Fatty Acid Composition and Glyceride Structure of Piglet Body Fat from Different Sampling Sites

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Abstract

Pigs were slaughtered at 16 weeks of age, and fat samples were obtained from outer and inner shoulder, outer and inner loin, and kidney. Fatty acid composition and glyceride type of distribution were determined. Glyceride structure was determined by pancreatic lipase hydrolysis. There were highly significant differences in fatty acid composition between sites. Fatty acids containing less than 18 carbon atoms were preferentially incorporated in the internal positions of the glycerides. The content of saturated fatty acids and fatty acids containing less than 18 carbon atoms at the 2-position of shoulder and loin glycerides was higher than in kidney glycerides. Differences in glyceride types were noted between sites.

Introduction

ANATOMICAL VARIATION in fatty acid composition and glyceride structure has not been studied extensively, probably because, up until a few years ago, analytical methods were not available for the investigation of small samples.

Hilditch and Williams (1) reported variation in the fatty acid composition of the trisaturated glycerides of pork fat which were obtained from different sites within the animal, and these findings have been supported by both Leat et al. (2) and Sink et al. (3). Savary et al. (4) found that, although fatty acid composition varied with the anatomical location of the pork sample, the preferential placing of palmitic acid at the 2-position was unaffected.

Privett et al. (5), when studying the glyceride structure of various rat tissues, noted a preferential incorporation of certain fatty acids into specific positions, which resulted in different structures of the triglycerides in various tissues. Barford et al. (6) found that the distribution of the principal glyceride types in the internal and external adipose tissue fats from the same pig was nonrandom, and the percentages of palmitic acid at the 2-position in these adipose fats were comparable; liver glycerides from this same animal differed strikingly from adipose glycerides. A study by Chacko and Perkins (7) on pork fat samples indicated that definite variations in the glyceride type of distribution existed according to the site from which the fat was obtained.

Experimental

Four piglets, 16 weeks of age, were slaughtered, and fat samples were taken from the following sites: outer shoulder, inner shoulder, outer loin, inner loin, and kidney.

The lipids were extracted in a Soxhlet apparatus with light petroleum ether (bp 30–60C) for 24 hr. The solvent was evaporated on a steam bath, and the samples were stored at −20C until used for analysis. Samples from the various sites of each piglet were analyzed individually.

Hydrolysis of the lipid samples with pancreatic lipase (E.C. 3.1.1.3) (Steapsin, Nutritional Biochemicals Corporation, Cleveland, Ohio) was carried out in a jacketed glass vessel under carefully controlled conditions of temperature, pH, and agitation. The temperature was maintained at 37C by circulation of water from a theromostat bath through the jacket, and the pH was maintained between 7.9 and 8.1 by a pH controller (Fermentation Design Inc., Edison, N.J.), which regulated the addition of 0.5 N NaOH. The mixture containing 2 g of lipid sample was agitated violently throughout the hydrolysis period by a high-speed mechanical stirrer. The extent of hydrolysis could be ascertained by the volume of 0.5 N NaOH added; hydrolysis was continued until approximately 15% of the ester bonds of the fat sample were hydrolyzed; this required about 2 min.

The lipids were extracted from the hydrolysis mixture with two 200-ml volumes of diethyl ether-light petroleum ether (50% v/v). The ether solution was filtered, washed with five 50-ml volumes of 1% $NaHCO_3$ solution, dried over sodium sulfate; the ether was evaporated on a steam bath.

Separation of mono-, di- and triglycerides was carried out by using a method similar to that of Carroll (8). The Florisil was deactivated with 7% deionized water, and 12 g of this were packed in chromatographic columns of 1.3 cm in diameter and 22 cm in length. The samples were dissolved in hexane to a concentration of 100 mg per milliliter, and 1 ml was added, by pipette, directly to the column. Since monoglycerides are not readily soluble in hexane, the mixture was shaken before 1 ml was removed. Triglycerides were eluted with 100 ml of 15% diethyl ether in hexane, diglycerides with 100 ml of 50% diethyl ether in hexane, and monoglycerides with 100 ml of 3% methanol in ether.

Separation of the lipid classes by thin-layer chromatography on Silica Gel G plates (9) was used to check the purity of the fractions eluted from the Florisil columns.

Methyl esters of the component fatty acids were made according to the method of deMan (10). Gas-liquid chromatographic analysis was carried out on an F & M (F & M Scientific Corporation, Avondale, Pa.) Model 720 dual column temperature-programmed, gas chromatograph with a thermal conductivity detector. The 10 ft × ¼ in. O.D. stainless steel columns were packed with 20% diethylene glycol succinate on 60/80 mesh firebrick. Methyl esters were chromatographed isothermally at 220C. The injection temperature was 275C, the detector was 240C, and the flow rate of helium gas was 50 ml per minute.

The areas under the peaks were measured by a planimeter and converted to weight percentage of methyl esters by using correction factors, established with pure compounds under identical conditions (10). The correction factors for 20:0, 20:1, 20:2, and 22:0 were established by extrapolation of the curve plotted from the previously established correction factors (11).

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TABLE I

Percentage of Fatty Acids Esterified at the 2-Position of Glycerides from the Outer and Inner Shoulder, Outer and Inner Loin, and Kidney of Piglets on a High Fat Diet, Calculated According to the Method of Mattson and Volpenhein (13).

Fatty acid		Outer shoulder	Inner shoulder	Outer loin	Inner loin	Kidney
14:0	Original	1.8	1.6	1.7	1.6	2.0
	2-monoglyceride	4.4	4.3	4.6	3.8	3.5
	% 2-position	84	87	90	83	58
16:0	Original	21.8	22.4	21.6	22.0	24.4
	2-monoglyceride	62.9	64.7	60.0	61.7	69.0
	% 2-position	96	96	92	94	94
16:1	Original	4.3	3.7	3.6	3.4	2.8
	2-monoglyceride	6.4	4.6	6.0	5.4	2.0
	% 2-position	50	41	54	54	24
18:0	Original	9.5	11.2	9.4	11.0	14.0
	2-monoglyceride	3.2	3.7	3.2	3.6	4.0
	% 2-position	12	11	12	11	10
18:1	Original	52.7	51.2	54.0	52.0	46.2
	2-monoglyceride	20.2	19.2	21.4	21.4	19.0
	% 2-position	13	12	13	14	14
18:2	Original	6.6	6.8	6.2	6.4	7.0
	2-monoglyceride	2.2	2.5	2.9	2.3	2.4
	% 2-position	11	12	16	12	12
20:0	Original	0.9	0.7	0.8	1.0	0.9
	2-monoglyceride	0.6	0.4	0.6	0.8	tr
	% 2-position	20	19	24	26	<33
20:1	Original	1.0	0.8	0.9	1.0	1.0
	2-monoglyceride	....	tr	tr	tr	....
	% 2-position	<33	<33	<33	<33	<33

Results and Discussion

The fatty acid composition of the porcine glycerides before hydrolysis and the composition of the purified monoglycerides, obtained after 15% hydrolysis with pancreatic lipase, are given in Table I.

An analysis of variance (12) of the component fatty acids of the original triglycerides, and the application of Duncan's multiple range test (12) on these data (Table II) revealed that there were highly significant differences in fatty acid composition between sites. In most cases kidney fat was found to be significantly different from the other sample sites; this was particularly evident with palmitic, stearic and oleic acids. When considering the depot fats taken from the shoulder and the loin, there was more difference between samples taken from the inner and outer layers than between samples taken from the shoulder and the loin. This was best illustrated with stearic and oleic acids; there was no significant difference between outer shoulder and outer loin or between inner shoulder and inner loin, but the difference between outer and inner sites was highly significant. The amount of palmitic acid in kidney fat was significantly different from the amount of this acid in the fat from other sites.

The fatty acid composition of the inner shoulder and inner loin fats was intermediate between the fatty acid composition of the kidney fat and the composition of the outer shoulder and outer loin fats. Specifically, greater amounts of palmitic and stearic acids and lesser amounts of oleic acid were found in kidney and inner body fats than outer body fats.

TABLE II

Results of Duncan's New Multiple Range Test on the Mean Fatty Acid Composition of Piglet Glycerides Obtained from Various Sites; Means with the Same Superscript Across the Rows Do Not Vary Significantly

Fatty acid	Outer shoulder	Inner shoulder	Outer loin	Inner loin	Kidney
14:0	1.8^{a}	1.8^{a}	1.7^{a}	1.8^{a}	2.0^{a}
16:0	21.8^{a}	22.3^{a}	21.6^{a}	22.3^{a}	24.9^{b}
16:1	4.2^{c}	3.6^{b}	3.8^{bc}	3.5^{ab}	3.0^{a}
18:0	8.9^{a}	11.4^{b}	9.2^{a}	11.1^{b}	13.9^{c}
18:1	53.4^{c}	50.7^{b}	54.2^{c}	51.6^{b}	46.1^{a}
18:2	6.6^{ab}	6.9^{b}	6.0^{a}	6.5^{ab}	6.8^{b}
20:0	0.8^{a}	0.8^{a}	0.8^{a}	0.9^{a}	0.8^{a}
20:1	0.8^{a}	0.8^{a}	1.1^{a}	0.9^{a}	0.9^{a}

These results agree with those of Leat et al. (2) and Sink et al. (3).

The percentage of each fatty acid at the 2-position in the triglycerides of each fat was calculated from the fatty acid composition of the original triglycerides and the 2-monoglycerides obtained after 15% hydrolysis with pancreatic lipase (13) (Table I). Smith et al. (14) have suggested that less than 28% of a particular fatty acid esterified at the 2-position indicates that the fatty acid is located preferentially at the 1- and 3-positions and that more than 38% at the 2-position indicates preferential attachment of the acid at the 2-position. The results indicate that myristic and palmitic acids were preferentially incorporated into the 2-position of all the porcine glyceride samples under study. Stearic, oleic, linoleic, arachidic, and gadoleic acids were esterified primarily at the 1- and 3-positions. Palmitoleic acid seemed to be preferentially esterified at the 2-position in the inner and outer shoulder and in the inner and outer loin fats but was located at the 1- and 3-positions in kidney fat.

Brockerhoff et al. (15) studied the positional distribution of fatty acids in the triglycerides of animal depot fat and concluded that the distribution between the internal and external positions was governed by chain length and unsaturation. The shorter[2] and more unsaturated fatty acids show a greater tendency to occupy the internal position. These authors maintained that this rule also applies to porcine fat, but in these glycerides the influence of chain length overrides that of unsaturation. To determine whether shorter chain fatty acids or saturated fatty acids have a greater preference for the internal position on porcine triglyceride molecules, comparisons were made between short- and long-chain fatty acids at the 2-position and between saturated and unsaturated fatty acids at the 2-position (Table III). Myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids were used in the comparisons, and the percentages at the 2-position were calculated by avearaging the component percentages listed in Table I. There was more of a preference for the shorter chain fatty acids than for the saturated fatty acids at the 2-position of the glycerides. These results support the theory of Brockerhoff et al. (15) that short-chain fatty acids are preferentially incorporated into the internal positions of porcine glycerides.

The results in Table III show that the proportion of saturated and shorter chain fatty acids at the 2-position of shoulder and loin glycerides was constantly higher than in kidney glycerides, which indicated that kidney fat showed a lesser tendency than

[2] In the context of this paper "short" is used for all fatty acids with less than 18 carbon atoms.

TABLE III

Proportions of fatty acids at the 2-position in the glycerides of outer and inner shoulder, outer and inner loin, and kidney fats of piglets

Sample site	Proportion at the 2-position based on unsaturation		Proportion at the 2-position based on chain length	
	saturated[a]	unsaturation[b]	shorter chains[c]	longer chains[d]
Outer shoulder	64	25	77	12
Inner shoulder	65	22	75	12
Outer loin	65	28	79	14
Inner loin	63	27	77	12
Kidney	54	17	59	12

[a] Saturated fatty acids included are 14:0, 16:0, and 18:0.
[b] Unsaturated fatty acids included are 16:1, 18:1, and 18:2.
[c] Shorter chain fatty acids included are 14:0, 16:0, and 16:1.
[d] Longer chain fatty acids included are 18:0, 18:1, and 18:2.

TABLE IV

Glyceride types calculated from pancreatic hydrolysis data according to Vander Wal (16)

Sample site	Composition: Types (weight %)				Composition: Isomers (weight %)			
	GS_3	GS_2U	GSU_2	GU_3	SUS	SSU	USU	UUS
Outer shoulder	1.4	17.8	59.0	21.7	0.6	17.2	51.8	7.2
Inner shoulder	2.1	20.8	56.1	21.1	0.9	19.8	47.2	8.9
Outer loin	1.6	18.2	57.1	23.2	0.7	17.4	48.8	8.3
Inner loin	2.0	20.7	58.2	19.0	0.7	20.0	50.7	7.5
Kidney	3.6	27.2	54.8	14.4	1.1	26.0	46.7	8.0

the adipose fats to esterify saturated fatty acids at the 2-position. This could mean that kidney glycerides may have a different structure from shoulder and loin glycerides.

Results of the calculation of glyceride types according to the method of Vander Wal (16) (Table IV) showed that some differences may exist in the glyceride types present in the piglet fats from the various sample sites. Kidney fat contained higher amounts of GS_3 and GS_2U, but lower amounts of GSU_2 and GU_3, than fats from the shoulder and loin. Kidney fat contained the least amount of the isomer USU in comparison with the other sites, but little difference existed between sites as far as their UUS content was concerned. A study of the glyceride types of inner and outer shoulder fat and the inner and outer loin fat revealed that inner shoulder and inner loin fats contained more GS_3 and GS_2U than outer shoulder and outer loin fat. Outer shoulder and outer loin fats seemed to contain more GU_3, but the amount of GSU_2 and its isomers in the shoulder and loin samples were varied.

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