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Metabolism of adipose tissue: incorporation of isoleucine carbon into lipids by slices of adipose tissue*

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SUMMARY

Slices from adipose tissue and liver were incubated with uniformly labeled C^{14}-isoleucine and the respiratory carbon dioxide, fatty acids, and nonsaponifiable lipids were isolated and analyzed for radioactivity. Organic acids were qualitatively identified by chromatography and autoradiography. Adipose tissue oxidized isoleucine to carbon dioxide at a rate greater than that of liver. Conversion to nonsaponifiable lipids was small for both tissues. Recovery of C^{14} from isoleucine C^{14} in fatty acids of adipose tissue was 50 to 100 times greater than recovery in liver slices. C^{14} was recovered in acetate, propionate, methylmalonate, and α-methylacetoacetate in adipose tissue after incubation with isoleucine. The role of acetate, propionate, methylmalonate, α-methylacetoacetate, and isoleucine in biosynthesis of fatty acids in adipose tissue is discussed.

Adipose tissue is now considered to be a metabolically active tissue containing most of the enzymes required to carry on reactions common to other mammalian tissues. Thus, many of the schemes that have been described for utilization, storage, and interconversion of carbohydrates and fats in muscle, liver, and kidney have also been described in adipose tissue. However, the quantitative differences previously observed (1, 2) for the conversion of certain metabolites to long-chain fatty acids suggest that adipose tissue contains a pathway involving branch-chain fatty acids, not found in other tissues.

Propionate, for instance, is believed to be converted to fatty acids by a path that includes methylmalonate as an intermediate:

$$\text{propionate} + (X) \rightarrow \text{methylmalonate} \rightarrow \text{fatty acids.}$$

When C^{14}-bicarbonate was tested as a possible (X) in slices of adipose tissue, no radiocarbon appeared in the synthesized fatty acids (2).

A possible role for isoleucine in connection with these studies was suggested by the work of Coon and co-workers (3). These investigators have shown that isoleucine is degraded in rat liver slices to propionate and other compounds by way of a series of reversible reactions. In order to understand the nature of this novel pathway for the biosynthesis of long-chain fatty acids, the present investigation was undertaken on the metabolism of isoleucine in adipose tissue.

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EXPERIMENTAL

Radioactive Substrates. Uniformly labeled C^{14}-L-isoleucine with a specific activity of 7.35 mc. per mmole was purchased from Nuclear-Chicago Corporation.

Preparation and Treatment of Slices. Male white Swiss mice were fed *ad libitium* an adequate diet of Purina laboratory chow until the time of sacrifice. The adult mice were killed by separation of the cervical vertebrae, and the liver and adipose tissue were rapidly removed. The preparation of slices and incubation procedures were identical with those employed in our previous investigations (1, 2). Further experimental conditions are described in the Tables.

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Analytical Procedures. Collection of CO_2, separation and isolation of lipid fractions, and radioassay methods have been described elsewhere (1, 2). After collection of CO_2, the slices and medium were transferred to a tube and centrifuged. The supernatant fluid was decanted. The tissue was extracted twice with 5 ml. of hot water, and the combined supernatant fraction was treated for chromatography as described below.

Hydrazone derivatives of keto-acids were prepared by grinding the tissue in a Potter-Elevehjem homogenizer in the presence of 2,4-dinitrophenylhydrazine (4). Five mg. of the hydrazine dissolved in 1 ml. of 6 N HCl was used per gram of tissue. The suspension was allowed to stand for 15 minutes and cell debris was separated by centrifugation. The aqueous solution was extracted with three, 15 ml. volumes of a chloroform-ethanol (80:20, v/v) mixture. The hydrazones were then extracted from this mixture with 15 ml. of 1 N Na_2CO_3 and the chloroform-ethanol layer was discarded. The Na_2CO_3 solution was washed with 10 ml. of chloroform-ethanol to remove any unchanged hydrazine. The Na_2CO_3 solution was acidified with 6 N HCl and the hydrazones extracted with three, 10 ml. volumes of chloroform-ethanol. The organic phase was then dried under a gentle stream of air. The hydrazones were separated and identified by paper chromatography as described below.

Radiochromatography. Procedures for identifying methylmalonic acid were described previously (2). Acetic and propionic acids were converted to their ammonium salts and chromatographed with 95 per cent ethanol—NH_4OH (100:1, v/v) as described by Kennedy and Barker (5). The hydrazones of the α-keto acids were dissolved in a minimum amount of 0.1 M glycine-NaOH solution, pH 7.4 and streaked on Whatman No. 2 paper, which had been impregnated previously with glycine solution and dried. The

TABLE 1. CONVERSION OF C^{14}-ISOLEUCINE TO FATTY ACIDS, NONSAPONIFIABLE LIPIDS, AND CARBON DIOXIDE BY SLICES OF ADIPOSE TISSUE AND LIVER*

Experiment	Tissue	C^{14} Recovered as			C^{14} per 100 mg. Fat-free Tissue Recovered as		
		Fatty Acids	Non-saponifiable Lipids	CO_2	Fatty Acids	Nonsaponifiable Lipids	CO_2
		total cpm.	*total cpm.*	*total cpm.*	*percentage*	*percentage*	*percentage*
1	Adipose	191,000	2,180	193,000	1.11	0.01	1.09
2	Adipose	83,000	2,910	103,000	0.38	0.01	0.46
3	Adipose	239,000	2,920	166,000	1.24	0.02	0.86
4	Adipose	94,000	2,730	216,000	0.50	0.01	1.15
5	Adipose	111,000	4,800	186,000	0.49	0.02	0.82
Mean					0.74 ± 0.16 †	0.01 ± 0.00	0.88 ± 0.11
6	Liver	6,720	5,450	141,000	0.01	0.01	0.24
7	Liver	5,280	7,020	145,000	0.01	0.01	0.24
8	Liver	4,730	4,210	117,000	0.01	0.01	0.20
9	Liver	9,010	7,510	122,000	0.02	0.01	0.26
10	Liver	7,500	5,960	120,000	0.01	0.01	0.19
Mean					0.01 ± 0.00	0.01 ± 0.00	0.23 ± 0.01

* 1460 ± 20 mg. of tissue slices were incubated for 3 hours at 37.5°C with 0.001 M C^{14}-isoleucine. The total activity added to each flask was 4.5×10^6 cpm. 14.0 ml. of Krebs-bicarbonate buffer enriched with 0.01 M succinate and 0.011 M glucose was used.

† Standard error of the mean.

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TABLE 2. EFFECT OF PROPIONATE ON CONVERSION OF C^{14}-ISOLEUCINE TO FATTY ACIDS, NONSAPONIFIABLE LIPIDS, AND CARBON DIOXIDE BY SLICES OF ADIPOSE TISSUE AND LIVER *

Experiment	Tissue	C^{14} Recovered as			C^{14} per 100 mg. Fat-free Tissue Recovered as		
		Fatty Acids	Non-saponifiable Lipids	CO_2	Fatty Acids	Nonsaponifiable Lipids	CO_2
		total cpm.	*total cpm.*	*total cpm.*	*percentage*	*percentage*	*percentage*
1	Adipose	2,820	240	6,920	0.08	0.01	0.21
2	Adipose	2,660	100	6,720	0.06	<0.01	0.16
3	Adipose	2,570	240	3,600	0.06	0.01	0.09
Mean					0.07 ± 0.01 †		0.15 ± 0.03
4	Liver	920	310	2,150	0.01	<0.01	0.02
5	Liver	830	300	6,460	0.01	<0.01	0.07
6	Liver	620	720	5,190	0.01	0.01	0.06
Mean					0.01 ± 0.00		0.05 ± 0.01

* 1430 ± 10 mg. of tissue slices were incubated for 3 hours at 37.5°C with 0.001 M C^{14}-isoleucine. The total activity added to each flask was 700,000 cpm. 14.0 ml. of Krebs-bicarbonate buffer enriched with 0.011 M glucose was used. Propionate was also added to a final concentration of 0.01 M.

† Standard error of the mean.

chromatogram was developed in tertiary amyl alcohol:ethanol:water (50:10:40, v/v/v) for 48 hours by the ascending method.

"No screen" X-ray films were used for the preparation of autoradiograms.

RESULTS

C^{14}-isoleucine Experiment. Isoleucine was readily metabolized by adipose tissue as shown by the data in Table 1. This tissue converted isoleucine to long-chain fatty acids in amounts comparable to those observed previously for acetate, propionate, and methylmalonate; a mean value of 0.74 per cent per 100 mg. of fat-free tissue was recovered as C^{14}-labeled fatty acids after incubation with C^{14}-isoleucine. This may be compared to values of 1.60 per cent for propionate-1-C^{14}, 1.25 per cent for acetate-2-C^{14} and 0.52 per cent for methylmalonate (1, 2). When compared to liver, the extent of conversion of isoleucine to long-chain fatty acids was 50 to 100 times greater in favor of adipose tissue.

Adipose tissue was also able to oxidize the isoleucine to CO_2 to a greater extent than liver: 0.88 per cent per 100 mg. fat-free tissue was the average value found for conversion of isoleucine to CO_2 by adipose tissue compared to 0.23 per cent for liver.

Small but detectable amounts of isoleucine were converted to nonsaponifiable lipids by both tissues.

C^{14}-isoleucine Carrier Propionate Experiment. Addition of carrier propionate or methylmalonate to C^{14}-isoleucine was next tested to determine whether these compounds could serve as trapping agents to depress conversion of isoleucine to fatty acids.

Addition of carrier propionate to a final concentration of 0.01 M reduced the conversion of C^{14}-isoleucine to fatty acids by adipose tissue from a value of 0.74 per cent (Table 1) to a value of 0.07 per cent (Table 2). A somewhat less marked depression of about sixfold was also noted in the conversion of isoleucine to carbon dioxide. In the case of liver slices, addition of carrier propionate depressed the conversion of isoleucine to carbon dioxide from a value of

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0.23 per cent to 0.05 per cent per mg. fat-free tissue. There was no corresponding depression in conversion to fatty acids.

C^{14}-isoleucine Carrier Methylmalonate Experiment. Addition of carrier methylmalonate also depressed the conversion of isoleucine to fatty acids in adipose tissue (Table 3), although to a lesser degree than that observed by the addition of carrier propionate. The value was found to be 0.38 per cent of added C^{14}, converted to fatty acids by adipose tissue with carrier methylmalonate as compared to a value of 0.74 per cent without added carrier. Only a slight depression in conversion of isoleucine to carbon dioxide with added methylmalonate was found (Table 3).

A possible explanation for the greater inhibition brought about by the addition of carrier propionate as compared to methylmalonate would be that the former penetrated the cell at a much faster rate than the latter. Or perhaps methylmalonate was converted to an activated form, probably the acyl coenzyme A derivative, at a much slower rate than propionate. A comparison of the rates of oxidation of these compounds (propionate-1-C^{14}, 6.90 per cent and methylmalonate with carboxyl-C^{14}, 0.08 per cent) renders either of these explanations feasible.

Addition of carrier methylmalonate caused no depression in conversion of isoleucine to fatty acid in liver. A slight depression in conversion to carbon dioxide was observed for this tissue (Tables 1 and 3).

Chromatography-Autoradiography. Figure 1 shows recovery of C^{14} in the methylmalonate spot after incubation of adipose and liver tissues with C^{14}-isoleucine. No radioactivity could be detected in the area where succinate would be expected to migrate. Figure 2 shows recovery of C^{14} in acetate and propionate spots for both tissues. α-Methylacetoacetate containing radiocarbon was found after incubation with isoleucine, as shown in the radiogram in Figure 3.

TABLE 3. EFFECT OF METHYLMALONATE ON CONVERSION OF C^{14}-ISOLEUCINE TO FATTY ACIDS, NONSAPONIFIABLE LIPIDS, AND CARBON DIOXIDE BY SLICES OF ADIPOSE TISSUE AND LIVER*

Experiment	Tissue	C^{14} Recovered as			C^{14} per 100 mg. Fat-free Tissue Recovered as		
		Fatty Acids	Non-saponi-fiable Lipids	CO_2	Fatty Acids	Nonsaponi-fiable Lipids	CO_2
		total cpm.	*total cpm.*	*total cpm.*	*percentage*	*percentage*	*percentage*
1	Adipose	5,630	410	9,230	0.23	0.01	0.38
2	Adipose	6,030	190	8,620	0.28	0.01	0.39
3	Adipose	9,820	210	13,900	0.44	0.01	0.62
4	Adipose	6,320	130	10,400	0.27	0.01	0.63
5	Adipose	16,400	320	22,800	0.70	0.02	0.98
Mean					0.38 ± 0.08 †	0.01 ± 0.00	0.60 ± 0.10
6	Liver	800	600	8,190	0.01	0.01	0.14
7	Liver	1,230	380	8,850	0.02	0.01	0.15
8	Liver	390	320	9,700	0.01	0.01	0.19
9	Liver	310	420	7,250	0.01	0.01	0.13
10	Liver	410	610	7,420	0.01	0.01	0.13
Mean					0.01 ± 0.00	0.01 ± 0.00	0.15 ± 0.01

* 1420 ± 10 mg. of tissue slices were incubated for 3 hours at 37.5°C with 0.001 M C^{14}-isoleucine. The total activity added to each flask was 43,000 cpm. 14.0 ml. of Krebs-bicarbonate enriched with 0.01 M succinate and 0.011 M glucose was used. Methylmalonate was also added to a final concentration of 0.01 M.

† Standard error of the mean.

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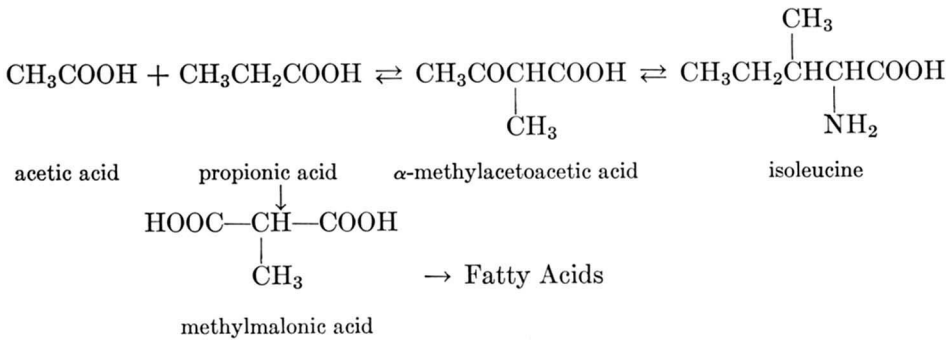

SCHEME 1

DISCUSSION

In a previous report (2) it was suggested that propionate might first be converted to methylmalonate en route to the formation of long-chain fatty acids in adipose tissue. Further evidence supporting this scheme has since been reported (6). C^{14}-methylmalonate has been recovered following addition of propionate-1-C^{14} to slices of adipose tissue. Methylmalonate is formed by a mechanism not utilizing condensation of bicarbonate with propionate as a major role. However, the recovery of C^{14}-succinate following incubation of C^{14}-bicarbonate with carrier propionate in slices of adipose tissue (2) indicates that the scheme described by Flavin and Ochoa for methylmalonate synthesis must still be considered (7).

These results raised the possibility that propionate condenses with a compound of chain length greater than 1 carbon in the formation of intermediates that are converted first to methylmalonate and secondly to long-chain fatty acids. Isoleucine was tested as a possible precursor in this pathway, since this compound has been shown by Coon and associates (3) to be degraded to a five-carbon keto-acid, α-methylacetoacetate, which in turn is in equilibrium with propionate and acetate.

Recovery of radioactivity in spots corresponding to acetate, propionate, and α-methylacetoacetate after incubation with C^{14}-isoleucine indicates that the series of reactions outlined by Coon *et al.* for other tissues likewise is operative in adipose tissue. The depression in conversion of isoleucine to fatty acids in adipose tissue after addition of high concentrations of carrier propionate supports the theory that isoleucine is degraded to this compound or to an intermediate that is in equilibrium with it.

These results are consistent with two possible schemes for describing a path that may play a major role in the over-all synthesis of lipids in adipose tissue. One possible scheme would be as shown in Scheme 1 (centered above), where isoleucine is first degraded to propionate, which in turn is converted to lipids. Methylmalonate is an intermediate in the last step.

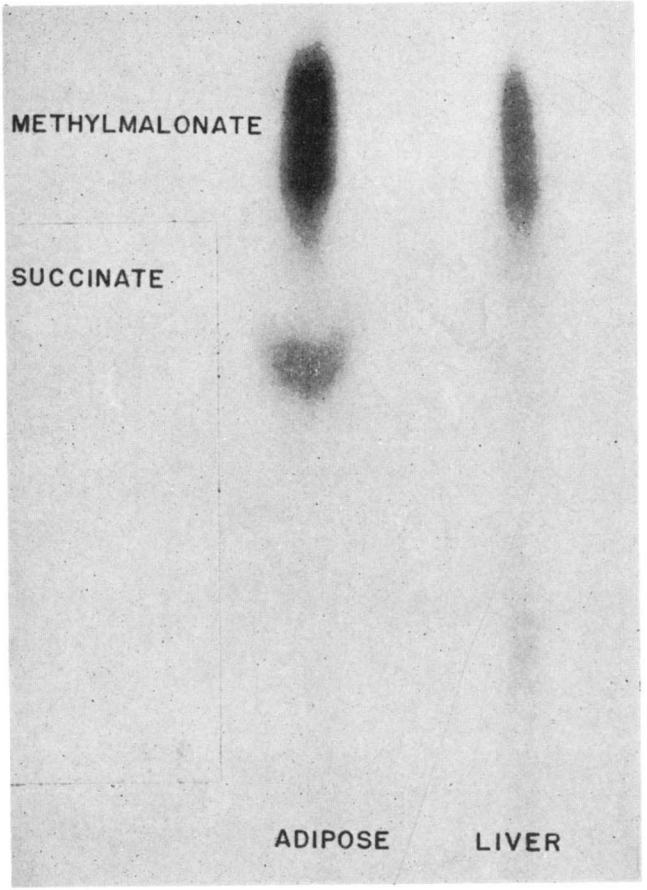

FIG. 1. Autoradiograms of adipose tissue and liver extracts from mice. Supernatant and combined washings were electrodialyzed, extracted with ether, and chromatographed for 20 hours with isoamyl alcohol-4 N formic acid. Tissues were incubated with C^{14}-isoleucine. R_f values obtained for controls at the points indicated are as follows: methylmalonate, 0.85 and succinate, 0.74.

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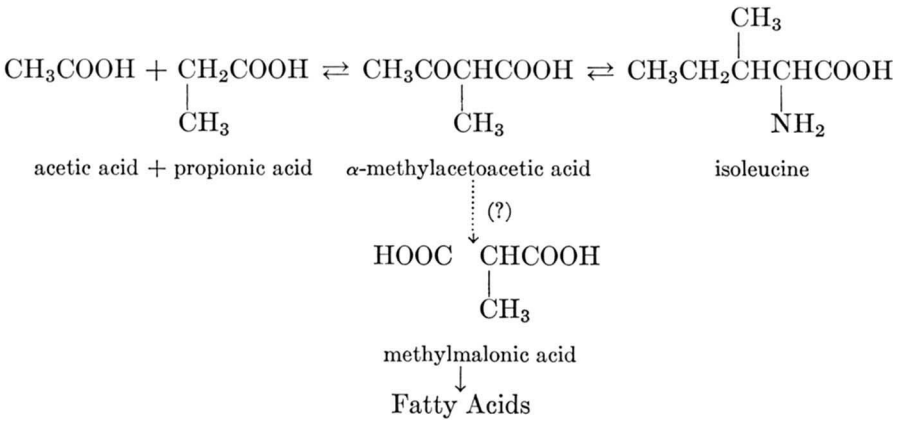

SCHEME 2

In this scheme isoleucine conversion to fatty acids would be depressed by the addition of carrier propionate and methylmalonate, which fits the facts.

Another scheme, which is purely hypothetical, would be as shown in Scheme 2 (centered above). This scheme is consistent with the result obtained in this paper. Furthermore, it explains why carbon-1 of acetate is incorporated to a greater extent than carbon-2 by slices of adipose tissue, results which were obtained and reported earlier (1).

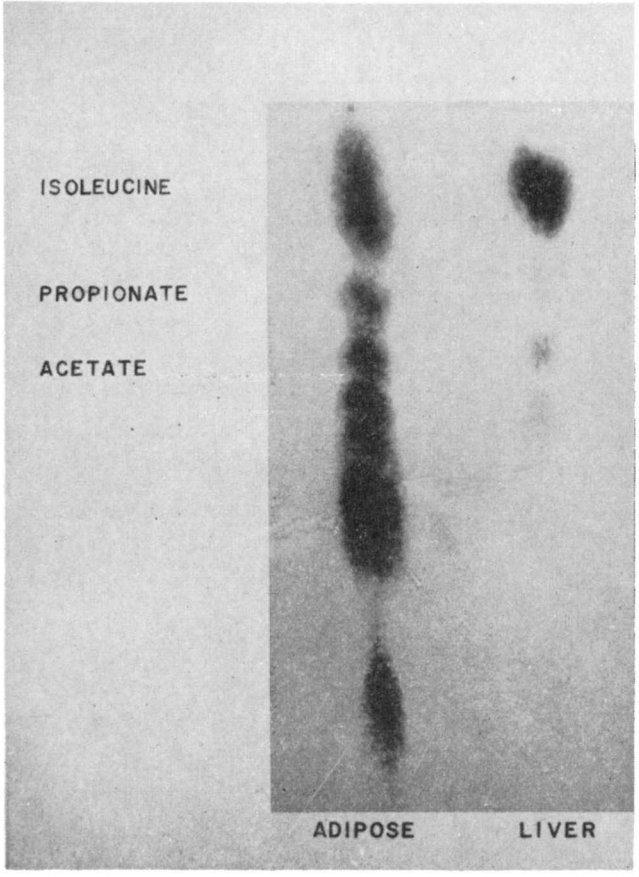

FIG. 2. Autoradiograms of adipose tissue and liver extracts from mice prepared as in Figure 1. The ammonium salts of the organic acids were chromatographed for 20 hours with ethanol—NH_4OH—H_2O. Tissues were incubated with C^{14}-isoleucine. R_f values obtained for controls at the points indicated are as follows: isoleucine, 0.58, propionate, 0.46, and acetate, 0.38.

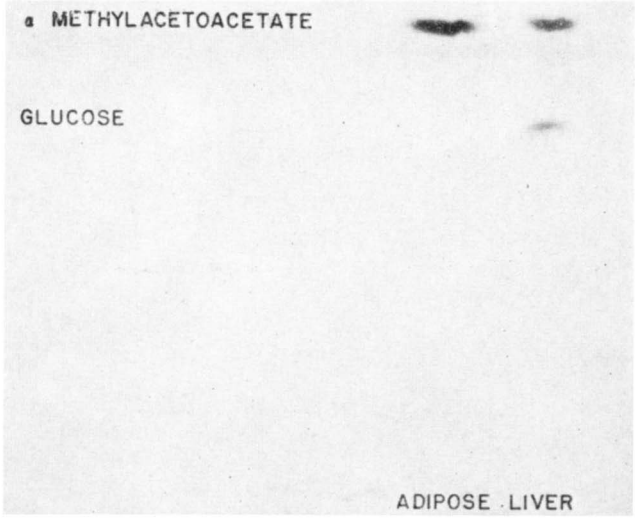

FIG. 3. Autoradiograms of hydrazones from adipose tissue and liver. Tissues were ground in a solution of 2,4-dinitrophenylhydrazine. After purification, the hydrazones were chromatographed for 48 hours with tertiary amyl alcohol-ethanol-water. Tissues were incubated with C^{14}-isoleucine. R_f values obtained for controls at the points indicated are as follows: α-methylacetoacetate, 0.96 and glucose, 0.78.

For the sake of brevity, the schemes do not include all the intermediates that would be expected to intervene between the compounds indicated, nor do they show the compounds in their possible activated forms. Other established paths for lipid synthesis, such as condensation of two acetate molecules, are omitted.

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Acetate, propionate, methylmalonate, and isoleucine have all been shown to be very active in their ability to form long-chain fatty acids. The last three substrates, in fact, have been shown to form fatty acids much more readily in adipose tissue than in liver. Thus the data suggest that adipose tissue might have a specific function in synthesizing fats from these metabolites and that the scheme shown above might play a major role in this activity. Investigation concerning the mechanism and intermediates in the pathway as well as the nature of the long-chain fatty acids formed are the subject of further study.

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