial enzyme in rabbit adipose tissue, whereas the reverse was true in the case of rat adipose tissue. The citrate carbon, which flows to isocitrate and hence to ketoglutarate in the cytosol, could be returned to the mitochondria either by direct diffusion of ketoglutarate (28) or indirectly by a transamination sequence (29). When pyruvate (or acetate) is supplied as the sole carbon source for fatty acid synthesis, the cytosolic isocitrate dehydrogenase may be the main source of NADPH. However, we are presented with another problem when pyruvate is the sole carbon source, as there is no apparent source of cytosolic NADH, required for the conversion of oxalacetate to malate. The question as to how the NADH is supplied for this reaction is also relevant in the case of conversion of acetate to fat in the absence of glucose by ruminant tissues. It is, however, a question that seems to have escaped attention (30). Some NADH could be produced in the glyceraldehyde-3-phosphate dehydrogenase reaction as a result of breakdown of endogenous substrates, but it is doubtful whether this is sufficient.

Rous and coworkers (31, 32) have suggested that oxalacetate might be directly converted to malonyl CoA via an oxidative decarboxylation analogous to the ketoglutarate ↔ succinyl CoA transformation. Support for the existence of such a pathway is somewhat speculative, and we have no evidence to either confirm or deny the occurrence of such a conversion in rabbit adipose tissue.

TABLE 4. Incorporation of [3-^{14}C]- and [2-^{3}H]malate into fatty acids by perirenal adipose tissue from rabbit and rat

	Ratio of [2-^{3}H]- to [3-^{14}C]Malate Incorporated
Rat	0.175 ± 0.022
Rabbit	0.099 ± 0.011

Incubations contained isotopically labeled DL-malate (1 mM) and pyruvate (40 mM). Adipose tissue from four rats (avg wt 255 g) and four rabbits (avg wt 2.3 kg) was incubated in triplicate. The average incorporation of label for each animal was calculated and used to derive the mean ± SEM for the isotope incorporation ratio for each group of animals.

From our results it is clear that the rabbit represents a group of animals that exhibit some of the metabolic characteristics of both ruminants and nonruminants. In common with the ruminants, the rabbit has a very low capacity for the conversion of glucose to fat and a high capacity for lipogenesis from acetate. However, whereas in the ruminant this block in glucose utilization has been attributed to the limited ability of ruminant tissue to transport acetyl equivalents from the mitochondria to the cytosol, this is not so in the rabbit. The rabbit can convert pyruvate to fat at a rate comparable to that observed in other nonruminants. Although the translocation of acetyl equivalents is mediated by the citrate cleavage enzyme in both rabbit and other nonruminants, the rabbit differs from other nonruminants in that the replenishment of mitochondrial oxalacetate is effected with minimal participation of malic enzyme and pyruvate carboxylase. We have compared the activities of most of the glycolytic enzymes in the adipose tissue of rabbit and rat and find that the most significant differences are in the activities of hexokinase and pyruvate kinase, which are approximately 10-fold higher in the rat. In contrast, the key enzymes of the pentose phosphate cycle, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are of similar activity in the adipose tissue of both species. We believe, therefore, that the low capacity of rabbit adipose tissue to convert glucose to fat is probably due to the low activity of two of the key glycolytic enzymes, hexokinase and pyruvate kinase. The relatively high activity of the enzymes of the pentose phosphate cycle ensures that a large proportion of the glucose metabolized flows in this direction.

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