

## COMPARISON OF THE TRIGLYCERIDE SYNTHESIS OF CARP AND RAT IN ADIPOSE TISSUE AND LIVER SLICES INCUBATED WITH 1—<sup>14</sup>C PALMITIC ACID

S. HERODEK

*Biological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary*

Received: March 31st 1966

A great progress can be stated in the studies on the metabolism of adipose tissue in the last few years. A detailed review on the subject was supplied by VAUGHAN (1961). The same problem was discussed by 2 Conferences, the matter of both being published under the titles: "Adipose tissue as an organ" (KINSELL ed. 1962) and "Fat as a tissue" (RODAHL ed., 1964) resp. In contrast to such extensive studies on the metabolism of the adipose tissue of homiotherm animals we are in the knowledge only of a single paper dealing with the metabolism of the adipose tissue of fishes. This work was carried out by T. FARKAS (1966) in the same laboratory where the present investigations took place. FARKAS investigated the release of free fatty acids from the adipose tissue into the medium. The subject of our study is the opposite process — the incorporation of the fatty acids into the adipose tissue and liver of the fish. For the sake of comparison the same investigations were carried out also with rats.

### Material and methods

The carps were males of 0.5 kg weight, originating from the fish ponds of Balatonlelle. For 1 day before the experiments they were kept in aquarium. The rats were Wistar males weighing 150—200 gr and were fed ad libitum on their standard diet.

The animals were killed by a blow on their head. Their liver and a piece of adipose tissue were separated. The adipose tissue was represented in fish by the intestinal adipose tissue, in the rat by the epididymal fat pad. The liver of both animals was cut into cca 1 mm thick slices and 1 g of them was incubated in 10 ml medium. 150 mg of adipose tissue was incubated in 5 ml medium.

Each incubation lasted for 10 min by gentle shaking. The tissues of fish and rat were incubated at 20° C and 37° C resp. The incubation medium for the rat tissues consisted of a KREBS-RINGER phosphate buffer (pH 7.4, Ca<sup>2+</sup>-omitted) which was 5 mM for glucose and contained 5% human serum albumin. The medium for fish differed only so far as instead of 0.9% it contained only 0.48% NaCl.

The specific activity of the 1—<sup>14</sup>C palmitic acid (Reanal, Hungary) was 1 mC/mmol. Its concentration in the medium was 2 μC/ml. The labeled palmitic

acid was complexed to albumin. For this purpose the fatty acid was neutralized with 0.1 N NaOH and warmed up and mixed thoroughly with the preheated albumin solution.

After incubation the tissue slices were rinsed quickly with 0.48% NaCl and 0.9% NaCl solution for carp and rat resp., in order to remove the adherent fatty acid activity. The tissues were homogenized in a glass POTTER with chloroform-methanol 2 : 1, and extracted with 25 ml of the same mixture. An aliquot of the filtrate was evaporated under CO<sub>2</sub> and dissolved in chloroform. This solution then was applied on thin layer plates. 1 mg/ml diglyceride and 1 mg/ml inactive palmitic acid were also added to the chloroform to enable on the chromatograms the detection of these compounds present in the tissues only in very small quantities.

The thin-layer chromatography was carried out on 0.3 mm thick silica gel plates with the developing solvent mixture of petroleum ether (b. r. 40—70°C)-diethyl ether — acetic acid 70 : 30 : 01. Detection by spraying with 0.2% Rhodamin B in ethanol. The location of the bands was marked under UV light, and they were separately scraped off. This way the lipid extract was separated into the following fractions: cholesterol esters, triglycerides, free fatty acids, diglycerides, monoglycerides and phospholipids.

The lipids were eluted from the silica gel by 2 hour's extraction with diethylether in a microsoxhlet apparatus. The radioactivity measurements were carried out by a Tri-Carb liquid scintillation spectrometer. The scintillation liquid consisted of 0.3% PPO and 0.04% POPOP in toluene.

### Results and discussion

The amounts of fatty acids incorporated from the medium into the three investigated lipid classes in the two different tissues of the two species are given in *Table 1*.

Of course the in vivo fatty acid uptake of these tissues can not be calculated from the data. The absolute intensity of the triglyceride synthesis is neither calculable because there are different fatty acid pools within the tissue and the specific activity of the triglyceride synthesis pool is unknown. There is no basis to compare the data of the adipose tissue and those of the liver.

*Table 1*

The incorporation of 1-<sup>14</sup>C palmitic acid into the various lipid fractions of the adipose tissue and liver of rat and carp

	Adipose tissue		Liver	
	rat	carp	rat	carp
Triglycerides . . . . .	5317 ± 319	93 ± 7	522 ± 63	151 ± 47
Diglycerides . . . . .	1527 ± 135	25 ± 2	429 ± 53	163 ± 6
Free fatty acids . . . . .	728 ± 42	2147 ± 623	1065 ± 287	1145 ± 87

The values are given in 10<sup>-10</sup> mol fatty acid/g wet tissue. Each value is the average of 3 animals ± standard error of the mean.

## 1. Táblázat

Az 1-<sup>14</sup>C palmitinsav beépülése a patkány és a ponty zsírszövetének és májának zsiradékaiába

	Zsírszövet		Máj	
	patkány	ponty	patkány	ponty
Triglicerid .....	5317 ± 319	93 ± 7	522 ± 63	151 ± 47
Diglicerid .....	1527 ± 135	25 ± 2	429 ± 53	163 ± 6
Szabad zsírsav .....	728 ± 42	2147 ± 623	1065 ± 287	1145 ± 87

A táblázat adatai 10<sup>-10</sup> mol zsírsav/g élő szövetet jelentenek. Minden érték három állat átlaga, ± az átlag középhibája.

A semiquantitative comparison of the 2 identical tissues of the two species seemed reasonable.

The triglycerides show a measurable radioactivity in all the four tissues, there is a triglyceride synthesis in all of them.

The diglycerides show also a radioactivity in all the four tissues, and in the same order of magnitude as that of the triglycerides. More detailed the two adipose tissues are similar, the diglycerides showing one fourth of the activity of triglycerides, whereas in the livers of both species the activity of diglycerides equals that of triglycerides. Regarding the distribution of the labeled fatty acids between the diglyceride and triglyceride fraction, the carp and rat showed no differences. We studied the behaviour of the radioactive diglycerides in the adipose tissue of rat in another work (HERODEK 1966) in details, where the adipose tissue first incubated with labeled palmitic acid as above was transferred in an inactive medium for a second 10 min incubation. During this incubation in inactive medium no significant decrease of the radioactivity of the diglycerides took place, indicating, that there must be at least two different triglyceride pools within the fat cells, — in one the diglycerides are rapidly transformed into triglycerides, in the other the diglycerides have a much lower turnover rate. Our present results, showing a high diglyceride/triglyceride activity ratio in both tissues of both animals, suggest the existence of diglycerides with a lower turnover rate to be a general phenomenon of the triglyceride synthesis. In this respect the time curve of the radioactive diglycerides in more tissues of more species seems to be worthy of study.

For free fatty acids it seems to be a regularity, that the more the labeled fatty acid is incorporated by the tissue, the less is the radioactivity of the free fatty acids of the tissue. The entrance of the fatty acids into the tissue is a physico-chemical process, its intensity is regulated by the concentration gradient.

Owing to the general lack of knowledge on the lipid metabolism of fishes, we are restricted in the interpretation of the intensity of the incorporation of the fatty acids into the triglycerides only to different hypothesis.

The triglycerides of the fish liver contained only one third of the radioactivity of the rat liver triglycerides. Here not so much this difference but the identical order of magnitude is perhaps to be emphasized. On the other hand in the radioactivity of the adipose tissue the carp is surpassed about fifty times by the rat, indicating some kind of basic difference between the adipose tissues of the two species. As mentioned above the mammalian tissues

were at 37° C, those of the fish at 20° C incubated as 37° C could scarcely represent a physiological temperature for a carp. The experiments of FARKAS (1966) were carried out also at 20° C, and under this condition the adipose tissue of the carp released even more free fatty acids than the rat adipose tissue at 37° C. And just as seen above also the two livers took up the fatty acid — in spite of the temperature difference — in the same order of magnitude. There is no reason to put the medium under suspect, as FARKAS (1966) applied the same in his experiments. Still to test the most physiological medium the adipose tissues of three carps were incubated in their own plasma with the addition similar to the synthetic medium of glucose and labeled fatty acid. Also this incubation did not result in more significant incorporation into the triglycerides. 92% of the activity uptake remained in the form of free fatty acids.

It is not probable that the slow incorporation is caused by absence of glucose because glucose was added to the medium in a quantity sufficient to restore completely the triglyceride synthesis in the adipose tissue obtained from starving rats. Moreover FARKAS (1966) found the adipose tissues of carps from the same catch to be rich in glycogen. The entrance of the fatty acids into the tissue is not involved in this problem as the radioactivity of the free fatty acids is higher in the adipose tissue of the carp than in that of the rat. The possibility might be brought up that the adipose tissue of the carp incorporates into triglycerides mainly fatty acids of endogenic origin, while the exogenic ones play only an unimportant role. The gas chromatographic demonstration of a high amount of linoleic acid in this tissue (ABDEL-HAY and HERODEK 1966) excludes this possibility as this fatty acid, similarly to the mammals, can not be synthesized by fish.

It might be supposed that the triglycerides get without previous hydrolysis into the adipose tissue of fishes from the circulation. Such mechanism was suggested earlier for mammals, however, according to the more recent experiments (RODAHL 1964) both the uptake and release of triglycerides are bound to hydrolysis. Yet it is not sure that the same is true for fishes. The uptake of intact triglycerides by the cell would render unnecessary an extensive triglyceride synthesis within the cell. The incubation of the tissue with triglycerides labeled by  $^{14}\text{C}$  in glycerol and  $^3\text{H}$  in fatty acid followed by the determination of the two isotopes in the tissue could give decisive answer to this question. Such experiment is in the present out of scope of our technical possibilities. The demonstration of the presence or absence of lipoprotein lipase could provide also important informations in this problem.

A permanent exchange between the fatty acids of triglycerides and free fatty acids in the adipose tissue of rat was demonstrated (VAUGHAN and STEINBERG 1963) by comparing the release of glycerol and free fatty acid. This means that part of the lipolyzed fatty acids instead of release into the medium is re-esterificated. Therefore the total triglyceride synthesis is higher than the net increase of the amount of triglycerides, going on also under conditions where the amount of triglycerides is unchanged or even decreasing. The lack of such a dynamic relation in fish could explain the difference observed in the incorporation of labeled fatty acids even if the net change of the amount of triglycerides is similar in the two animals. According to CAHILL's suggestion (KINSELL 1962) the energy requiring re-esterification of the fatty acids can play an important role in the heat economy of the homoiothermic animals,

The relatively low oxygen consumption of the adipose tissue of homoiotherms, at least under in vitro conditions, speaks against this theory. But if CAHILL is right it could be supposed that in a poikilothermic animal, where the heat production has no meaning, we do not find an extensive re-esterification.

### Summary

Adipose tissue and liver slices of the carp and rat were in vitro incubated in media containing labeled palmitic acid. The lipid fractions were separated by thin layer chromatography, and their radioactivity was determined.

Triglyceride synthesis took place in each tissue. Its intensity had similar order of magnitude in the liver slices of the two species. On the other hand the synthesis was fifty times lower in the adipose tissue of the carp than in that of the rat under the experimental conditions. For interpretation we are restricted to hypothesis.

The diglycerides showed in each tissue a radioactivity of the same order of magnitude to that of the triglycerides. Probably besides the diglycerides, rapidly transformed to triglycerides, there exists a diglyceride pool of much slower turn over.

### REFERENCES

- ABDEL-HAY, A., S. HERODEK (1966): Comparative study on the fatty acid composition of the tissue lipids in the fish *Cyprinus carpio* L. — *Annal. Biol. Tihany*, **33**.
- FARKAS, T. (1966): The effect of catecholamines and ACTH on the blood and adipose tissue FFA levels by the fish *Cyprinus carpio* L. — in *Progress in Biochemical Pharmacology* Vol. 2. (in press) S. KARGER *Verlagsbuchhandlung für Medizin, Basel, Switzerland*.
- HERODEK, S. (1966): The distribution of labeled palmitic acid in the diglycerides and triglycerides of rat adipose tissue. — *in prep.*
- KINSELL, L. W. ed. (1962): Adipose Tissue as an Organ — *Proceedings of the Deuel Conference on Lipids*. — CHARLES C THOMAS Publisher Springfield — Illinois, USA.
- RODAHL, K. ed (1964): Fat as a Tissue. — *Proceedings of a Conference Held at The Lankenau Hospital 1962*. MCGRAW-Hill Book Company, New York.
- VAUGHAN, M. (1961): The metabolism of adipose tissue in vitro. — *J. Lipid Research* **2**, 293–316.
- VAUGHAN, M., D. STEINBERG (1963): Effect of hormones on lipolysis and esterification of free fatty acids during incubation of adipose tissue in vitro. — *J. Lipid Res.* **4**, 193–199.
- VAUGHAN, M., D. STEINBERG, R. PITTMAN (1964): On the interpretation of studies measuring uptake and esterification of 1-<sup>14</sup>C palmitic acid by rat adipose tissue in vitro. — *Biochim. Biophys. Acta* **34**, 154–166.

A PONTY (*CYPRINUS CARPIO L.*) ÉS A PATKÁNY  
TRIGLICERID SZINTÉZISÉNEK ÖSSZEHASONLÍTÁSA  
1-<sup>14</sup>C PALMITINSÁVVAL INKULBÁLT ZSÍRSZÖVET ÉS MÁJ METSZETEKBEN

*Herodek Sándor*

**Összefoglalás**

Patkány és ponty zsírszövetéből és májából készített metszeteket jelzett palmitinsavat tartalmazó közegben inkubáltuk. A lipid-frakciókat rétegekromatográfia segítségével szétválasztottuk, és mértük radioaktivitásukat.

Mindegyik szövetben volt triglicerid szintézis. Ennek erőssége a májszövetekben azonos nagyságrendű, a ponty zsírszövetében viszont az adott kísérleti feltételek mellett ötvenszer alacsonyabb, mint a patkányéban. Ennek magyarázatára csak feltevésekre szorítkozhatunk.

A digliceridek az összes szövetben a trigliceridekéhez hasonló mértékű radioaktivitást mutattak. Feltehető, hogy a gyorsan trigliceriddé alakuló digliceridek mellett, egy hosszabb életű diglicerid raktár is van.

A szabad zsírsavak annál alacsonyabb százalékát tartalmazták az összes aktivitásnak, mennél magasabb volt ez az összes aktivitás az egyes szövetekben.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ СИНТЕЗА ТРИГЛИЦЕРИДОВ В ЖИРОВОЙ И ПЕЧЕНОЧНОЙ ТКАНЯХ, ИНКУБИРОВАННЫХ С 1—С<sup>14</sup>-ПАЛЬМИТИНОВОЙ КИСЛОТОЙ, У КАРПА И КРЫСЫ

Шандор Херодек

Срезы из жировой и печеночной тканей карпа и крысы инкубировали в среде, содержащей 1—С<sup>14</sup>-пальмитиновую кислоту. Измеряли радиоактивность липидных фракций, полученных с помощью тонкослойной хроматографии.

В каждой из тканей обнаружен синтез триглицеридов. Интенсивность синтеза в тканях печени одинакова, а в жировых тканях у карпа, при данных экспериментальных условиях, в 50 раз ниже, чем у крысы. Этому явлению пока не нашли объяснения.

Во всех тканях диглицериды проявляли такую же активность, как триглицериды. Предполагается, что помимо диглицеридов, быстро превращающихся в триглицериды, имеется более стабильное депо диглицеридов.

Активность свободных жирных кислот тем ниже, чем выше в данной ткани тотальная активность.