Biol. Neonate *34:* 61–67 (1978)

Relationship between Fatty Acids and Lipid Peroxidation in Lungs of Neonates

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Key Words. Lung · Fatty acids · Triglycerides · Lipid peroxidation · Rat milk lipids · Neonates

Abstract. Triglycerides from the lungs of neonatal rats and mice were found to contain large amounts of the polyunsaturated fatty acids, arachidonate (20:4) and docosahexaeonoate (22:6). These fatty acids were diminished or absent in the triglycerides from the lungs of adult rats and mice and both neonatal and adult guinea pigs. No age-related changes were observed in the fatty acid composition of lung phospholipids in any of these species. The presence of arachidonic acid and docosahexaenoic acid in lung triglycerides correlated with the ability of these lungs to peroxidize lipids *in vitro* in all species. Depletion of lung triglycerides in neonatal rats by fasting abolished this lipid peroxidizing activity.

Introduction

Changes in the fatty acid composition of triglycerides from liver (3, 12), heart (15) and lung (3, 8) have been reported in developing rats. The fatty acid composition of phospholipids from these organs did not change. In rat lung triglycerides, the quantity of arachidonic acid (20:4) and docosahexaenoic acid (22:6) diminishes as the animal matures (8). This decrease may be related to the dietary intake of fatty acids. The fatty acid composition of rat milk has been reported (3, 14) but no information is available on any alterations during lactation. The first purpose of this study was, therefore, to analyze the fatty acid composition of rat milk during lactation and relate any alterations to the changing composition of lung triglycerides.

The polyunsaturated fatty acids found in neonatal rat lungs are highly susceptible to *in vitro* lipid peroxidation (8, 9). Lungs from neonatal mice, but not neonatal guinea pigs also exhibit this lipid peroxidizing activity (9). The second purpose of this study, therefore, was to analyze the fatty acid composition of lung lipids from neonatal and adult mice, guinea pigs and rats with the intention of comparing collected data with the previously determined ability of these lungs to peroxidize lipids.

Materials and Methods

Female Sprague-Dawley rats were obtained from ARS Sprague-Dawley (Madison, Wisc.) and bred in our laboratory as described elsewhere (1). Pregnant Swiss-Webster mice were obtained from Arthur Sutter (Springfield, Mass.). Pregnant guinea pigs were an inbred Hartley strain (West Branch, Iowa). Lung tissues were obtained from adult females and neonates of both sexes. Lung phospholipids and triglycerides were isolated from total lipid extracts by thin-layer chromatography and their fatty acid composition determined by gas chromatography as described previously (8). Rat milk was obtained from nursing mothers who were maintained on Purina Laboratory Chow (Ralston Purina, St. Louis, Mo.). These animals were anesthetized with pentobarbital (35 mg/kg, i.p.) and then injected with 1 U of oxytocin (Sigma, St. Louis, Mo.). 5 min later milk was collected from all nipples by aspiration. Lipids were extracted from the milk by the method of *Folch et al.* (4) and the fatty acids identified by gas chromatography following transesterification with 14% borontrifluoride in methanol (8, 11). Neonatal rats were fasted by placing them in a 32 °C incubator with a humidified atmosphere for 40 h. Lipid peroxidation was measured following homogenization of neonatal lung tissue in a Sorvall Omni-mixer as described by *Kehrer and Autor* (9). Protein concentrations were measured by the microbiuret method (6).

Results

A previous study on the fatty acid composition of rat lung lipids included data obtained from rats 1, 5, and 12 days after birth as well as from adult animals (8). The fatty acid composition of lung lipids from fetal (day 21 of gestation) and 19-day-old rats and is reported in table I. The fatty acid composition of the triglyceride fraction from fetal rat lungs was similar to that of rats 1 and 5 days after birth (8). The percentage of arachidonic acid and docosahexaenoic acid in the triglycerides from lungs of 19-day-old rats was lower than that in fetal lungs and appeared to be intermediate between that found in 12-day-old and adult rat lungs. The fatty acid composition of the phos-

Table I. Fatty acid composition of rat lung lipids[1]

Fatty acid[2]	Triglycerides		Phospholipids	
	fetal[3]	19 day	fetal[3]	19 day
12:0	0.2	4.8	0.0	0.0
14:0	2.7	7.5	2.6	3.6
14:1	0.5	0.8	4.5	7.8
16:0	27.2	26.5	27.4	21.0
16:1	6.4	2.3	8.1	2.3
18:0	6.8	9.0	10.3	13.8
18:1	24.3	25.9	14.8	13.4
18:2	9.3	14.9	3.8	6.4
20:4	8.3	4.3	20.0	26.4
22:6	14.6	4.1	8.5	5.7

[1] Data are expressed as the percentage by weight of the total fatty acid methyl esters measured.
[2] Carbon atom chain length:double bonds.
[3] Lungs were removed from fetuses on day 21 of gestation.

pholipids from fetal and 19-day-old rat lungs was similar to that seen at all other ages.

Of all the polyunsaturated fatty acids identified in rat lungs, arachidonic acid (20:4) and docosahexaenoic acid (22:6) are present in the greatest quantity (8). Both fatty acids have been shown to be susceptible to lipid peroxidation (2, 10). Figure 1 shows that in the rat lung, these two fatty acids show age-related quantitative changes in these two fatty acids in the triglyceride fraction which parallel the age-dependence of lung lipid peroxidizing activity (9). Little change in fatty acid composition with age was seen in the phospholipid fractions from rat lungs (fig. 1).

The cause of the age-related changes in the fatty acid composition of rat lung triglycerides is not clear. It has been suggested that in the liver (12) and brain (13) lipids of neonatal rats these polyunsaturated fatty acids have a dietary origin. This implies that changes in the fatty acid composition of the diet or the absorption of polyunsaturated fatty acids may result in the alterations seen in the fatty acid composition of tissue lipids. Rats ingest milk exclusively until about 15 days after birth. The major changes in the fatty acid composition of rat lung triglycerides occur 5–19 days after birth.

Analysis of the fatty acid composition of rat milk at various days postpartum revealed minimal changes in the content of all fatty acids except arachidonic acid and docosahexaenoic acid which declined from 1 to 12 days postpartum (table II).

Like the rat neonatal, but not adult mouse lung lipids are susceptible to *in vitro* peroxidation (9). Analysis of the fatty acid composition of mouse lung lipids revealed that the triglyceride fraction from neonatal but not adult lungs contained the peroxidizable fatty acids, arachidonate and docosahexaenoate (table III). No differences were evident in fatty acid composition of phospholipids between 6-day-old and adult mouse lungs (table III).

Lung homogenates from either neonatal or adult guinea pigs are not susceptible to *in vitro* lipid peroxidation (9). Analysis of the fatty acid composition of lipids from the lungs of

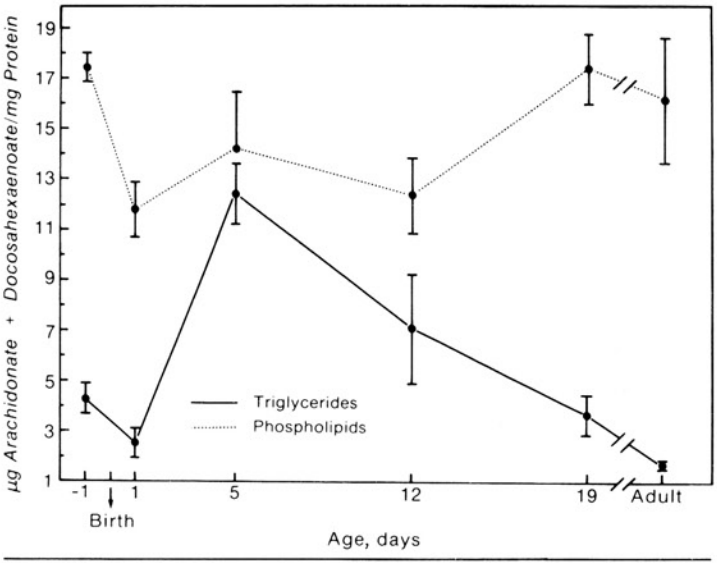

Fig. 1. The quantity of the methyl ester derivatives of arachidonic acid and docosahexaenoic acid in lung triglycerides and phospholipids as a function of age.

Table II. Fatty acid composition of rat milk[1]

Fatty acid[2]	Days postpartum							
	1	2	3	4	5	8	11	12
8:0	3.9	5.9	6.2	4.6	5.9	3.3	4.5	6.1
8:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
10:0	9.6	14.8	16.2	12.2	17.2	10.7	13.1	16.3
10:1	0.2	0.5	0.3	0.4	0.6	0.3	0.4	0.5
12:0	5.7	9.0	8.8	9.7	13.0	11.1	10.8	11.7
12:1	<0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.3
14:0	3.4	5.2	4.6	8.0	9.8	9.5	9.3	9.4
14:1	0.5	0.6	0.3	0.8	0.8	0.5	0.5	0.9
16:0	17.5	13.9	14.3	16.3	15.0	18.0	16.9	15.6
16:1	4.0	3.2	3.1	2.7	2.2	2.9	2.2	2.2
17:0	0.0	0.0	0.0	0.7	0.6	0.0	0.4	0.8
17:1	0.3	0.3	0.2	0.5	0.4	0.3	0.3	0.5
18:0	4.4	3.9	4.0	4.0	3.2	4.2	4.2	3.5
18:1	25.3	20.0	21.3	20.2	15.6	20.4	19.1	16.8
18:2	15.4	13.5	12.2	13.4	11.1	13.5	12.7	11.5
18:3	1.9	2.0	2.0	1.9	1.4	1.9	1.9	1.6
20:4	5.4	4.4	3.8	2.7	2.2	2.1	2.1	1.4
22:6	2.8	2.7	2.4	1.6	1.2	1.4	1.3	1.0

[1] Data are expressed as the percentage by weight of the total fatty acid methyl esters measured.
[2] Carbon atom chain length:double bonds.

Table III. Fatty acid composition of mouse lung lipids[1]

Fatty acid[2]	Triglycerides		Phospholipids	
	6 day	adult	6 day	adult
12:0	0.7	0.0	0.0	0.0
14:0	3.0	2.7	2.1	1.2
14:1	0.2	<0.1	3.3	6.4
16:0	28.9	32.5	25.7	27.1
16:1	4.1	6.3	2.5	5.7
18:0	5.8	9.1	12.3	13.0
18:1	25.4	36.7	12.1	10.1
18:2	11.9	11.3	4.7	5.4
20:4	7.9	1.4	20.7	18.1
22:6	12.0	0.1	16.6	12.9

[1] Data are expressed as the percentage by weight of the total fatty acid methyl esters measured.
[2] Carbon atom chain length: double bonds.

Table IV. Fatty acid composition of guinea pig lung lipids[1]

Fatty acid[2]	Triglycerides		Phospholipids	
	5 day	adult	5 day	adult
14:0	3.9	4.3	1.6	2.4
14:1	0.8	0.9	5.0	3.2
16:0	37.6	37.6	26.9	36.1
16:1	3.3	3.4	3.1	3.4
18:0	8.6	8.1	13.6	11.9
18:1	32.2	35.6	19.6	20.7
18:2	11.2	9.8	11.6	12.0
20:4	2.6	0.4	18.0	10.2
22:6	0.0	0.0	0.6	0.1

[1] Data are expressed as the percentage by weight of the total fatty acid methyl esters measured.
[2] Carbon atom chain length: double bonds.

Table V. Effect of fasting on rat lung lipids and lipid peroxidation: total quantity of lung fatty acids

Triglycerides[1]		Phospholipids[1]		Lipid peroxidation[3]	
fed	fasted[2]	fed	fasted	fed	fasted
56.8 ± 13.6	1.0 ± 0.3	44.0 ± 11.3	32.0 ± 10.7	0.12	0.02

[1] Values are expressed as micrograms of total fatty acid methyl esters per milligram of lung protein.
[2] Neonatal rats were fasted for 40 h by placing them in a 32 °C incubator with a humidified atmosphere.
[3] The extent of malondialdehyde formation was measured in the 900 *g* supernatant fraction of a lung homogenate. Data are expressed as the change in absorbance at 535 nm/mg protein following a 30-min incubation at 37 °C.

5-day-old and adult guinea pigs revealed no age-related differences in either the triglyceride or phospholipid fractions (table IV). Furthermore, the triglyceride fraction from this species contained only small amounts of arachidonic acid and no docosahexaenoic acid (table IV).

It has been reported that fasting will decrease the triglyceride content of neonatal rat lungs (5). Table V indicates that fasting neonatal rats for 40 h does deplete neonatal lung triglycerides and that this treatment greatly deminishes the ability of these lungs to undergo *in vitro* lipid peroxidation.

Discussion

The age-related changes in lipid peroxidizing activity of neonatal rat lung tissue appears to be caused by changes in the polyunsaturated fatty

acid content of lung triglycerides (8). The data in this paper support this hypothesis by demonstrating that fasting, which depletes lung triglycerides in neonatal rats, diminishes the amount of measurable lipid peroxidation. In addition, lungs from fetal and 19-day-old rats, which are not susceptible to lipid peroxidation, contain only small quantities of arachidonic acid and docosahexaenoic acid esterified as triglycerides. Previous data indicated that lung tissue from neonatal mice, but not neonatal guinea pigs were capable of generating lipid peroxides (9). This activity arises from the polyunsaturated fatty acid moieties of lung triglycerides which are also present in neonatal mouse lungs but lacking in adult mouse lungs and both neonatal and adult guinea pig lungs.

The source, complete function and explanation for age-related changes in the polyunsaturated fatty acid moieties of neonatal lung triglycerides is not yet clear. Examination of the fatty acid composition of rat milk suggests that the diet may contribute to these changes, although the modifications in the composition of lung triglycerides occur more rapidly than those in the milk. A second possibility is an alteration in the absorption of fatty acids in the neonate as a function of age. Such differences have been reported in the rat but do not appear to occur at the same rate as changes in pulmonary fatty acids (5). This suggests, therefore, that these age-related compositional changes are occurring in response to alterations in the manner by which polyunsaturated fatty acids are utilized by the lung. Neonatal rats and mice undergo rapid alveolar growth and cellular proliferation during the neonatal period while neonatal guinea pigs are born with relatively mature lungs (7, 16). These developmental changes will require large quantities of polyunsaturated fatty acids for membrane phospholipid synthesis. Lung triglycerides may function as a reservoir of unsaturated fatty acids for membrane biosynthesis, thus permitting continuous growth in spite of intermittent nutrition.

Acknowledgments

Dr. *Anne P. Autor* is the recipient of a Public Health Service Research Career Development Award (HD00068) from the National Institute of Child Health and Human Development. *James P. Kehrer* was supported by a Pharmacology Predoctoral Training Grant (5T 32GM07069-03) from the National Institute of General Medical Sciences. This work was also supported by USPHS grant NIGMS 12675.

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