## The distribution of fatty acids in the triglycerides of the Artiodactyla (even-toed animals)

F. H. MATTSON, R. A. VOLPENHEIN, and E. S. LUTTON

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

SUMMARY The distribution of fatty acids in the triglycerides of a number of Artiodactyla (animals with an even number of toes) has been determined. In confirmation of earlier reports, palmitic acid was found predominantly in the 2-position of the triglycerides of the domestic pig. The same pattern was found to exist in the European and American wild boar and also in two species of peccary. In the other members of this group of animals, the palmitic acid was either approximately randomly distributed among all three positions or the 1- and 3positions contained more of this acid than did the 2-position. In all species, only small amounts of stearic acid were esterified at the 2-position. Myristic acid, although present in only small amounts, tended to concentrate in the 2-position. No apparent pattern of distribution of the unsaturated acids was noted. The relationship of the distribution of palmitic acid to the origin of the various families of animals is discussed.

SBMB

JOURNAL OF LIPID RESEARCH

HE ARTIODACTYLA, although commonly referred to as cloven-hooved animals, are, more properly, animals with an even number of toes. Among the animals in this order is the domestic pig. Several years ago it was reported (1-3) that the palmitic acid in the triglyceride of pigs was found almost exclusively in the 2-position. In the few other species that have been studied, the distribution of saturated acids differed sharply from that in the pig (3-5). However, in many of these studies the saturated fatty acids were reported as a class, no differentiation of the individual acids being made. To determine whether the domestic pig is indeed unique in the manner in which it deposits palmitic acid, we have determined the fatty acid distribution in the triglycerides of a number of species of the artiodactyla.

## EXPERIMENTAL METHODS

Fats from the following animals were used in this study. The numbers correspond to those given in Table 1.

Domestic pig, Sus scrofa. 1. From local slaughter house. 2. From a domestic pig raised on a diet containing 25% of safflower seed oil.

Wild boar, Sus scrofa. 3. From Switzerland. 4. From New Hampshire.

Peccary. 5. Peccari angulatus bangsi from Panama. 6. Peccari tajacu from Texas.

Hippopotamus. 7. Hippopotamus amphibius from Kenya.

Camel. 8. Camelus from New York Zoo.

White-tailed deer. 9. Odocoileus virginianus from Michigan.

Sheep. 10. Ovis musimou from local slaughter house.

Beef. 11. Bos taurus from local slaughter house.

Samples of both subcutaneous and peritoneal fat were obtained from the Panamanian peccary. The fatty acid composition and distribution were essentially the same in both samples. These results are in agreement with those in an earlier report (5) that the anatomical location from which adipose tissue is taken does not influence the distribution of fatty acids on the triglyceride molecule.

Adipose tissue obtained from the animals listed above was extracted with ethyl ether. Triglycerides were isolated from this extract by silica gel chromatography (6). The distribution of fatty acids in the triglyceride molecule was determined by the selective hydrolysis of the ester linkages at the primary positions with pancreatic lipase (7). Fatty acid compositions of the resulting monoglyceride and the original triglyceride were determined on the methyl esters by gas-liquid chromatography under the following conditions: liquid phase, 12%~(w/w) of ethylene glycol adipate polyester on 60-80 mesh, acidwashed and neutralized Chromosorb W; column length, 200 cm; temperature 200°; He flow rate, 50 ml/min, standard temperature and pressure; sample size, 0.5-5  $\mu$ l; detector, thermal conductivity. Quantitative results with National Heart Institute fatty acid ester standards showed that values from 1 to 5% have a maximum relative error of 10%; those above 5%, a maximum relative error of 5%. The over-all relative error of the analytical values is about 7%. Since we are concerned with the distribution pattern of the major fatty acids, any fatty acid that constituted less than 1% of the total is not reported. The sum of these unreported acids varied among the species, but in no instance constituted more than 3% of the total.

## RESULTS

The fatty acid distribution in the triglycerides of these various fats is shown in Table 1. For each fat, the first line gives the composition of the whole triglyceride, the second line gives the composition of the fatty acids in the 2-position of the triglyceride, and the third line (Proportion) reports the percentage of each fatty acid that is in the 2-position. If a fatty acid is randomly distributed among all three positions in the triglyceride molecule, the proportion value will be 33%. The occasional proportion value that is in excess of 100% is attributable to the large relative error where a fatty acid is present in very small amounts.

Lard (sample 1) clearly shows a high concentration of palmitic acid in the 2-position. The feeding of a large amount of safflower seed oil to a pig (sample 2) resulted in an increase in the linoleic acid content of the lard and a decrease in the level of the other acids. In spite of the decrease in the content of palmitic acid, this acid was still esterified predominantly in the 2-position.

Domestic pigs probably originated from the selective breeding of the wild boar. The distribution of palmitic acid in the wild boar, both European (sample 3) and American (sample 4), show that this specific positioning of the palmitic acid in the domestic pig is not the result of selective breeding.

The peccary, although similar in appearance to the pig, is usually assigned to a separate family. It is likely that the pig and peccary arose from a common suoid stock which differentiated into the two families during the late oligocene period. This common origin is supported by the preponderance of the palmitic acid that is esterified with the 2-position in the triglyceride of the peccary (samples 5 and 6).

Of the still existing species, the hippopotamus is probably the one most nearly related to the pig and the peccary. The hippopotamus is believed to have differentiated much earlier in time, probably near the end of the eocene period. In this species the suoid pattern of palmitic acid distribution does not occur.

The remaining species in Table 1 are of other families of the artiodactyla. In none of these is there the specific

TABLE 1		FATTY ACID COMPOSITION OF WHOLE TRIGLY-							
CERIDES A	ND	of Fa	тту Ас	IDS AT	THE	2-Pos	TION;	AND	Pro-
PORTION O	OF	Еасн	Fatty	Acid	THAT	IS AT	THE	2-Post	TION

		14:0	16:0	18:0	16:1	18:1	18:2	18:3	
		mmole %							
1.	Domestic pig								
	Triglyceride	1	28	15	3	42	9	2	
	2-Position	5	72	4	4	12	3	0	
	Proportion*	167	86	9	44	10	11	0	
2.	Domestic pig,								
	"unsaturated"								
	Triglyceride	1	13	8	1	20	54	3	
	2-Position	2	36	3	2	12	42	2	
	Proportion*	67	92	12	67	20	26	22	
3.	Wild boar, Switzer-								
	land								
	Triglyceride	1	28	15	1	45	10	0	
	2-Position	3	74	4	2	12	4	0	
	Proportion	100	88	9	67	9	13		
4.	Wild boar, U.S.A.								
	Triglyceride	1	25	14	3	43	13	1	
	2-Position	3	61	4	5	20	7	Ō	
	Proportion	100	81	10	56	16	18	Ō	
5.	Peccary, Panama								
	Triglyceride	1	23	12	3	51	8	2	
	2-Position	5	58	4	6	20	6	1	
	Proportion	167	84	11	67	13	25	17	
6.	Peccary, U.S.A.		•					• ·	
	Triglyceride	2	22	16	6	42	7	3	
	2-Position	4	61	6	9	17	3	1	
	Proportion	67	92	12	50	14	14	11	
7.	Hippopotamus							••	
	Triglyceride	4	27	21	5	38	3	2	
	2-Position	3	15	6	6	63	2	4	
	Proportion	25	19	10	40	55	22	67	
8.	Camel		•••						
	Triglyceride	5	31	31	4	28	1	Tr.	
	2-Position	3	9	7	4	70	4	2	
	Proportion	20	10	8	33	83	133		
9.	White-tailed deer		••	Ũ	00	00			
	Triglyceride	3	24	31	3	36	2	1	
	2-Position	4	27	23	2	40	3	1	
	Proportion	44	38	25	22	37	50	33	
10	Sheen		50	40		5.			
	Triglyceride	5	27	27	3	35	2	1	
	2-Position	6	14	9	5	58	6	2	
	Proportion	40 <sup>°</sup>	17	11	55	55	100	67	
11.	Beef		• ·	••				0,	
	Triglyceride	4	30	25	5	36	1	0	
	2-Position	8	14	8	6	61	3	õ	
	Proportion	67	16	11	40	-56	100		
		~ .							

\* Percentage of fatty acid type esterified with the 2-position, i.e., mole % 14:0 in 2-position

 $\frac{1100}{9} \times \text{mole } \frac{\%}{14:0 \text{ in triglyceride}} = \text{percentage 14:0 in triglyceride}$ ide that is located in 2-position.

distribution of palmitic acid that is seen in the pig and the peccary.

Besides palmitic acid, only stearic and myristic acids show patterns of distribution. In all species of the present study, stearic acid is esterified predominantly at the 1and 3-positions of the triglyceride. Although palmitic acid also tends to concentrate in the 1- and 3-positions, except in the pig and peccary, this effect is not as marked as for stearic acid. Thus the statement that saturated fatty acids are esterified predominantly with the 1- and 3positions of nonsuoid animal triglycerides applies more clearly to stearic than to palmitic acid. Certainly myristic

JOURNAL OF LIPID RESEARCH

ASBMB

acid does not follow such a pattern, for it is found mainly in the 2-position.

Except in the pig and the peccary, there is a tendency for the unsaturated acids to be concentrated in the 2position of the triglycerides. However, this specificity does not approach that seen with palmitic acid in the 2position of the suoids or with stearic acid in the 1- and 3positions of all the species that have been examined. Whether there is a pattern of distribution of the unsaturated acids would require the examination of many more species.

We wish to thank the following individuals for supplying the samples used in these studies: E. H. Ahrens, Jr., New York; M. Further, Basle, Switzerland; C. P. Gandal, New York; F. Grande, Minneapolis, Minn.; C. M. Johnson, Panama City, Panama; I. Mann, Kabeta, Kenya; U. A. Nolen, Corpus Christi, Tex.; L. K. Sowls, Tucson, Ariz.; and, particularly,

R. S. Harris of Cambridge, Mass., who approached many of these people for us.

Manuscript received January 17, 1964; accepted February 27, 1964.

## References

- 1. Hilditch, T. P., and W. J. Stainsby. Biochem. J. 29: 90, 1935.
- 2. Meara, M. L. J. Chem. Soc. no vol: 23, 1945.
- 3. Quimby, O. T., R. L. Wille, and E. S. Lutton. J. Am. Oil Chemists' Soc. 30: 186, 1953.
- 4. Mattson, F. H., and E. S. Lutton. J. Biol. Chem. 233: 868, 1958.
- 5. Savary, P., J. Flanzy, and P. Desnuelle. Biochim. Biophys. Acta 24: 414, 1957.
- 6. Quinlin, P., and H. J. Weiser, Jr. J. Am. Oil Chemists' Soc. 35: 325, 1958.
- 7. Mattson, F. H., and R. A. Volpenhein. J. Lipid Res. 2: 58, 1961.

Downloaded from www.jir.org by guest, on June 19, 2012

MATTSON, VOLPENHEIN, AND LUTTON Triglyceride Structure 365