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FATTY ACID UPTAKE AND ESTERIFICATION IN ADIPOSE TISSUE*

B. SHAPIRO, I. CHOWERS AND G. ROSE

Department of Biochemistry, The Hebrew University, Hadassah Medical School, Jerusalem (Israel)

The rate of mobilization of fat from adipose tissue and its deposition is not determined solely by diffusion of these substances from the tissue to the blood and vice versa. Thus, increased mobilization during hunger[1] or after treatment with pituitary extracts[2] is not brought about by decreased fat concentration in the blood, but rather takes place in spite of increased blood fat levels.

Evidence for the participation of tissue metabolism in the transport of fat into the cells was obtained by studying the uptake of fat by adipose tissue *in vitro*. It was found[3,4] that isolated adipose tissue removes fat from serum and other lipid media, provided that fat-depleted tissue is used. This uptake of fat disappears when heat-killed tissue is used or when metabolic poisons, such as fluoride or cyanide are added. Also, uptake was observed only with neutral fat and fatty acids but not with phospho-

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lipids or cholesterol esters. The uptake of the esters was markedly inhibited by fluoride while that of the free fatty acids was not affected by this poison. In view of this difference in susceptibility, it was postulated that the ester linkage is of importance in the metabolic activity, which facilitates the transport.

In the present paper experiments are reported, in which adipose tissue was incubated in a medium containing radiostearic acid. The dependence of the uptake of this acid on the nutritional state of the animal was examined and the fate of the stearic acid incorporated was studied.

EXPERIMENTAL

Mesenteric adipose tissue was obtained from three groups of rats, (a) normally fed rats, (b) rats starved for 5–6 days until a weight loss of about 30% and (c) rats starved as in group b and refed for 24 or 48 hours on a standard diet consisting of: 70% carbohydrates, 20% protein and 10% fat and minerals. The rats were killed by dislocation of the neck, bled and the mesenterium was stripped off the intestine and dissected free of pancreatic tissue and lymph nodes. The tissues were rinsed in ice cold 0.9% sodium chloride solution, dried on filter paper and placed into the incubation medium.

The incubation medium consisted of 2 micromoles/ml carboxyl-labelled sodium stearate, 5% bovine serum albumin (fraction V) dissolved in Krebs-Ringer phosphate solution (pH 7.4)[5] (calcium omitted). The last solution was composed as follows: 0.9% NaCl, 100 parts; 1.15% KCl, 4 parts; 3.8% $MgSO_4 \cdot 7H_2O$, 1 part; a mixture of 40 ml of 0.25 *M* Na_2HPO_4 and 2 ml *N* HCl brought to 100 ml and adjusted to pH 7.4, 21 parts.

The mesenterium of one rat was incubated in 1 ml of medium at 37° C in a water bath with continuous shaking. Samples were taken after 5 minutes and 4 hours (unless otherwise stated) and were dried on lens paper on a planchet and counted with a thin end-window Geiger-Mueller counter (Tracerlab, TGC-2) and the counts corrected for self-absorption by the sample.

Another sample of the medium was extracted with a 3:1 alcohol-ether solution. The extract served for counting and for titration of the fatty acids. At the end of the incubation time, the tissue was removed from the medium, rinsed shortly in a 0.1 *N* NaOH solution followed by 3 washings with 0.9% NaCl and was extracted by boiling alcohol-ether solution.

Fatty acid estimation was carried out by acidifying the alcohol-ether extract, adding water and washing the ether layer several times with water, until neutral. The ether was evaporated and the residue dissolved in ethanol, which had previously been neutralized with *N*/10 NaOH by bringing it to a faint pink colour in the presence of phenolphthalein. The solution was then titrated with *N*/20 or *N*/50 NaOH with a microburette with a stream of nitrogen passing through the solution.

Separation of free fatty acids from neutral fat. The alcohol-ether extract, of the tissue or medium was evaporated to dryness and the lipids were dissolved in dry acetone. The acetone solution was passed through a column of magnesium oxide, containing an equal weight of celite[6]. In preliminary tests with radiostearate and unlabelled neutral fat, about 1% of the radioactivity together with over 90% of the ester were found in the first 25 ml of the effluent. By adding carrier stearic acid to the effluent and recycling, the radioactivity in the effluent could be reduced to negligible amounts.

$^{14}CO_2$ *determination* was carried out according to ROSE AND SHAPIRO[7].

RESULTS

With all three groups of tissues examined, a considerable uptake of radiostearate from the medium was observed. However, marked differences were found between these groups (Table I). The lowest uptake was found with tissues of starved animals (group b) while those taken from animals refed after the starvation period (group c) showed the highest uptake (63.7%). The tissues of normally fed animals (group a) were intermediary (38.6%). This finding shows that the state of the tissue is a major factor in the assimilation of fatty acids. The "net uptake" measured by titrating the fatty acids, remaining in the medium, was about the same as the uptake found by radio-

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activity measurement, except in group c. It seems likely that an exchange between fatty acids in the medium and a fatty acid compound in the tissue takes place in this group in addition to the net uptake.

The data presented in Table I show no correlation between initial substrate concentration and uptake, when expressed in percents. In Table II experiments are presented, in which identical tissue samples were incubated for varying times and with varying substrate concentrations. It is evident that the uptake follows a first order reaction rate and percentage uptake does not vary markedly with substrate concentration. It can be concluded that the rate of uptake is proportional to substrate concentration. When higher fatty acid concentrations were tested, dispersion of the substrate became difficult and the uptake decreased. It is not clear whether this decrease is due to bad dispersion or to poisoning of the tissue with high fatty acid concentrations.

TABLE I

UPTAKE OF STEARIC ACID BY ADIPOSE TISSUE *in vitro*
For conditions see EXPERIMENTAL.

Nutritional state of rat	*Original concentration in medium*		*% uptake of acid*	
	cts/min/ml	*μequiv./ml fatty acid*	*by radioactivity*	*by titration*
a. Normally fed				
1.	6315		19.5	
2.	3345		48.5	
3.	3655	2.16	54.0	68.2
4.	14260	1.675	40.3	47.3
5.	14460	1.675	30.9	21.0
			Mean: 38.6	45.5
b. Starved				
1.	54480	1.76	31.3	41.7
2.	53420	0.993	20.0	
3.	64000	1.2	11.1	15.6
4.	62670	1.29	12.9	8.6
5.	29250	0.66	23.1	27.7
6.	8580	2.24	19.0	0.0
			Mean: 19.6	18.7
c. Refed for 24 hours				
1.	58260	1.76	86.0	83.2
2.	57360	0.993	55.0	
3.	110000	2.31	58.5	32.0
4.	64000	1.2	44.8	65.6
5.	63430	1.29	36.0	15.4
6.	15810	0.66	62.0	41.6
7.	5890		49.2	
Refed for 48 hours				
8.	5870		77.8	
9.	6040		91.5	
10.	15630		68.2	
11.	15630	1.1	81.8	75.0
12.	8570	2.12	54.0	39.0
			Mean: 63.7	50.0

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In order to elucidate the fate of the fatty acids assimilated by the tissue, the alcohol-ether extract of the tissue was analyzed for total radio-activity and was partitioned into free fatty acids and fatty acid esters by passing through a MgO column. In addition, in some experiments, $^{14}CO_2$ was collected and counted. The results of these measurements, summarized in Table III, show that in group c, in which the fatty acid uptake was the highest, most of the fatty acids were found in a form, not retained by the MgO column. The radioactive material in the tissues could not be separated from the neutral fat by repeated chromatography on the MgO column. It seems likely that the fatty acids in the tissue were converted mainly into triglyceride-fatty acids.

In group b, in which the fatty acid uptake was much decreased, a considerably lower percentage of the assimilated fatty acids were found to be in a bound form and most of it behaved as free fatty acid.

$^{14}CO_2$ production, on the other hand, was much higher in the tissue from starved animals (group b) than that from refed ones (group c). The higher $^{14}CO_2$ production may be related to the larger activity of the free acids in the tissues of the starved animals.

TABLE II

TIME AND CONCENTRATION DEPENDENCE OF FATTY ACID UPTAKE

Conditions as in Table I, except for time and substrate concentration; refed rats. In experiment a, mesenteria of 3 rats were divided each into 3 equal parts, so that every flask contained one third of each mesenterium. In experiment b, every flask contained one half of each of 2 mesenteria.

Expt.	*Time of incubation (hours)*	*Initial concentration (μequiv./ml)*	*% uptake*	*Relative velocity constant**
a	1	2	52	0.32
	2	2	77	0.37
	4	2	92.5	0.28
b	4	2	84	
	4	4	88	

* Calculated for a first order reaction: $k = 1/t \log a/(a-x)$.

TABLE III

UPTAKE OF STEARATE-1-^{14}C BY ADIPOSE TISSUE

Conditions as in Table I.

	% uptake from medium	*% of fatty acid taken up found as*	
		$^{14}CO_2$	*ester in tissue*
Refed rats	76	1.4	87
	81	1.1	90
	82	1.3	85
	72	1.4	70
	38	2.4	27
Starved rats	27	16.5	34
	25	4.2	27
	15		8
	51		26
	49		15

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The introduction of fatty acids into the neutral fat fraction in the tissue could be visualized as a result of a lipase activated exchange between free fatty acids and triglycerides. However, results obtained so far are not in agreement with this assumption. It could be shown that while fatty acids were rapidly introduced into esters linkages, glycerol was esterified only to a small extent (Table IV). About 25% of the radioglycerol added to the medium was taken up by the tissue. Out of this quantity only negligible amounts were transformed into ether-soluble compounds or burned to $^{14}CO_2$. In addition, nonlabelled glycerol, added to the fatty acid experiments, did not increase the uptake of fatty acid, nor did it change the portion of the acid transformed into ester (Table V). Finally it was found that fatty acid uptake was almost abolished by cyanide, while fluoride was without effect (Table V).

TABLE IV

UPTAKE AND ESTERIFICATION OF GLYCEROL

Conditions as in Table I. 1-^{14}C-glycerol was added to incubation medium instead of radio-stearic acid.

	Initial concentration of glycerol (μequiv./ml)	*Uptake %*	*% of radio glycerol taken up found in*	
			$^{14}CO_2$	*ester*
Refed rats	2	23.5	2.5	5
	2	21.2	0.5	2
Starved rats	2	25.7	0.5	2.4
	2	22.5	0.4	1.0

TABLE V

INFLUENCE OF ADDITIONS ON UPTAKE AND ESTERIFICATION BY ADIPOSE TISSUE

Conditions as in Table I; starved rats.

Addition	*Uptake %*	*Esterification %*
None	68	33
0.1 % Glycerol	54	33
None	49	15
0.1 % Glycerol	58	19
None	67	33
M/500 Cyanide	6	2
None	31	
M/20 Fluoride	32	
Incubation at 0° C	2	

DISCUSSION

The uptake of fats or fatty acids itself does not necessarily require an active process, since these substances in the process of being transferred from an aqueous suspension into the fat droplets of the cells move in a direction which results in the equilibrium state of a fat/water system. However, previous results, where the uptake of fat was shown to be inhibited by metabolic poisons and by lowering the temperature, and the

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results obtained in the present experiments with free fatty acids, point to a facilitated transfer mechanism; a mechanism in which the attainment of the above equilibrium is accelerated by enzymic processes. These enzymic processes involve esterification of the free fatty acid moving into the cells. The capacity of the tissue for esterification changes with the nutritional state of the animal. It is lowest in the starved animal and highest in the animal recovering from starvation, that is after refeeding for two days. Together with the decrease in esterifying capacity, the facilitated transfer of fatty acids is also decreased. It thus seems likely that esterification is involved in the facilitated transfer.

The conditions favouring esterification indicate a mechanism differing from a lipase activated exchange. Most likely, an enzymic system similar to that found in liver by TIETZ AND SHAPIRO[8] requiring ATP and other cofactors, is responsible for the esterification of the absorbed fatty acids in adipose tissue. This assumption is corroborated by the finding that a higher fatty acid activating system requiring ATP, CoA and Mg^{++} is present in adipose tissue[9].

It may be visualized that the fatty acid entering the cell boundary becomes bound to the activating enzyme which enables it to cross the cell boundary and enter the cytoplasm surrounding the fat droplet. The fatty acid may then be transferred from the enzyme complex to an acceptor in the cytoplasm forming the fatty acid ester, which can subsequently diffuse into the fat droplet of the cell.

SUMMARY

1. Mesenteric adipose tissue when incubated in stearate-1-^{14}C enriched medium was found to take up fatty acid from the medium.

2. The uptake was dependent upon the nutritional state of the animal from which the tissue was obtained. It was found to be lowest in the "starved" and highest in the "refed" animals. Tissues from normally fed animals exhibited intermediary values.

3. The uptake was found to be proportional to the fatty acid concentration.

4. Most of the fatty acids assimilated by the "refed" tissues was found inside the tissue in a bound form, unseparable from the triglyceride fraction. Esterification was found to be much reduced in starved tissues.

5. ^{14}C-Glycerol was esterified only to a very small extent, and additional glycerol caused no change in the uptake and esterification of fatty acids.

6. The uptake and esterification were almost abolished by $M/500$ cyanide but were not affected by $M/20$ fluoride.

7. The significance of the results from the aspect of the mechanism of fat uptake by adipose tissue is discussed.

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