LIPID ABSORPTION AND TRANSPORT IN THE SUCKLING RAT

by

Germain Jithendra Fernando

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOCHEMISTRY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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Fernando, Germain Jithendra

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The University of Arizona PH.D. 1982

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Germain Jithendra Fernando

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	Date 10/12/82
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SIGNED: Germain Fernando

THIS DISSERTATION IS DEDICATED TO MY MOTHER AND FATHER

AND TO MY GRANDFATHER AND UNCLE ANNESLEY

ACKNOWLEDGMENTS

First I must thank Dr. Wells from the bottom of my heart for all the help and advise he gave me during the course of this research work. It was a priviledge to work under his guidance. Dr. Wells is not only a great scientist but a person with an extremely kind heart.

I must also thank Joan and Mary Lou for helping in many ways and for their interesting discussions, and Patrick, Katie, Helen, Nancy, Raja, Cheryl, Susan, Celia, Wally, and all the others who made my stay in Tucson a real pleasure.

I am indebted to my wife Rosarine, who shared my difficulties during the time this dissertation was being written.

I am grateful to my dissertation committee and especially to Dr. Godfrey Getz, The University of Chicago for reading the manuscript and for the valuable comments offered.

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ABSTRACT

The suckling rat obtains about 70% of its energy from the catabolism of lipid, compared to only about 25% in the adult rat. In addition, lipids are in great demand for membrane synthesis in the rapidly growing tissues. Thus the suckling rat represents an important system in which to study lipid metabolism.

In this dissertation research the following experiments were performed on rats during the suckling period: (1) Determination of the concentration and fatty acid composition of triacylglycerols, cholesteryl esters, phospholipids, and free fatty acids in dam's milk, and in suckling rat lymph, portal plasma, vena cava plasma and liver; (2) Determination of positional distribution of fatty acids among the three different positions in triacylglycerols of milk, lymph, plasma and liver; (3) Isolation of plasma and mesenteric lymph lipoproteins by density gradient centrifugation, and identification of the individual classes of lipoproteins by measurement of their density and by polyacrylamide gel electrophoresis; (4) Determination of the concentration of each lipoprotein by refractometry and determination of the concentration and fatty acid composition of different lipid components in each lipoprotein fraction; (5) Studies of physical properties of the lipoproteins using analytical ultracentrifugation; (6) Determination of apoprotein composition and concentration in each lipoprotein fraction.

The results show that the suckling rat consumes a diet which is rich in medium chain fatty acids (35% of total milk fatty acids). Mesenteric lymph triacylglycerols carry a significant amount of these medium chain fatty acids, unlike the adult in which they would be transported as free fatty acids via the portal vein. Medium chain fatty acids are enriched at the sn-3 position in all triacylglycerols. Lymph contains chylomicrons, very low density lipoproteins, and high density lipoproteins, but no low density lipoproteins. Plasma contains all classes of lipoproteins but at concentrations higher than found in the adult, especially low density lipoproteins which are present at levels six times that found in the adult. The plasma very low density lipoproteins have a lipid composition which resembles that of chylomicron remnants. Apoprotein, lipid composition, and electrophoresis patterns show that the plasma lipoproteins in the suckling rat are heterogeneous.

INTRODUCTION

The suckling period is an important period for lipid metabolism. Neonatal rats obtain about 70% of their energy from the catabolism of milk triacylglycerols. In contrast, adult rats on a normal diet obtain only about 25% of their energy from lipids. Furthermore, lipids are required as the structural components of tissues which are growing rapidly during the suckling period. Therefore it is interesting to study whether lipids are metabolized by the neonate similar to adults or whether different pathways are used. In addition, the suckling rat provides a good model to study adaptation of an organism to a high fat diet. Although lipid absorption and transport have been extensively cstudied in the adult rat, these processes remain to be fully characterized in the suckling rat.

As a first step in studying lipid metabolism in the suckling rat, it is necessary to know the fatty acid composition and concentrations of lipid components in different body fluids. Since milk is the sole dietary source of lipids in the suckling rat, it was analysed in detail. The fatty acid composition of rat milk had been published (Helander and Olivecrona 1970; Smith, Watts, and Dils 1968; Rees, Shuck, and Ackermann 1966), but a complete analysis, especially of the major polyunsaturated fatty acids, had not been done prior to my work (Fernando-Warnakulasuriya et al. 1981).

All of these data show a high medium chain (8:0 to 12:0)[1] fatty acid content in rat milk triacylglycerols.

Complete plasma lipid fatty acid compositions for the suckling rat had not been reported, although plasma lipid concentrations had been published (Foster and Bailey 1976; Sherman, Guthrie, Wolinsky, and Zulak 1978; Cryer and Jones 1978). Lymph is the major carrier of lipid from the intestine to plasma. Therefore the fatty acid composition and concentrations of different lipids in lymph were analysed. Liver lipids were also analysed in order to determine how liver acts on lipid substrates.

The vehicle of lipid transport in blood is lipoproteins. Lipids are water insoluble substances. In order to transport lipids in the aqueous environment of the organism, the lipids are complexed with proteins to form water soluble particles which are called lipoproteins. The lipoproteins form in such a way that the nonpolar lipids (triacylglycerols and cholesteryl esters) are segregated into the core of the particle away from water, whereas the polar lipids (free cholesterol and phospholipids) and proteins are in the periphery of the particle facing the aqueous environment. This arrangement stabilizes the structure. Lipoproteins are synthesized by both the liver and the intestine.

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1. The notation for fatty acids are given as the number of carbon atoms and the number of double bonds separated by a colon. e.g. 16:0 for palmitic acid, and 18:1 for oleic acid.

Lipoproteins are subdivided into classes depending on their densities, namely, chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (Table 1). These different classes of lipoproteins not only have different densities, lipid compositions and protein components, but also have different metabolic functions. Chylomicrons are the major lipoproteins involved in dietary lipid transport from the intestine via lymph to the blood stream where the triacylglycerols are hydrolyzed and the liberated fatty acids are utilized by tissues. VLDL, which are synthesized in the liver and intestine, are carriers of endogenously synthesized triacylglycerols from liver and intestine into the blood stream. LDL mainly carry cholesterol from the liver to the peripheral cells for utilization. HDL transport excess cholesterol from extrahepatic tissues to the liver. These metabolic functions are determined by the protein components associated with the lipoprotein particle concerned. The protein moieties are called apolipoproteins or apoproteins. The major classes of apoproteins present in rat lipoproteins are apoA-I, apoA-IV, apoB, apoC, and apoE (Table 2). None of these lipoproteins or their apoproteins had been characterized in the suckling rat. Therefore I have determined the lipid compositions and concentrations of the different classes of lipids in suckling rat lipoproteins. Some work has also been done on the composition of apoproteins.

Table 1. Properties of rat lipoproteins[a]

Fraction	Source	Density kg/l	S_f[b]	Diameter nm	protein %	lipid %
CM	Intestine	<0.96	400-9000	75-600 av=120	2	98
VLDL	Liver & Intestine	0.96-1.006	20-400	28-75 av=58	14	86
LDL	CM, VLDL & Liver	1.006-1.063	0-20	15-30 av=21	30	70
HDL_1	Liver (Intestine)[c]	1.020-1.090	?	14-29 av=19	33	67
HDL_2	Liver (Intestine)[c]	1.063-1.125	3.5-9[d]	10-20 av=14	40	60
HDL_3	Liver (Intestine)[c]	1.125-1.21	0-3.5[d]	5-40 av=5.5	50	50

[a] CM = Chylomicrons; VLDL = Very Low Density Lipoproteins; LDL = Low Density Lipoproteins; HDL = High Density Lipoproteins.

[b] S_f = Floatation rate in svedberg units at a density of 1.063.

[c] = Intestinal HDL has not been assigned to a specific subclass.

[d] = $F^o_{1.2}$ values for human lipoproteins. These values are floatation rates in svedberg units at a density of 1.2.

Table 2. Apoproteins of rat lipoproteins[a]

Apo-protein	molecular weight	Function	component of: Lymph	component of: Plasma	Sites of synthesis
A-I	28,000	LCAT Activation	CM,VLDL,HDL	HDL	Intestine Liver
A-II	8,000	?	CM,VLDL	HDL	Liver?
A-IV	46,000	Intestinal TG transport?	CM,VLDL,HDL	HDL d>1.21	Intestine Liver
B_{335k}	335,000	CM & VLDL secretion; TG transport	CM,VLDL	LDL VLDL	Liver
B_{240k}	240,000	CM & VLDL secretion; TG transport	CM,VLDL	VLDL	Liver Intestine
C-I	7,000	Inhibition of CM uptake by liver	CM,VLDL	HDL	Liver
C-II	8,000	activation of lipase; Inhibition of CM uptake	CM,VLDL	HDL,VLDL	Liver>> Intestine
C-III	11,000	Inhibition of lipase? & of CM uptake	CM,VLDL	HDL,VLDL	Liver>> Intestine
E	35,000	binds to liver CM-receptor	CM,VLDL	HDL,VLDL d>1.21	Liver

[a] LCAT = Lecithin Cholesterol Acyl Transferase; CM = Chylomicrons; VLDL = Very Low Density Lipoproteins; LDL = Low Density Lipoproteins; HDL = High Density Lipoproteins; d = density in kg/l.

Diet and digestion

The only source of food for the neonatal (1-15-day old) rat is milk. Milk is very rich in fat (12%) and about 97% of the lipid is present as triacylglycerols (TG). In fact, 50% of the diet (dry weight) of the suckling rat consists of TG, whereas less than 10% of the adult diet is TG (Henning 1981). This amount of TG would account for about 70% of the energy intake in the suckling rat (Rokos et al. 1963). In spite of the importance of lipid digestion, absorption, and transport during the suckling period, few studies have been done to elucidate these processes.

Some of the earliest work on lipid metabolism in the neonatal rat was done by O. Koldovsky, P. Hahn, and associates at the Czechoslovak Academy of Sciences in the early nineteen sixties. They studied the changes in blood cholesterol, fatty acids, and acetoacetic acid during postnatal development (Grafnetter et al. 1960). This study showed that there is a change in all the lipid components measured at the time of weaning around 20 days. They concluded this was due to the change of the lipid content of the diet at the time of weaning. In another study (Rokos et al. 1963) it was shown that in 10-day-old rats, pancreatic lipase activity was only one-eighth of that in the adult rat, in spite of the fact that the suckling rat consumes a diet which contains more than four times the fat content. These observations led to a few studies as to how the suckling rat digests and absorbs fat efficiently. Helander and Olivecrona (1970) showed considerable hydrolysis of triacylglycerols in the suckling rat stomach. Hamosh and Scow (1973) showed the presence of a lipase secreted by the serous glands of the tongue. This lingual lipase was shown to hydrolyze triacylglycerols in the suckling rat stomach. This was confirmed by Staggers, Fernando-Warnakulasuriya, and Wells (1981) in a later study. This study also showed preferential hydrolysis of medium chain fatty acids from milk TG by lingual lipase. The absorption of these medium chain fatty acids via the portal vein was shown by Aw and Grigor (1980).

The composition of rat milk has been published (Helander and Olivecrona 1970; Smith, Watts, and Dils 1968; Rees, Shuck, and Ackermann 1966), but a complete fatty acid analysis, particularly of the major polyunsaturated fatty acids had not been reported. All these data show a high medium chain fatty acid content in rat milk triacylglycerols. Stereospecific analysis of cow milk triacylglycerol by Pitas, Sampugna, and Jensen (1967) had shown that shorter chain fatty acids were selectively esterified at the sn-3 position in the triacylglycerol molecule. Later studies by Marai, Breckenridge, and Kuksis (1969) on goat and sheep milk, and studies on pig milk by Christie and Moore (1970) indicated that this selectivity is a common feature in animal milk. Since stereospecific analysis of rat milk triacylglycerols has not been previously reported, I analysed them and the results have been published (Staggers et al. 1980). This study showed that triacylglycerols of rat milk also show a selectivity for medium chain fatty acids at the sn-3 position. This analysis has helped in our study (Staggers et al. 1980) to elucidate the mechanism of action of lingual lipase.

Composition and concentrations of lymph, plasma, and liver lipids

As a first step in studying lipid metabolism in the suckling rat, it is necessary to know the the fatty acid composition and concentrations of lipid components in mesenteric lymph, plasma, and liver. Dobiasova, Hahn and Koldovsky (1964) had published the fatty acid composition of triacylglycerols and phospholipids from liver of the suckling rat at different ages. This study showed that liver triacylglycerols increase dramatically within one day after birth whereas liver phospholipid increase is more gradual. These postnatal increases of lipid levels were attributed to the high fat content of milk. They also showed that the medium chain fatty acid composition of liver triacylglycerols reflects the composition of those fatty acids in the diet, but this effect was not seen in the phospholipid composition. Their results show a significant decrease of arachidonic acid (20:4) in the liver phospholipid fraction with increasing age. In a more detailed study on the variation of plasma free fatty acids and triacylglycerols with age, Forster and Bailey (1976) showed there is considerable day to day variation of these lipids in the blood of suckling rats. The reason for these changes is not clear though they presumed it to arise from the effect of hormones.

Lipoproteins

Most of the work on lipoprotein characterization has been done on human plasma. This is due to the assumption that studying lipoproteins helps in the understanding of cardiovascular disease in man. Recently more work has been done on other animals especially rats, because they are being used as experimental models for lipid and lipoprotein metabolism in man. However no studies had been published on suckling rat lipoproteins. In spite of the fact that only small amounts of samples can be obtained from the suckling rats, it is potentially one of the most interesting systems to study as far as lipid metabolism is concerned, because of the huge influx of lipids into the suckling rat system.

Adult rat lipoprotein levels have been measured by several groups and this has been reviewed by Chapman (1980), and I quote "Comparison of the available and rather meager data in the literature on the distribution of serum lipoproteins in the rat reveals the disturbing finding that large variations in lipoprotein levels have been found in the animals of the same strain.". He attributed these variations to a problem inherent to the pooling of small blood samples from several animals. For Sprague-Dawley rats, de Pury and Collins (1972) have reported the concentrations of VLDL, LDL and HDL to be 107, 56 and 240 mg/100ml, respectively, whereas Lasser et al. (1973) reported values of 46, 16 and 80 mg/100ml, respectively. These differences may be due to differences in sex and/or age of the animals used. The chemical composition of rat plasma lipoproteins also showed variations between different studies (Chapman 1980): VLDL: triacylglycerols (TG) 50-75%, cholesteryl esters (CE) 1.9-17%, free cholesterol (CH) 2.6-8.9%, phospholipids (PL) 9.6-18.3%, and protein (PR) 5.4-13.8%; LDL:TG 5.5-31.8%, CE 12.8-39.8%, CH 4.8-19.1%, PL 18.7-28.7%, and PR 12.6-28.0%; HDL:TG 0.6-7.6%, CE 10.5-33.6%, CH 3.1-14.8%, PL 15.1-29.8%, and PR 32.8-61.0%. These variations are probably due to the different density intervals used by various investigators in isolating LDL and HDL fractions from rat plasma.

The concentrations of each lipid component and the overall fatty acid composition of Wistar rat lipoproteins were determined by Malhotra and Kritchevsky (1978). They studied rats 1.5 months and 24 months old, and the results show that there is a significant difference in the distribution and chemical composition of lipids between lipoproteins of young and old rats. The concentrations of plasma lipoprotein CE, CH, TG, and free fatty acids (FFA) in older rats were higher than in young rats. The amount of sphingomyelin (SP) when compared to phosphatidylcholine (PC), is higher in older rats. Furthermore, unsaturated fatty acids predominate in young rat plasma lipoproteins, and saturated fatty acids predominate in older rat lipoproteins. They reported the ratio of 18:2, 18:3 and 20:4 fatty acids to 16:0 and 18:0 fatty acids to be 0.89 for the young and 0.55 for the older rat. In the phospholipid fractions of both the young and the older rats, PC was the predominant phospholipid in all lipoprotein classes representing at least 60% of the total. The percentage of SP in all the phospholipid fractions was less than that of the human lipoproteins as reported by Skipski et al. (1967). Studies by Tall et al. (1979), and Lusk et al. (1979) agree with the above reported value, that SP constitutes only about 18% of the total phospholipids in rat lipoproteins. A complete study of the fatty acid compositions of all the different classes of phospholipid present in rat plasma lipoproteins has not been performed by any of these authors. The cholesteryl ester fatty acid composition of rat lipoproteins shows a high percentage (~40%) of arachidonic acid

(20:4) (Mookerjea, Park, and Kuksis 1975). Rat HDL CE contains less 18:2 (~25%) and more 20:4 (~40%) than in humans (Nichols 1967). These data suggest that rat lipoprotein CE might play an important role in the transport of essential fatty acids especially 20:4 in blood. Furthermore these results indicate either some difference between the substrate specificity of lecithin cholesterol acyl transferase (LCAT) activity in rat and man, or differences in the fatty acid composition of lecithin in rat and man, or alternatively, that lipoprotein CE have differing origins in the two species (Chapman 1980). Triacylglycerol fatty acid composition of all lipoprotein fractions in the adult rat show that it is mainly composed of 16:0 (~20%), 18:1 (~40%), and 18:2 (~22%) (Mookerjea et al. 1975).

Apoproteins

Since the apoprotein composition determines the metabolic fate of lipoproteins, it is important to study the apoprotein composition of lipoproteins. The first two groups to investigate rat lipoprotein apoproteins were Koga, Horwitz, and Scanu (1969), and Bersot et al. (1970). Both of these studies showed heterogeneity of the apoproteins. Koga et al., reported the sedimentation properties, molecular weights, immunological properties, and amino acid composition of apoproteins. Bersot et al. studied the apoproteins of rat lipoproteins by gel filtration and ion-exchange chromatography, and they confirmed the heterogeneity of rat apoproteins and the fact that some apoproteins were shared by VLDL, LDL, and HDL, as shown by Koga et al. They also noted the similarity between some of the rat and human apoproteins, and the presence of an apoprotein fraction of high molecular weight (>150,000) in LDL and VLDL, which was later shown to be analogous with human apoB (Krishnaiah et al. 1980). In 1974 Swaney, Reese, and Eder analyzed rat HDL apoproteins by SDS-gel electrophoresis, which gave better separation than the methods used by the previous authors. They showed the presence of three major and several minor protein components in rat HDL apoprotein fraction. The component present in the greatest amount (50-60%) was shown to be a 27,000 molecular weight protein which is comparable to apoA-I in human HDL. The other two major proteins were shown to be of molecular weights 46,000 and 35,000; which the they termed apoA-IV and "arginine-rich" apoprotein (or ApoE), respectively. They could not detect these apoproteins in human HDL, although recently apoA-IV was shown to be present in human VLDL (Beisegel and Utermann 1979). The amino acid composition of these three major apoproteins has also been determined by Swaney et al (1974). The minor components were composed of low molecular weight apoC polypeptides and apoA-II. Herbert et al. (1974) analyzed this low molecular weight fraction by gel filtration and ion-exchange chromatography and their results indicated this fraction consisted of four polypeptides representing homologues of the human A-II, C-I, C-II, and C-III apoproteins. The apoA-II which is normally present in humans as the dimeric form, was shown to exist in the monomeric form in the rat. However rat and human apoA-II closely resembled each other in amino acid composition. The apoC fraction of the rat was also shown to be similar to human apoC fraction. The apoC-I and C-II of rat and man were alike in amino acid composition, and these lacked carbohydrate. Rat apoC-III was resolved into two polymorphic forms, C-III-0 and C-III-3 depending on the carbohydrate content. C-III-0 lacked sugar residues whereas C-III-3 contained one galactosamine and three sialic acid residues per mole. Humans have C-III-0 as a minor form, and C-III-I and C-III-2 as the major forms (Kane et al. 1975). Some dissimilarities between human and rat C-III amino acid composition have also been observed. Swaney and Gidez (1977) used isoelectric focusing in further analyses of these low molecular weight fractions. The A-II, C-II, C-III-0, and C-III-3 components focused as single bands with isoelectric points of 4.83, 4.74, 4.67, and 4.50, respectively. SDS-gel electrophoresis of these components indicates their molecular weights to be 8,000, 8,000, 10,000, and 11,000, respectively. ApoC-I was shown to have a molecular weight of 7,000, but the authors failed to get a satisfactory resolution of this basic peptide by isoelectric focusing even at a high pH range (7-9).

The very high molecular weight fraction (>150,000) called apoB, which is a major protein constituent of the triacylglycerol-rich lipoproteins, is insoluble in water after delipidation (Schaefer, Eisenberg, and Levy 1978). Studies of humans with abetalipoproteinemia, a genetic absence of apoB, indicate that it plays an indispensable role in the intracellular transport and secretion of TG-rich lipoproteins (Herbert, Gotto, Fredrickson 1978). It also serves a functional role in the metabolism of LDL by binding to high affinity cell surface receptors in various tissues and thereby initiating LDL internalization and catabolism (Goldstein and Brown 1977). In the rat, apo-B is synthesized in the liver and intestine, and in no other organ (Wu and Windmueller 1979). Recent studies of rat apoB on 3.5% SDS polyacrylamide gels by

Krishnaiah et al. (1980) show that the circulating lipoproteins in the rat contain at least two immunologically distinct forms. The higher molecular weight form which has a molecular weight of 335,000 predominates in plasma VLDL and LDL, and the smaller form which has a molecular weight of 240,000 is the major form in lymph lipoproteins and VLDL of fed rats. This distribution and the increased plasma concentration of the smaller form after the rat is fed with fat, indicates the origin of the smaller form to be the intestine. Studies by Wu and Windmueller (1981) using isotope labeling methods show that rat small intestine produces mainly the smaller form of apoB with only trace amounts of larger form being produced. The liver was shown to produce both forms. The circulating half-life of the hepatic larger form was 2.4 times that of hepatic smaller form, indicating independent catabolism of the two forms. This suggests different metabolic properties for the two forms. The half life of smaller form from the intestine was found to be even shorter than the smaller form from the liver.

Mesenteric (Intestinal) Lymph Lipoproteins

Intestinal lymph contains all of the lipoproteins found in plasma (Borgstrom and Laurell 1953). The origin of these lipoproteins, especially their apoproteins, poses an interesting problem. These intestinal lipoproteins are transported via lymph to the blood stream where they are catabolized. Using lymph-cannulated rats, Ockner, Hughes, and Isselbacher (1969) showed that the intestine can synthesize VLDL and contribute TG to the plasma pool via lymphatics in fasted animals. Intestinal lymph lipoprotein apoproteins contain apoA-I, A-IV, B, C, and E. Recently the presence of another apoprotein in the rat lymph chylomicrons and VLDL has been reported (Fidge and McCullagh 1981). This protein has a molecular weight of 59,000, and has been named as apoA-V. Because of its association with lymph chylomicrons, it is thought to play some important role in the exogenous TG transport. It was uncertain whether these proteins are present as the result of de novo intestinal synthesis or transfer from plasma. Synthesis of apoA-I by intestine is indicated by the experiments done by Glickman and Kirsch (1973) using intraperitoneal injection of radioactive amino acid precursor. This was confirmed by their later study (Glickman and Green 1977), using duodenal injections of the precursor into rats whose secretion of hepatic lipoproteins were reduced by the administration of 4-aminopyrazolopyrimidine. Studies using intestinal perfusions done by Wu and Windmueller (1978) show the capability of intestine to synthesize apoB, apoA-I, and apoA-IV. Under these conditions, only limited synthesis of apoC was carried out by the intestine, and only apoC-II and apoC-III-0 fractions were involved. This study also indicates no apo-E or apoA-II is synthesized by the rat intestine. These results were confirmed by Krause et al. (1981) in their study of rats treated with ethinyl estradiol which produces hypolipidemia in the rat. They also showed that apoA-IV is the most inducible of the apoproteins during fat absorption, which indicates it to be important in the formation of TG-rich lipoproteins by intestine. However no one has published any data on apoproteins of suckling rat lipoproteins.

Lipoprotein Metabolism

Dietary fat consists mostly of triacylglycerols. After digestion and absorption, dietary TG and other lipids are repackaged into lipoprotein particles in the intestine. These lipoproteins, mainly consisting of chylomicrons and VLDL, are transported via the thoracic lymph duct into the blood stream. Being in contact with plasma, the lymph chylomicrons acquire apoC and apoE from plasma HDL (Imaizumi, Fainaru, and Havel 1978; Blum 1980). Chylomicrons are then acted upon by lipoprotein lipase, an enzyme present in capillary endothelium of most extrahepatic tissues, and most of the TG in the core of the particles is hydrolyzed (Nestel, Havel, and Bezman 1962). The newly acquired apoC of chylomicrons contains apoC-II which activates lipoprotein lipase (Fielding and Havel 1977); and also the apoC inhibits chylomicron removal by the liver, enabling peripheral catabolism to occur (Shelburne et al. 1980; Windler, Chao, and Havel 1980a). Chylomicron TG hydrolysis results in the formation of partial glycerides which may be taken up by the tissues (Scow, Blanchette-Mackie, and Smith 1976) or hydrolyzed in plasma (Fielding 1981), and fatty acids which are removed by binding to plasma albumin (Fielding and Havel 1977). During hydrolysis, the surface components viz., phospholipids, apoA-IV, apoA-I, and apoC, of these lipoproteins are transferred to plasma HDL (Tall et al. 1979, and Redgrave and Small 1979). This hydrolysis results in the formation of chylomicron remnants which are rich in cholesteryl esters and phospholipids, and these remnants are rapidly removed by the liver (Redgrave 1970). During remnant formation, there is considerable loss of chylomicron mass (~96%) which involves the loss of TG, PL, and apoproteins. The major apoproteins of the remnants are apoB (intestinal) and apoE. During remnant formation, apoC is transferred back from the chylomicrons to the HDL fraction, allowing the remnant to be removed by the liver. The removal of the chylomicron remnants by the liver is a rapid, receptor mediated process (Sherrill and Dietschy 1978, Carrella and Cooper 1979). Several studies indicated that apoE is the major chylomicron determinant for uptake by the liver receptor (Shelburne et al. 1980; Windler et al. 1980a; and Windler et al. 1980b). Studies by Windler et al. (1980b) on animals treated with ethinyl estradiol to lower their apoE level, showed that even apoE deficient chylomicrons were rapidly removed by the liver, suggesting that intestinal apoB may also be a recognition factor. Intestinal VLDL or small chylomicrons probably are metabolized as chylomicrons (Green and Glickman 1981). No studies have been done to determine the fate of lymph HDL. It is thought that this discoidal HDL which is known to be an excellent substrate for lecithin cholesterol acyl transferase (Hamilton et al. 1976), will acquire cholesteryl ester and contribute to plasma HDL.

Plasma lipoproteins are involved mainly in endogenous lipid transport. Plasma contains VLDL, LDL, and HDL; and these interact dynamically within the vascular system. Many of the processes involved in lipoprotein metabolism are regulated by enzymes which will influence plasma lipoprotein levels. Plasma VLDL is synthesized in the liver and intestine, and is the primary vehicle for the transport of endogenously synthesized TG. The TG carried in VLDL is transported to sites of storage (adipose tissue) and utilization (heart, muscle, mammary gland, lung, etc.). The availability of fatty acids of either endogenous or exogenous origin and the rate of de novo synthesis of fatty acid in the liver, have been shown to affect VLDL formation (Eisenberg and Levy 1975). VLDL catabolism proceeds by the initial hydrolysis of TG by extrahepatic lipoprotein lipase, the remnant formed from this hydrolysis is converted to plasma LDL or taken up by the liver and degraded. Recent studies by Berry et al. (1981) on the role of liver in the degradation of VLDL indicates the ability of hepatic lipoprotein lipase to hydrolyze VLDL TG and PL. However at present, the exact mechanism by which VLDL is taken up by liver cells is uncertain. Some LDL is formed by the conversion of VLDL into LDL in the circulation (Fidge and Poulis 1975). Unlike man, in the rat most VLDL is not converted to LDL in plasma. This may explain the lower concentrations of LDL in the rat compared with man. In 1978, Fidge and Poulis showed that there is a plasma LDL subfraction (Sf 0-5 or d= 1.040-1.063 g/ml) which is derived independently of VLDL catabolism in the adult rat. Whether this LDL fraction comes directly from the liver is not known. Studies done by Marsh (1974) with perfused rat liver, failed to find de novo LDL secretion. LDL delivers cholesterol to the peripheral tissues, where specific cell surface receptors bind, internalize, and degrade the LDL particles providing the cell with cholesterol (Goldstein and Brown 1977). It is assumed all LDL return to liver and get degraded. However there is little evidence in the literature to support this assumption. Studies by Stein et al. (1977) indicate that extrahepatic tissues also play an important role in LDL degradation.

Plasma HDL consists of spherical particles in which an apolar core of CE and TG is stabilized by a shell of PL and apoproteins. Plasma HDL is derived from a precursor HDL of different structure and chemical composition. The precursor HDL is derived either from secretion by the liver or during the lipolysis of the TG-rich lipoproteins (chylomicrons and VLDL) (Tall and Small 1980). Hepatic secretion of HDL has been inferred from its presence in perfusates of rat liver. Marsh (1976) showed that the noncirculating liver perfusate contains a CE-poor, PL-rich lipoprotein in the density 1.06 to 1.21 g/ml range, with apoE as its main constituent in contrast to plasma HDL which had apoA-I as its major apoprotein. Hamilton et al. (1976) confirmed the above finding; they showed that the liver secretes disc-shaped lipid bilayer particles which represent the nascent form of HDL. Their studies also show that the precursor HDL is the preferred substrate for LCAT. LCAT is the enzyme responsible for esterification of most of the cholesterol in plasma (Glomset 1968), and is activated by apoA-I (Fielding et al. 1972). This enzyme catalyzes the transfer of an acyl group (fatty acid) from the 2-position of lecithin (ie. PC) to the 3-hydroxyl of cholesterol. When LCAT acts on these discoidal precursor HDL, the cholesterol present in the surface of these particles is converted to cholesteryl ester, which being an apolar material, forms a core expanding the bilayer into a sphere. Tall and Small (1978) suggested a mechanism for the formation of discoidal HDL from chylomicrons and VLDL. When chylomicrons and VLDL are acted upon by lipoprotein lipase, their apolar lipid core is diminished and this exerts pressure on its polar core and causes it to break away as bilayer discs. Such folds of bilayer membrane may be seen arising from the chylomicron surface when treated with lipase in vitro (Blanchette-Mackie and Scow 1976). These discs may be converted to spherical plasma HDL, secondary to the action of LCAT. Plasma HDL plays an important role as an acceptor of PL and apoA-I from chylomicrons and VLDL. In vitro studies show HDL can accept cholesterol from cell membranes and the action of LCAT may enhance the cholesterol transporting capacity of HDL. Whether or not these factors are involved in the cholesterol removal in vivo remains to be demonstrated (Tall and Small 1980). In the rat, HDL is removed predominantly by the liver, and possibly by the small intestine (Eisenberg et al 1973).

Statement of Problem

The purpose of the research to be described in this dissertation was to investigate lipid absorption and transport in the suckling rat. This was accomplished by studying the following: (i). Measuring the concentrations of TG, PL, FFA, CE, and CH in milk, lymph, portal plasma, vena cava plasma, and liver. (ii). Determining the fatty acid composition of TG, PL, FFA, and CE in milk, lymph, liver, portal and vena cava plasma. (iii). Analyzing the stereospecific fatty acid distribution in TG of milk, lymph, plasma, and liver. (iv). Separating the lipoproteins into different density classes and determining the density of each fraction. (v). Determining the concentration of different classes of lipids present in the different fractions of lipoproteins. (vi). Determining the fatty acid composition of different classes of lipids present in the different fractions of lipoproteins. (vii). Measuring the amounts of apoproteins present in each fraction of lipoproteins. (viii). Studying the physical properties of lipoproteins by analytical ultracentrifugation.

METHODS

Sprague-Dawley rats were obtained from a breeding colony maintained by the Division of Animal Resources of the College of Medicine. At 2 days after birth the litters were reduced to ten pups. Nine to ten day old rats were used in all the studies except in the lipoprotein studies, where fourteen to fifteen day old rats were used. These pups were used within 30 minutes after removal from the mother, in order to maintain the nearly continuous fed-state characteristic of suckling rats.

Milking of rats

Rats were milked under ether or pentobarbital anesthesia after intraperitoneal injection of 200 milliunits of oxytocin.

Collection of mesenteric lymph

All surgical procedures were carried out under pentobarbital anesthesia (40 ug/g body weight, i.p.). Lymph was collected by cannulating the mesenteric lymph duct with PE-10 polyethylene tubing using the procedure of Warshaw (1972). The cannula was filled with a heparin solution (1000 units/ml) and the lymph collected into tubes in ice buckets. Generally lymph was collected for 4 hours while the animals were maintained under anesthesia in a warm box at 30^{o} C. Lymph from animals was pooled, diluted with an equal volume of NaCl solution (density=1.006), and centrifuged in a Brinkmann Eppendorf 3200 centrifuge for 2 minutes to remove any fibrin clots and blood cells. The supernatant was used in the analyses.

Collection of blood plasma

Blood was collected under diethyl ether anesthesia via aortic puncture using Monoject tubes (Sherwood Medical, St. Louis, MO.) containing EDTA, and kept at 4^{o} C until all samples were collected. Plasma was separated by centrifuging the blood in a clinical centrifuge for 20-30 minutes at 4^{o} C. An attempt was made to assess the role of the liver in altering the fatty acid composition of blood lipids by comparing portal and vena cava blood, as follows. A suture was used to tie off the portal vein just below the liver. The animal was then exsanguinated through a cut in the vein which was made between the suture and the gastrointestinal tract. For the vena cava sample, the suture was placed between the liver and the heart and the animal was exsanguinated through a cut in the vena cava between the liver and the suture. However, it should be realized that the blood obtained from the vena cava is not only of hepatic drainage, but also represents drainage from other viscera and the lower limbs. Due to the technical difficulty of cannulating the hepatic vein, I have assumed that the vena cava blood has a composition similar to that of the hepatic vein

Lipid extraction from samples

Plasma, milk, and liver lipids were extracted by the method of Wells and Dittmer (1965), omitting the acidified chloroform methanol extraction. Livers were freeze-clamped (using clamps precooled in liquid nitrogen) immediately after removal, and the frozen tissue was powdered before extraction. In the analyses of free fatty acids in plasma, lymph, and liver, the lipid extracts were not washed, in order to prevent the loss of medium chain fatty acids. All the lipoprotein fractions except chylomicrons were extracted by the method of Bligh and Dyer (1959). The chylomicron fractions were extracted by the method of Folch, Lees, and Sloane Stanley (1957).

Separation of lipid classes

Neutral lipids were separated into cholesteryl ester, triacylgycerol, cholesterol, and phospholipid on silica gel G thin layer plates, using hexane-diethyl ether-formic acid 80:20:2 (Christie, Noble, and Moore 1970). Phospholipids were separated into phosphatidylethanolamine, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine, on silica gel G plates using chloroform-methanol-acetic acid-water 50:30:8:3 (Skipski, Peterson, and Barclay 1964). Lipids were detected by spraying the plates with a 0.1% aqueous solution of ANS (Gitler 1972). Each band was scraped from the plate and extracted by a modification of the method of Arvidson (1968), in which ammonium hydroxide replaced acetic acid and the extracts were washed with 0.9% saline. In the case of the free fatty acid band, the original method was used except that the extracts were washed with saline.

Quantitative analysis of lipids

Phospholipids were quantitated by measuring the amount of phosphorous in the lipid extracts by the method of Bartlett (1959) after digestion with 70% perchloric acid. Cholesterol was measured by the method of Bowman and Wolf (1962), reducing the total volume of the assay to 1 ml. However due to the high content of TG in lipoproteins, the following method was used instead in the total cholesterol determination of lipoproteins. The lipoprotein lipid extracts were dried in a screwcapped tube, 50-100ul of stigmasterol (1ug/ul) in ethanol was added as an internal standard followed by 500ul of 2M sodium methoxide solution in methanol. The mixture was allowed to react at room temperature for 90 minutes. After adding 1 ml of water and 2 ml of isooctane, the isooctane layer was analyzed using a Hewlett-Packard 403 gas chromatograph equipped with a 3380A integrator. The columns (glass, 20 in., i.d. 1/8 in.) were filled with 3% JXR 100/120 Gas Chrom Q (Applied Science Labs, State College, PA.) and the separations were carried out at 230^{o} C. The carrier gas used was helium at a flow rate of 100ml/min. Free cholesterol was quantitated by the addition of the stigmasterol standard to the lipid extracts prior to their separation on silica gel plates; then extracting the spot corresponding to these two sterols and analyzing in the gas chromatograph. Triacylglycerols (TG), free fatty acids (FFA), cholesteryl ester (CE), and phospholipids (PL) were quantitated by methylating their fatty acids in the presence of an internal standard and running in the gas chromatograph. The following are the internal standards used: For TG and PL, tritridecanoin; for FFA, tridecanoic acid; for CE, cholesteryl eicosadienoate. The TG, FFA, and

CE standards were added to the lipid extracts, prior to their separation on silica gel plates.

Preparation of methyl esters for GLC analysis

Methyl esters were prepared by transmethylating the acyl lipids according to a modification of the method of Doss and Oette (1968). Lipids (0.01 to 2mg) were dissolved in 100ul of dichloromethane and 100ul of 2M sodium methoxide in anhydrous methanol was added; the mixture was kept at room temperature under nitrogen in the dark for 5 minutes (for cholesteryl esters the incubation time was prolonged to 25 min). The reaction was stopped with 100ul of acetic acid, followed by 1.5ml of water, and the methyl esters were extracted with 5ml of hexane. The hexane layer was washed twice with 1ml of 0.9% NaCl solution and dried with anhydrous $MgSO_4$. The hexane layer was concentrated to about 100ul under a stream of nitrogen, and 1-2ul was analyzed by GLC. Free fatty acids were methylated with diazomethane, according to the method of Schlenk and Gellerman (1960). Sphingomyelin fatty acids were methylated using a modification of the method of Nelson (1962). The sphingomyelin samples (10-200nmol) were dissolved in 500ul of anhydrous methanol, which was mixed with 35ul of concentrated sufuric acid. The tubes were tightly capped after flushing with nitrogen, and incubated at 60-63^{o} C for 12-16 hours. Then 1.5ml of water was added followed by 5.0ml of hexane, the mixture was vortexed thoroughly. The hexane layer was washed thrice with 1ml of 0.9% NaCl solution, and evaporated to dryness under a stream of nitrogen.

GLC analysis of methyl esters

Gas liquid chromatography was performed on a Shimadzu GC mini 1 gas chromatograph equipped with a Shimadzu C-R1A recording data processor. Columns (glass 1.8m, i.d. 2.6 mm) were filled with 10% Silar 10C on 100/120 Gas-Chrom Q packing (Applied Science Labs, State College, PA.). A linear temperature program from 130 to 230^{o} C at a rate of 4^{o} C/min was used.

Stereospecific analysis of triacylglycerols

Stereospecific analysis of TG was performed by the method of Brockerhoff (1965), as modified by Christie and Moore (1969). The *sn*-2-monoacylglycerols were generated by the method of Mattson and Volpenhein (1964) as described by Myher and Kuksis (1979). The *rac*-1,2-diacylglycerols were generated from TG as described by Myher and Kuksis (1979), except hexane:diethyl ether 1:1 (v/v) was used as the solvent system in the tlc separation of diacylglycerols. Phosphatidylphenols were prepared by the procedure of Brockerhoff (1965), as described by Breckenridge (1978), and these were stereospecifically hydrolyzed by phospholipase A_2, according to the method of Christie and Moore (1969). The phospholipase A_2 (EC 3.1.1.4) was purified from *Crotalus adamanteus* venom by the method of Wells (1975). The fatty acid composition of the *sn*-3 position was calculated from the composition of the hydrolysis products.

Lipoprotein isolation

Solutions of different densities were prepared according to Redgrave, Roberts, and West (1975). Chylomicrons were separated from plasma and lymph by centrifugation using a Beckman SW60 rotor (35,000rpm for 25 min. or 3×10^6 g-min.) at 5^oC, and the centrifuge was stopped without using brakes. The tubes were punctured at the bottom and the chylomicron-free plasma was removed. The chylomicrons remaining in the tubes were carefully transferred into vials using the solution of density 1.006. The chylomicron suspension was mixed with 0.5 volumes of the solution of density 1.019, overlaid with the 1.006 solution, and centrifuged as described above. The chylomicrons, free of plasma contaminants, were collected from the top meniscus of the solution. The chylomicron-free plasma was subjected to density gradient centrifugation according to a modification of the method of Redgrave et al. (1975). Three milliliters of chylomicron-free plasma were placed in the bottom of a centrifuge tube (SW41) and mixed with 0.975g of KBr. This solution was successively overlaid with 3.2 ml of density 1.063, 3.2 ml of density 1.019, and 2.6 ml of density 1.006 solutions. The sample was centrifuged at 34,000rpm for 44-48 hours at 5^oC, and stopped without using brakes. One ml fractions were collected starting from the top of the tube. The fractions were dialyzed against 4 liters of 0.15M NaCl, 1mM EDTA (pH=7.0) at 4^oC overnight using a microdializer (Bethesda Research Labs, Gaithersburg, MD.). The density of each fraction from the density gradient was measured using an 1 ml pycnometer at 5^o using samples from the gradients run without plasma.

Lipoprotein analysis

The lipoproteins were characterized by polyacrylamide gel electrophoresis according to the method of Naito et al. (1973). The refractive index of each lipoprotein fraction was measured relative to the dialysate using a Brice-Phoenix Differential Refractometer (Virtis, Gardiner, NY.), in order to determine the concentration of lipoproteins (Lindgren 1974). Proteins were assayed according to the method of Kashyap, Hynd, and Robinson (1980) using bovine serum albumin as the standard. Furthermore in the assay of chylomicron containing samples, all tubes were extracted with diethyl ether after color development to minimize the turbidity.

Analytical ultracentrifugal studies

Total plasma lipoproteins (minus chylomicrons) were isolated by mixing 0.975g KBr in 3.0 ml of chylomicron-free plasma, overlayering with 1.21 density KBr solution and centrifuging for 48 hours at 35,000 rpm (SW 41) at 5^{o}C. The top 1 ml was removed and dialysed against either a 1.21 density NaBr or 1.063 density NaCl solution. Analytical ultracentrifugation was carried out in a Beckman model E equipped with electronic speed control at 25^{o}C and 52,000 rpm. Schlieren photographs were taken and data analyzed as described by Lindgren (1974).

Preparation of apoproteins

Chylomicrons were delipidated with diethyl ether according to Wu and Windmueller (1978). Chylomicron suspension was vortexed with 20 volumes of diethyl ether for 2 minutes at room temperature. After centrifugation, the aqueous layer was reextracted with 10 volumes of ether. A gentle stream of nitrogen was directed into the aqueous layer containing apoproteins to remove any traces of ether. The other lipoprotein fractions were extracted by a slight modification of the method of Lux, John, and Brewer (1972). All the lipoprotein gradient fractions were lyophillized, each protein residue was moistened with a drop of saline, and 5 ml of chilled $CHCl_3$:CH_3OH (2:1 v/v) was added and mixed. After incubating this mixture for 30 minutes at $4^{o}C$, 1mg of Dextran T-500 was added and the suspension vortexed. Then 5 ml of diethyl ether was added and the mixture was centrifuged at $4^{o}C$ in a Sorvall SS-34 rotor at 12,000rpm for 20 min. The protein pellet was dried under a gentle stream of nitrogen.

Apoprotein analysis

Apoproteins were analyzed by SDS gel electrophoresis according to the method of Weber and Osborn (1969). The gels used were 10% acrylamide. The concentration of TEMED used was half the recommended value, and the persulfate concentration was reduced to 11 mg/ml, so that the gels would polymerized in about 20 min. The protein in the gels were stained with a solution of 0.1% Coomassie Brilliant Blue R 250 in 50% methanol and 12% acetic acid overnight. Gels were destained by several changes of the destaining solution which consists of 5% methanol and 7.5% acetic acid in water. These gels were scanned at 633nm to determine the relative concentrations of individual apoproteins present.

RESULTS

The composition of rat milk is shown in Table 3. Rat milk is composed of 97% TG, while DG, CH, PL, CE, and FFA account for the remainder; no MG was detected in these samples. These results differ from those reported by Helander and Olivecrona (1970) especially with regard to TG content (97% vs 76%) and the presence of MG (9.5%). This variation may be due to the diet and/or methods used for obtaining milk.

Although studies have been done previously on the fatty acid composition of rat milk TG (Helander and Olivecrona 1970; Smith, Watts, and Dils 1968; Rees, Shuck, and Ackermann 1966), a complete analysis, particularly of the major polyunsaturated fatty acids, has not been reported. Therefore I analyzed the fatty acid composition of the major components of milk, and the results are reported in the Table 4. The fatty acid composition of rat milk TG agrees well with the values reported by others. An unusual feature of rat milk TG is the high content of its medium chain fatty acids (8:0 to 12:0). Milk TG consists of about 35% medium chain fatty acids.

The compositional data show that rat milk also contains polyunsaturated fatty acids (PUFA) (20:4, 22:5, 22:6). Furthermore, rat milk is a rich source of the essential fatty acid 18:2 which is the precursor of 20:4. The dietary 18:2 and 18:3 may be converted into PUFA in the suckling rat liver, brain, or other organs; where it is happening and how PUFA are transported to the developing tissues like brain, is not clear at present. This is one of the reasons which lead me to study

Table 3. Composition of rat milk[a]

Lipid	Concentration in mmol/l	mole%
Triacylglycerol (TG)	140.1 ± 69.5	97.3 ± 2.2
Diacylglycerol (DG)	1.6 ± 0.7	1.11 ± 0.03
Monoacylglycerol(MG)	N.D.	---
Cholesterol (CH)	1.2 ± 0.7	0.83 ± 0.02
Phospholipid (PL)	0.6 ± 0.2	0.42 ± 0.01
Cholesteryl ester (CE)	0.2 ± 0.1	0.14 ± 0.005
Free fatty acids (FFA)	<0.3	<0.2

[a] The results presented are mean ± S.D. for at least 4 determinations.

Table 4. Fatty acid composition of rat milk triacylglycerol (TG) and phospholipid (PL).

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
							mole%[a]						
TG	5.8	16.5	13.4	12.4	20.9	1.7	2.2	12.3	12.7	0.9	0.7	0.2	0.4
	(0.6	1.7	1.2	1.0	2.3	0.5	0.2	1.7	1.6	0.1	0.1	0.1	0.1)
PL	0.3	0.8	1.5	3.9	18.0	1.1	23.1	14.6	21.2	0.3	12.4	0.9	2.1
	(0.2	0.5	0.6	0.5	1.0	0.2	1.5	0.6	0.4	0.1	1.0	0.3	0.3)

[a] Mean value for at least three different samples. Standard deviation in parentheses.

suckling rat lipoproteins, which are the transport vehicles of fatty acids between different tissues of the body.

Composition of mesenteric lymph

The majority of lipid present in lymph is TG (85%) which carries a significant amount of 8:0 and 10:0 (~8%) fatty acids (Tables 5 and 6). In addition, a large amount of 12:0 (14.3%) is present. In the adult animals, most of the medium chain fatty acids are presumed to be absorbed via the portal system. The high content of medium chain fatty acids in the suckling rat lymph, may be due to the large amounts of fat being absorbed in these animals. Phospholipid comprises about 9% of lymph lipid and it has a high content of 18:2 (30.5%) and 20:4 (12.6%) (Table 7). This high amount of essential fatty acids present in lymph PL may be originating from bile PL and/or milk PL, and these fatty acids could be an important source of PUFA for the suckling rat. Indeed about 80% of the PL entering the small intestine is derived from bile (Staggers, Frost and Wells; results submitted for publication).

Comparison of lipids in portal and vena cava plasma, and of liver

An attempt was made to assess the role of the liver in altering the fatty acid composition of plasma lipids by comparing the lipids obtained from portal and vena cava blood. The comparison of lipids present in portal and vena cava plasma do not show significant differences except for the FFA fraction (Table 8). In portal blood, the FFA fraction has an increased content of medium chain fatty acids as compared to vena cava blood. These data support the notion of direct absorption of these fatty acids from the intestinal lumen after

Table 5. Quantitative analysis of lipid components[a] of 9-10 day old rats[b].

Lipid	Lymph mmol/kg	Portal Plasma mmol/l	Vena Cava Plasma mmol/l	Liver mmol/kg wet wt.
TG	85.3 ± 14.2	1.1 ± 0.2	1.0 ± 0.4	8.9 ± 2.3
PL	9.3 ± 4.1	2.8 ± 0.3	2.5 ± 0.2	23.4 ± 5.0
FFA	2.7 ± 0.7	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1
CE	2.0 ± 0.9	1.6 ± 0.6	1.6 ± 0.4	1.2 ± 0.2
CH	1.7 ± 0.9	1.1 ± 0.1	1.2 ± 0.1	6.1 ± 0.6

[a] TG=Triacylglycerols; PL=Phospholipids; FFA=Free Fatty Acids; CE=Cholesteryl Esters; CH=Cholesterol.

[b] The results presented are mean ± S.D. for at least 3 determinations.

Table 6. Fatty acid composition of Triacylglycerols (TG) from 9-10 day old suckling rats.[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2 w6	18:3[b] w3	20:4 w6	22:5 w3	22:6 w3
							mole%[c]						
LY	0.9	6.9	14.3	14.1	25.3	2.5	2.3	16.1	14.8	1.2	0.8	0.2	0.6
	(0.2	0.9	1.7	2.8	3.1	0.6	0.2	3.4	1.7	0.2	0.2	0.1	0.2)
PP	0.7	4.6	10.8	12.2	26.2	2.2	2.4	15.8	16.9	1.0	2.7	1.1	3.6
	(0.4	0.6	1.0	1.3	1.9	0.2	0.2	0.8	1.4	0.1	0.9	0.5	1.2)
LV	0.8	3.8	4.7	5.8	25.0	1.8	3.2	20.2	23.0	0.8	3.6	1.7	5.6
	(0.3	1.5	1.7	1.5	0.8	0.2	0.3	1.8	2.4	0.1	0.5	0.1	1.2)
VP	0.7	4.8	9.3	9.5	24.1	2.4	2.6	17.6	18.3	0.9	3.8	1.1	4.9
	(0.2	0.9	2.3	2.7	1.9	0.5	0.4	3.5	1.2	0.1	1.8	0.5	2.6)

[a] TG=Triacylglcerols; LY=Lymph; PP=Portal Plasma; LV=Liver; VP=Vena Cava Plasma.

[b] Value given is for 18:3 + 20:1.

[c] Mean for at least three different samples of ten pups each. Standard deviation is in parentheses.

Table 7. Fatty acid composition of phospholipids (PL) from 9-10 day old suckling rats[a]

	8:0	10.0	12.0	14.0	16.0	16.1	18.0	18.1	18.2 w6	18.3[b] w3	20.4 w6	22.5 w3	22.6 w3
							Mole%[c]						
LY	0.1	0.3	0.5	1.5	24.3	1.2	19.5	5.5	30.5	0.3	12.6	0.7	3.2
	(0.2	0.2	0.3	0.4	2.0	0.4	1.9	0.8	0.7	0.1	0.5	0.3	0.2)
PP	0.0	0.1	0.2	1.4	28.1	tr	16.8	4.7	24.9	tr	15.6	1.0	7.1
	(	0.2	0.1	0.2	0.8		0.3	0.5	1.6		1.6	0.7	0.4)
LV	0.0	tr	0.1	0.7	26.4	0.2	18.1	4.8	11.2	0.1	22.4	0.8	14.5
	(		0.1	0.3	1.5	0.2	0.9	0.3	0.5	0.1	0.6	0.4	1.0)
VP	0.0	0.1	0.2	1.2	27.8	0.1	16.7	5.0	24.0	0.1	16.6	0.9	7.4
	(	0.1	0.1	0.3	2.6	0.1	0.6	0.7	0.6	0.1	2.0	0.4	1.4)

[a] PL=Phospholipids; LY=Lymph; PP=Portal Plasma; LV=Liver; VP=Vena Cava Plasma.

[b] The value given is for 18:3 + 20:1.

[c] Mean for at least three different samples of ten pups each. Standard deviation in parentheses.

Table 8. Composition of free fatty acids (FFA) from 9-10 day old suckling rats.[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2 w6	18:3[b] w3	20:4 w6	22:5 w3	22:6 w3
						Mole%[c]							
LY	0.5	3.9	11.2	8.1	25.5	1.6	8.0	21.4	11.9	2.3	4.1	2.4	0.3
	(0.4	2.2	6.3	2.3	11.5	1.3	3.4	2.6	3.0	1.6	2.9	3.3	0.5)
LV	1.2	3.9	5.6	5.3	28.3	1.9	10.5	14.8	9.0	0.6	8.1	3.1	7.7
	(1.3	0.7	0.6	0.7	6.6	0.2	1.3	5.1	1.3	0.2	2.3	1.6	1.2)
PP	3.7	14.9	14.7	8.8	18.3	2.0	5.0	11.7	10.4	1.1	3.8	1.0	4.6
	(1.3	1.6	2.5	0.5	1.1	0.4	0.6	2.1	0.9	0.3	0.4	0.5	0.6)
VP	2.4[d]	6.1[e]	9.6[d]	8.5[f]	21.5[f]	2.6[f]	8.7[e]	15.4[f]	9.7[f]	1.8[f]	6.2[e]	1.1[f]	1.7[d]
	(0.5	3.9	4.6	1.4	0.9	0.7	1.9	4.7	1.6	1.2	1.7	0.4	2.1)

[a] FFA=Free Fatty Acids; LY=Lymph; LV=Liver; PP=Portal Plasma; VB=Vena Cava Plasma.

[b] Value given for 18:3 + 20:1.

[c] Mean for at least three different samples of ten pups each. Standard deviation is in parentheses.

[d] P<0.1

[e] P<0.05

[f] Not significantly different for portal-vena cava samples.

hydrolysis of TG in stomach or in intestine (Aw and Grigor 1980) and suggest rapid removal by the liver. Liver TG shows a lower 10:0 to 14:0 fatty acid content compared to plasma, which indicates that the liver may be using these preferentially for energy production. This preferential utilization of medium chain fatty acids for ketogenesis by the liver was shown in a recent study by Frost and Wells (1981). The amounts of 18:0, 18:1, 18:2, and 22:6 are somewhat higher in liver TG than in plasma. This might be due to the overall reduction of medium chain fatty acids in liver TG. In the PL fraction, the major differences between liver and plasma are in the content of 18:2, 20:4, and 22:6 (Table 7). The liver PL 18:2 content is less than half that of plasma, whereas the 20:4 and 22:6 content is very much increased. The cholesteryl ester fraction also shows differences in fatty acid composition between liver and plasma. The concentrations of 16:0, 18:0, and 18:1 are much higher in liver compared to plasma, whereas 18:2 and 20:4 are lower (Table 9). This indicates that plasma CE may be a carrier of these two essential fatty acids.

Stereochemical analyses of triacylglycerols

It has been recognized for some time that the fatty acids in natural TG are not randomly distributed among the three hydroxyl positions. In general, the distribution of fatty acids between the three positions of animal milk TG is extremely different. The long chain fatty acids are esterified predominantly to the sn-1 and sn-2 positions, and the medium chain fatty acids are predominantly esterified to the sn-3 position of the glycerol backbone. The results of Lin,

Table 9. Fatty acid composition of cholesteryl esters (CE) from 9-10 day old suckling rats[a]

	8:0	10.0	12.0	14.0	16.0	16.1	18.0	18.1	18.2 w6	18.3[b] w3	20.4 w6	22.5 w3	22.6 w3
							mole%[c]						
LY	tr	1.7	2.1	3.3	17.9	3.7	4.4	27.9	19.9	0.9	17.1	0.6	0.5
	(	0.1	0.3	0.2	2.8	0.2	2.3	4.1	2.1	0.2	6.4	0.1	0.8)
PP	0.0	tr	0.6	2.7	16.8	2.8	1.4	9.7	24.9	0.9	38.0	tr	2.3
	(		0.1	0.4	1.6	0.3	0.3	1.8	2.0	0.6	3.6		0.5)
LV	tr	0.6	0.9	2.7	22.7	3.2	4.3	28.5	18.8	0.9	14.9	0.3	2.2
	(	0.4	0.2	0.7	3.6	0.2	0.5	2.8	2.0	0.3	0.3	0.3	0.7)
VP[c]	0.0	0.1	0.3	2.5	15.8	3.0	1.4	11.6	24.2	0.4	38.7	0.1	2.2
	(	0.1	0.1	0.4	1.3	0.3	0.1	1.5	2.4	0.1	2.9	0.1	0.3)

[a] CE=Cholesteryl Esters; LY=Lymph; PP=Portal Plasma; LV=Liver; VP=Vena Cava Plasma.

[b] Value given is for 18:3 + 20:1.

[c] Mean for at least three different samples of ten pups each. Standard deviation in parentheses.

Smith, and Abraham (1976) and mine agree well on the distribution of fatty acids in rat milk TG. Fig. 1A shows the positional distribution of the major fatty acids in TG from milk. The 8:0, 10:0, and 12:0 fatty acids are enriched in position 3. This enrichment is greatest for 8:0 (~90%), then 10:0 (~70) and 12:0 (~50), indicating that the enzymes involved in the acylation of position 3 have an affinity for the shorter length fatty acids. Altogether these three medium chain fatty acids account for one third of the acids in the milk TG and over one half of the position 3 acids. There is some enrichment of 18:3, 20:4, and 22:5 also in position 3. It may be that the affinity of the enzymes are governed by the polarity of the fatty acid substrates. Position 2 is enriched with 14:0, 16:0, 16:1, 22:5, and 22:6. The reason for this enrichment is not clear. Filer, Mattson, and Fomon (1969) have shown that TG with 16:0 enriched in position 2 is better absorbed by human infants than TG with 16:0 enriched in the other positions. Position 1 is enriched in 18:0, 18:1, and 18:2 fatty acids, and these fatty acids account for over one half of the acids in the position 1.

The assimilation of large amounts of milk TG by the gastrointestinal tract of the suckling rat produces a high concentration of TG in the intestinal lymphatic fluid. The stereospecific fatty acid distribution of lymph TG (Fig. 1B) is also non-random and differs from that of the milk TG. Lymph TG also have position 3 enriched in 10:0 and 12:0 fatty acids as in milk TG. When compared to position 3 of milk, position 3 of lymph TG has a higher concentration of 18:1, 18:2, and 22:6 fatty acids, and a lower concentration of 18:0, 20:4, and 22:5 fatty acids. Position 1 and 2 also have some differences compared to

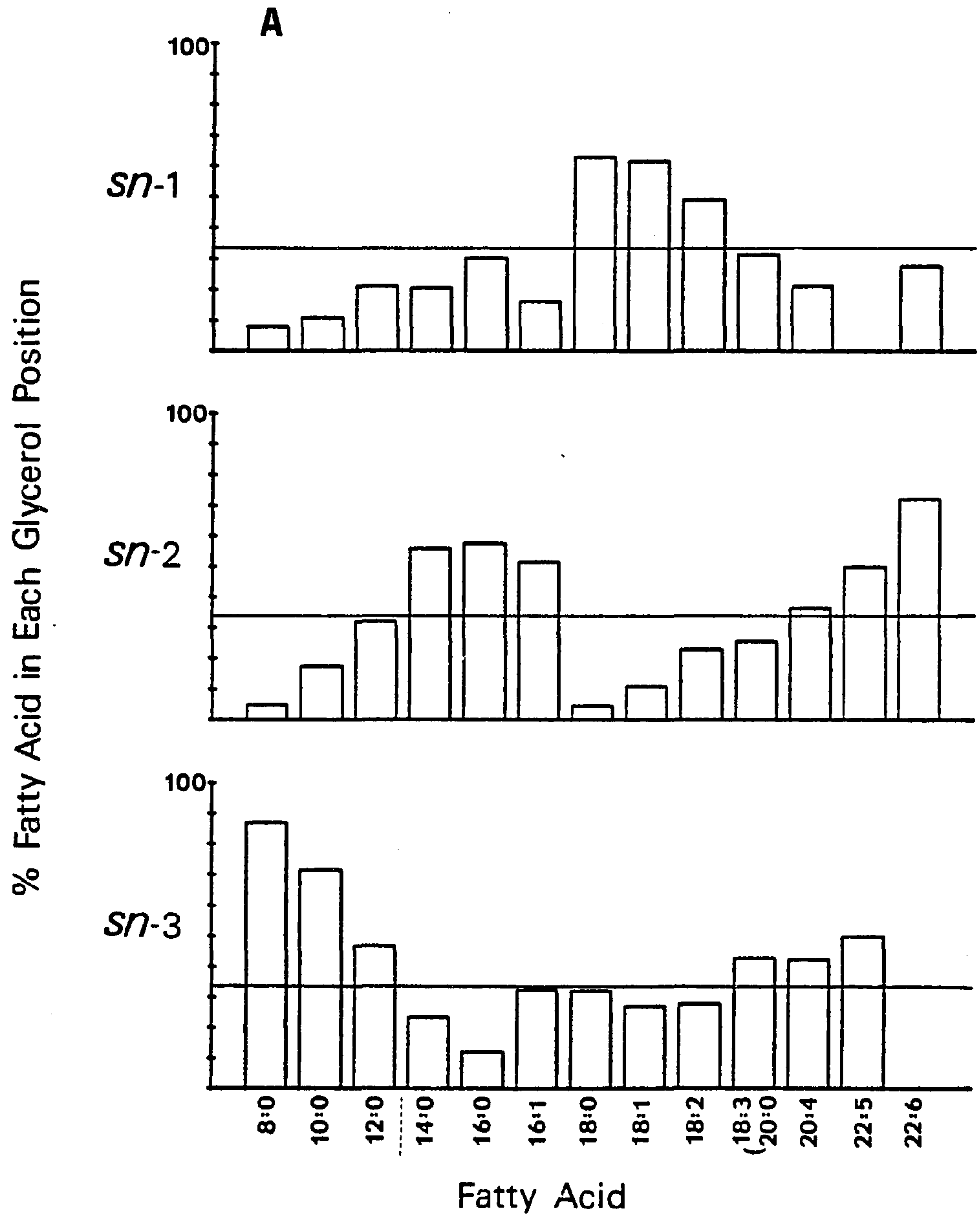

Figure 1A. Stereospecific composition of milk triacylglycerols. Vertical bars represent the percent of each fatty acid at each glycerol position (sn-1, sn-2, sn-3). The horizontal line indicates 33 1/3 percent, the proportion expected on the basis of a random positional distribution.

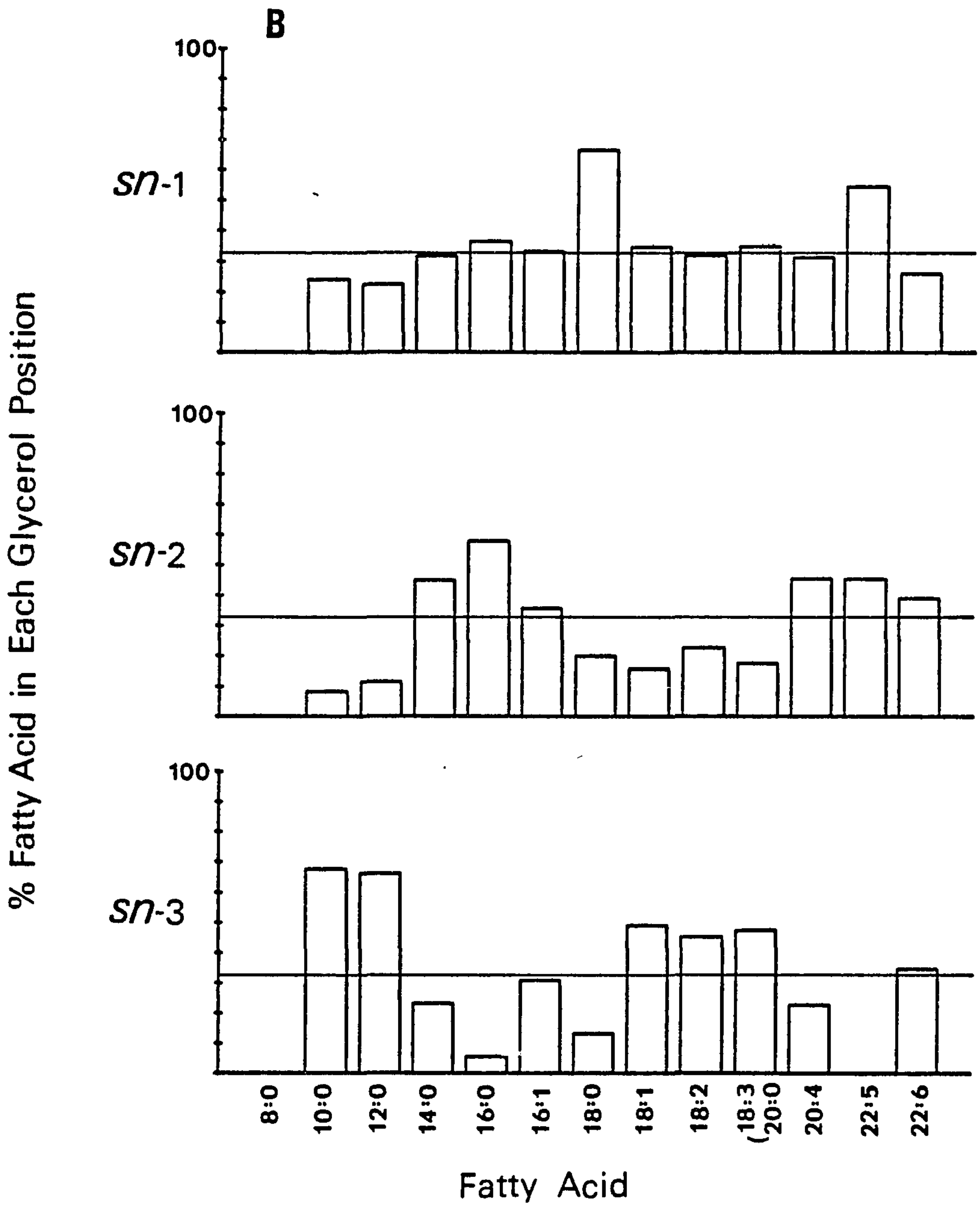

Figure 1B. Stereospecific composition of lymph triacylglycerols. Vertical bars represent the percent of each fatty acid at each glycerol position (sn-1, sn-2, sn-3). The horizontal line indicates 33 1/3 percent, the proportion expected on the basis of a random positional distribution.

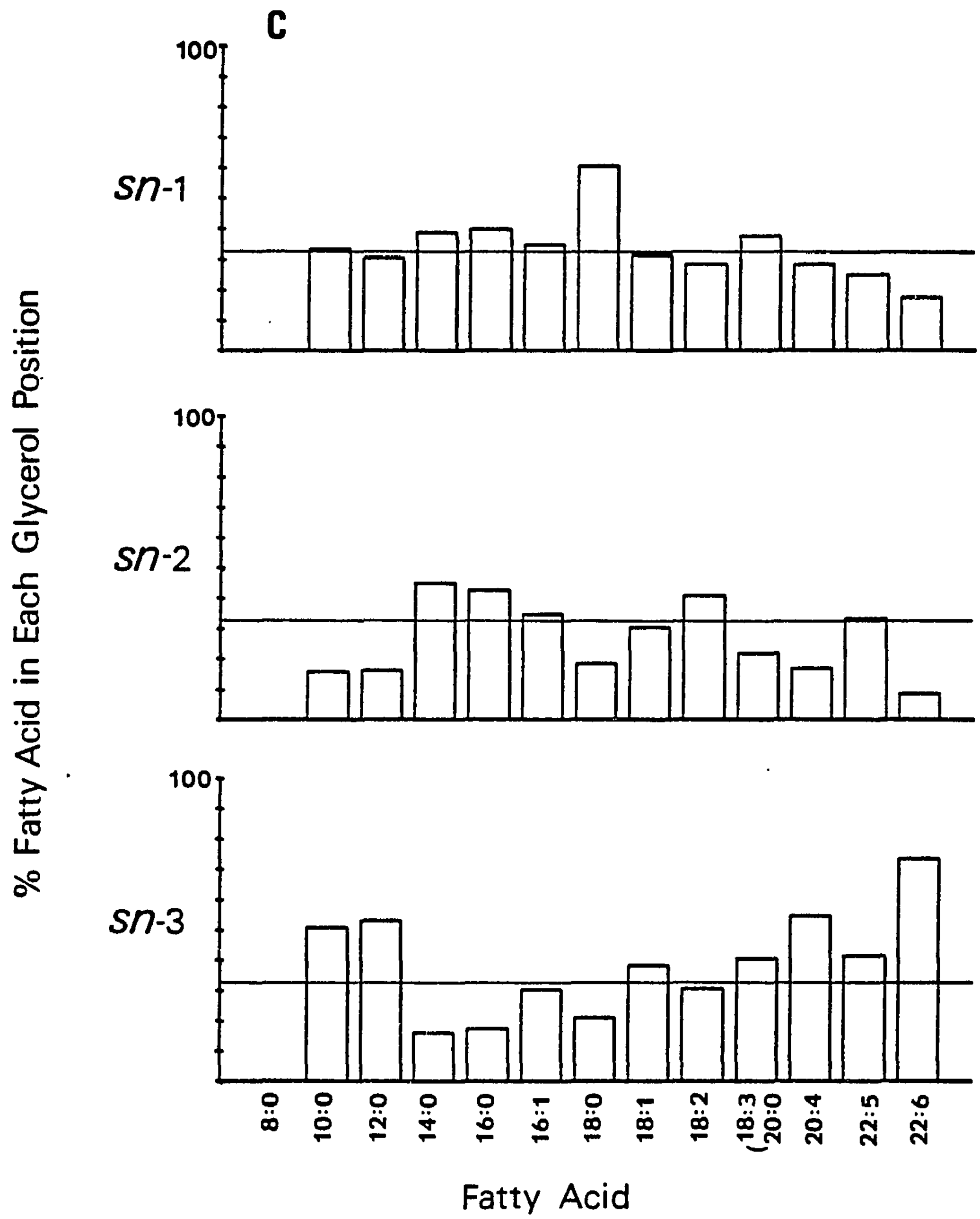

Figure 1C. Stereospecific composition of plasma triacylglycerols. Vertical bars represent the percent of each fatty acid at each glycerol position (sn-1, sn-2, sn-3). The horizontal line indicates 33 1/3 percent, the proportion expected on the basis of a random positional distribution.

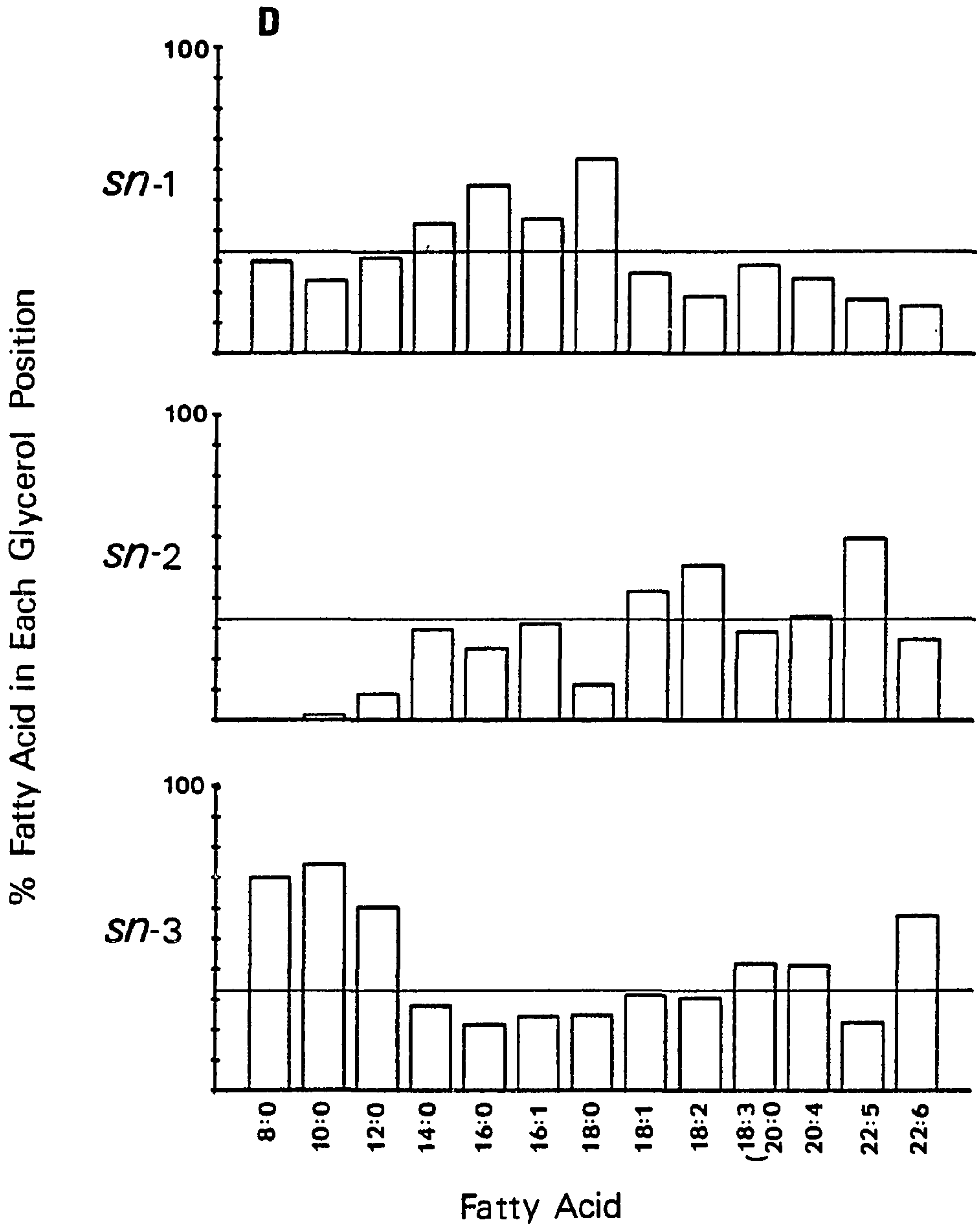

Figure 1D. Stereospecific composition of liver triacylglycerols. Vertical bars represent the percent of each fatty acid at each glycerol position (sn-1, sn-2, sn-3). The horizontal line indicates 33 1/3 percent, the proportion expected on the basis of a random positional distribution.

milk TG, which shows the lymph TG to be different although it has medium chain enriched position 3. The major differences between lymph and milk TG reflect a redistribution of long chain fatty acids into the position 3 to replace the medium chain fatty acids lost during digestion.

Plasma TG fatty acid distribution (Fig. 1C) is similar to lymph TG, except for an increased content of PUFA (20:4,22:5,22:6) which are enriched in position 3. This enrichment may be due to preferential hydrolysis of medium chain fatty acids from the position 3 in extrahepatic tissues and in liver. This enrichment may help in the transport of PUFA to the various tissues. Liver TG also has most of its medium chain fatty acid in the position 3 (Fig. 1D). The fatty acid distribution of liver TG is different from the others, especially the position 2 which is enriched in longer chain fatty acid.

In all the TG of the suckling rat, 18:0 is enriched in position 1. Position 2 is enriched with 14:0 and 16:0, except for the liver TG. The fatty acid 22:5 is also concentrated in position 2 of all TG except in plasma. Position 3 of all the TG is enriched with medium chain fatty acids and 18:3+20:1 fatty acids, and in all the TG analyzed, the fatty acids were found to be non randomly distributed. The small amount of 8:0 present in these samples precluded analysis by these methods.

<u>Results of the density gradient centrifugation of plasma lipoproteins</u>

Chylomicrons were separated by centrifugation at $3x10^6$ g-min. The chylomicron free plasma was separated into classes according to their densities in a NaCl/KBr gradient as described in the methods. Since plasma lipoproteins from suckling rats had not been previously investigated, I used density gradient centrifugation to allow the most complete characterization possible. The VLDL fraction floated to the top, and it is completely separated from the other fractions as shown by the polyacrylamide gel electrophoresis pattern of the fractions obtained from the gradient separation (Fig 2). The separation of LDL and HDL is good but not complete. Other investigators of rat lipoproteins have also had difficulty with separation of LDL and HDL (Chapman 1980). Table 10 shows the density of the fractions from the gradient and their lipoprotein concentrations as measured by refractometry. The LDL appear in the fractions 4, 5, and 6; this corresponds to a density between 1.028 and 1.050. Most of the LDL appeared in the fraction 5 which corresponds to a density 1.037. Fraction 6 has some LDL as well as a band which has an intermediate electrophoretic mobility, which I presumed to be some sub class of HDL. HDL appear in the fractions 7 to 11, the last two fractions having low concentrations. The density range for HDL is between 1.055 and 1.173. From the electrophoresis pattern one can see that HDL is composed of at least three different species. Based on the electrophoretic behavior of the lipoprotein fractions separated by the gradient centrifugation and the concentration of lipoproteins in each fraction, the concentrations of lipoproteins in suckling rat plasma are given as follows: VLDL (density < 1.015) = 112 ± 17 mg/100ml plasma; LDL (density 1.028-1.050) = 144 ± 18 mg/100ml plasma; HDL (density 1.050-1.173) = 385 ± 43 mg/100ml plasma. Chylomicron concentration could not be measured by refractometry due to its turbidity. However, a relative concentration for chylomicrons can be

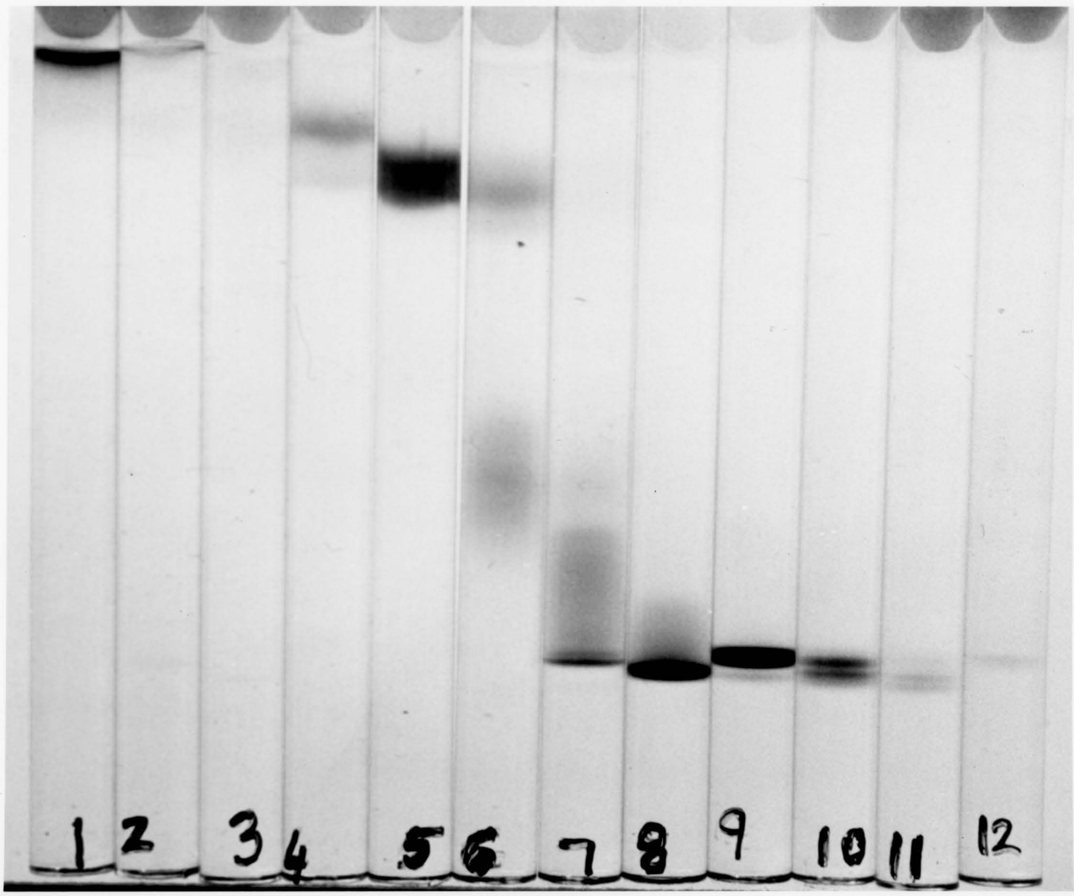

Figure 2. Polyacrylamide gel pattersn of plasma density gradient fractions.

Table 10. Density of Gradient Fractions and Lipoprotein Concentration

Fraction #	Fraction Density	Lipoprotein Content[a] (mg/100 ml plasma)
1	1.015	88.7 $\pm$ 8.8
2	1.018	12.7 $\pm$ 5.6
3	1.022	10.2 $\pm$ 2.5
4	1.028	22.4 $\pm$ 4.4
5	1.037	86.0 $\pm$ 6.8
6	1.050	71.9 $\pm$ 13.5
7	1.066	78.7 $\pm$ 8.6
8	1.087	125.5 $\pm$ 6.3
9	1.113	85.6 $\pm$ 7.2
10	1.141	34.4 $\pm$ 5.4
11	1.173	24.6 $\pm$ 9.0

[a] Concentration measured by refractometry assuming Δn/mg/100 ml=1.54 (Lindgren 1974). The values represent means $\pm$standard deviation for at least three different samples of ten pups each.

inferred by comparing the TG content to that of VLDL, which gives a concentration of chylomicrons of about 200mg/100ml plasma.

The concentration of VLDL (110mg/100ml) and HDL (385mg/100ml) in suckling rat plasma are at the upper range of values reported (Chapman 1980) for the adult rat, which are 36-107 and 80-246mg/100ml respectively. However the concentration of LDL in suckling rat plasma (144mg/100ml) is 3 to 9 fold higher than reported for the adult which is 16-62mg/100ml (Chapman 1980). These high values of lipoproteins reflect the high influx of dietary lipid into the system. The comparatively high value of LDL may facilitate the transport of cholesterol to developing tissues. Because of the high lipoprotein content, the suckling rat may be a good model to study the effects of high fat diets.

<u>Results of the density gradient centrifugation of lymph lipoproteins</u>

Lymph, after the removal of chylomicrons, was subjected to density gradient centrifugation and the fractions were collected as described in the methods. The fractions were subjected to polyacrylamide gel electrophoresis and the results are presented in the Fig 3. The results show that chylomicron free lymph contains only VLDL and HDL. LDL seems to be absent in suckling rat lymph. The concentration of VLDL in lymph is 831$\pm$5mg/100ml, and the concentration of HDL in lymph is 497$\pm$18mg/100ml. These values were obtained by refractometry of the density gradient fractions. The concentration of chylomicrons cannot be determined by refractometry due to the its turbidity. However a relative concentration for chylomicrons can be

Figure 3. Polyacrylamide gel patterns of lymph density gradient fractions.

inferred by comparing TG content to that of VLDL, which gives a concentration of chylomicrons about 3200mg/100ml lymph.

Only small amounts of LDL has been isolated from the adult rat lymph (Riley et al. 1980). This is consistent with the formation of normal LDL from VLDL within the plasma compartment and the low rate of filtration into lymph. The presence of VLDL and HDL in lymph obtained from the adult rat was shown by several investigators (Green and Glickman 1981). This is not unexpected because there is evidence suggesting that some VLDL and HDL are synthesized by the intestine (Green and Glickman 1981).

Concentrations of lipid components in lipoproteins

The concentrations of lipid components in the different lipoprotein fractions are shown in the Table 11. It can be seen from the table that lymph has a very high content of TG rich lipoproteins in accord with the earlier compositional studies on whole lymph (Table 5). The HDL present in lymph have a composition similar to what is reported for adult rat plasma HDL (Chapman 1980), except that lymph HDL is comparatively high in TG. The concentration of TG is high in chylomicrons and VLDL as expected. In lymph, CE is carried almost equally among chylomicron, VLDL, and HDL fractions. However free cholesterol and PL are mostly carried by lymph chylomicrons and VLDL. Plasma chylomicrons carry significant amounts of TG. Plasma LDL, which has elevated levels in suckling rat plasma, also carries a significant amount of TG. This fraction was shown to be rich in essential fatty acids. Although LDL contains only 8.2% of the plasma TG, it contains

Table 11. Lipoprotein Component Concentrations[a]

	TG	CE	CH	PC	PE	SP	LPC	PR
LCHY	40180 (8270)	410 (10)	660 (20)	3590 (510)	620 (120)	ND	490 (280)	1705 (135)
LVLDL	6300 (510)	390 (0)	293 (28)	1290 (100)	200 (60)	ND	ND	421 (93)
LHDL	50 (20)	280 (20)	78 (31)	450 (150)	70 (50)	ND	ND	292 (26)
PCHY	750 (108)	28 (12)	30 (6)	52 (1.2)	7.6 (5.2)	3.7 (1.5)	6.1 (3)	226 (37)
PF#1	328 (71)	29.2 (6.6)	100 (25)	124 (11.8)	13.1 (9.2)	16.6 (10.8)	4.6 (1.5)	94 (6)
#2	74 (14)	12.9 (0.7)	12.2 (6.7)	41.6 (14.5)	2.0 (0.9)	3.7 (1.6)	1.7 (1.2)	4.3 (1.0)
#3	12.0 (6.3)	16.6 (3.8)	11.6 (3.1)	28.5 (1.6)	1.5 (1.4)	3.8 (3.3)	2.6 (0.8)	11.2 (4.0)
#4	16.3 (4.8)	86.7 (30.7)	37.0 (2.8)	41.0 (9.0)	4.0 (1.9)	11.0 (7.7)	3.2 (1.1)	14.9 (6.3)
#5	34.9 (10.8)	371 (151)	180 (17)	247 (120)	5.9 (0.1)	36.0 (22.8)	10.4 (2.7)	164 (3.0)
#6	18.7 (6.8)	265 (108)	181 (27)	297 (169)	17.1 (11.9)	38.0 (24.3)	10.1 (3.0)	132 (2.8)
#7	4.4 (2.1)	220 (69)	159 (26)	315 (178)	18.0 (10.3)	40.0 (22.2)	10.7 (3.1)	184 (21)
#8	2.7 (0.7)	250 (35)	171 (8)	478 (208)	24.7 (14.4)	37.0 (16.8)	22.4 (12.9)	398 (10)
#9	1.9 (1.6)	153 (35)	85 (15)	372 (116)	10.4 (3.3)	30.0 (3.4)	11.7 (2.8)	302 (18)
#10	0.7 (0.6)	44.8 (10.0)	14.5 (3.9)	99 (60)	2.5 (1.3)	5.6 (2.7)	8.8 (8.3)	84.8 (2.2)
#11	0.6 (0.4)	13.2 (2.2)	3.4 (1.3)	31.0 (22.0)	ND	3.7 (1.9)	2.1 (1.9)	40.4 (9.8)

Table 11.--Continued

[a] Concentration for lipid components is umol/l, and protein content is in mg/l. Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. ND= Not detected. L=lymph, CHY=chylomicron, P=plasma, and F#`s refer to density gradient fractions.

46% of all plasma TG PUFA. Whether this suggests an important role for LDL TG in transport of PUFA in the suckling rat, remains to be investigated. The plasma HDL fraction is rich in CE, CH, PL, and PR.

For comparison to previously reported compositional studies on adult rat lipoproteins (Chapman 1980), the composition of suckling rat lipoproteins in terms of weight % has been calculated (Table 12). Lymph chylomicrons, VLDL, and HDL have compositions rather similar to those reported for adults. Plasma chylomicrons, LDL, and HDL also have compositions close to those reported for the adults (Chapman, 1980). However suckling rat plasma VLDL have a composition distinctly different from adult plasma; in particular the low levels of TG and high levels of PL. In fact the composition is similar to that of adult rat chylomicron remnants (Mjos et al. 1975). Considering the large influx of TG rich lipoproteins from lymph, it is not unreasonable to assume that the bulk of plasma VLDL could be remnants. Plasma chylomicrons have a composition similar to that of lymph chylomicrons except for a higher protein content.

Analytical ultracentrifugal studies of lipoproteins

Further characterization of suckling rat plasma lipoproteins was carried out using analytical ultracentrifugation. Chylomicrons and VLDL were not detectable even when the rotor speed was reduced to 20,000 rpm and/or the density of the solution adjusted to 1.006. Whether this results from aggregation of the TG rich lipoproteins during isolation or is an inherent property of these lipoproteins is unknown. When plasma lipoproteins were chromatographed on BioGel A50-m (exclusion limit 50

Table 12. Suckling Rat Lipoprotein Composition (% by weight)[a]. The values in parenthesis represent average values for adult plasma lipoproteins taken from Chapman (1980).

Lipoprotein	TG	CH	CE	PL	PR
A. LYMPH					
Chylomicrons	85.7±3.1	0.6±0.1	0.6±0.1	9.0±1.3	4.1±0.3
VLDL	73.7±1.9	1.5±0.1	3.4±0	15.8±2.0	5.6±1.2
HDL	3.1±1.0	4.6±1.2	18.9±2.1	43.2±4.2	30.2±3.1
B. PLASMA					
Chylomicrons	71.1±2.1	1.1±0.1	1.7±0.2	5.2±0.3	20.9±1.7
VLDL[b]	48.8±3.6	6.4±0.5	4.2±1.0	26.0±2.0	14.6±1.5
	(69.0)	(4.2)	(4.6)	(12.0)	(10.2)
LDL[c]	3.3±0.8	9.6±1.2	28.2±2.1	37.9±2.4	21.0±2.1
	(15.4)	(12.4)	(26.4)	(23.7)	(22.1)
HDL[d]	0.4±0.1	5.5±1.1	14.9±2.0	42.5±3.1	36.7±3.1
	(2.5)	(6.9)	(24.9)	(25.6)	(40.1)

[a] Calculated from compositional data assuming TG=triolein, CE=cholesteryl oleate, and PL=dioleoyl phosphatidylcholine.

[b] Gradient fractions 1-3.

[c] Gradient fractions 4-7.

[d] Gradient fractions 7-11.

million), all the TG rich lipoproteins eluted in the void volume. This result also suggest a very large size, but does not eliminate the possibility of aggregation during isolation. However, plasma LDL and HDL were characterized by analytical ultracentrifugation and the results are presented in Table 13. The properties of LDL, HDL_2, and HDL_3 from the suckling rat plasma are close to those reported for the adult rat (Chapman, 1980). Electron microscopic studies of adult rat lipoproteins by Pasquali-Ronchetti (1975), indicated the size of LDL (density=1.019-1.063) to be 200 Å which agrees well with the value of 210 Å obtained for the suckling rat by ultracentrifugal methods. Their values for HDL_1 (density=1.063-1.125) and $HDL_{2,3}$ (density=1.125-1.21), i.e., 136 Å and 55 Å agree with the values 120 Å and 80 Å obtained for the suckling rat. The concentrations of LDL and HDL calculated from ultracentrifugal studies are in good agreement with those determined from the refractometry of the density gradient fractions: For LDL 202 vs 144 mg/100ml, For HDL 332 vs 385 mg/100ml.

Gel chromatographic fractionation of lipoproteins

An attempt was made to separate lymph lipoproteins on a 2% BioGel A50-m 100-200 mesh column. The column used was 90x1.5cm, and it was eluted at room temperature with 0.2M NaCl, 0.001M EDTA (pH=7.0), and NaN_3 (200mg/l). The chylomicrons appeared in the void volume of the column, and the HDL fraction was not detectable due to the large volume dilution. When plasma lipoproteins were separated using this column, chylomicrons and VLDL appeared in the void volume and LDL and HDL appeared in later fractions as a mixture. A column with BioGel A5-m was

Table 13. Properties of Plasma Lipoproteins from Suckling Rat Plasma as Determined by Analytical Ultracentrifugation.

Lipoprotein	mg/100ml plasma	S_f^{oa}	D(A)[b]	M.W.[c]	σ[d]
LDL	202 ± 30[e]	8.45	210	30	1.028
HDL_1	108 ± 5[e]	2.25	120	5.6	1.034
$HDL_{2,3}$	224 ± 10[f]	1.0	80	1.7	1.034[g]

[a] Calculated from second moment of schlieren pattern and corrected to ρ=1.063.

[b] Diameter (D) calculated from $S_f^o = D^2(1.063-\sigma)/184.7$ (Lindgren 1974).

[c] Molecular Weight $(M.W.) \times 10^{-5} = 0.3153 D^3 \sigma$ (Lindgren 1974).

[d] Hydrated density (σ) determined from a plot of $\eta_t F^o$ vs ρ (Lindgren 1974)

[e] Average of runs at ρ=1.20 and 1.063.

[f] Run at ρ=1.20 only.

[g] Assumed (Lindgren 1974).

used subsequently in the separation of plasma lipoproteins. Chylomicrons and VLDL also appeared in the void volume of the column as a mixture. In the later fractions VLDL and LDL appeared as a mixture, followed by pure LDL and then LDL, HDL mixtures. Since this method was not successful in separating lipoproteins it was not further investigated.

Triacylglycerol fatty acid composition of lipoproteins

The TG fatty acid composition of lipoproteins are shown in the Table 14. Chylomicrons and VLDL carry significant amounts of medium chain fatty acids; the chylomicrons carrying the highest amount. In other fractions, the level of medium chain fatty acids are very low. This indicates rapid preferential removal of medium chain fatty acids by the liver and/or by lipoprotein lipase of extrahepatic tissues. This chas been shown in the study of Frost and Wells (1981). It is interesting to note that the TG composition of lymph and plasma chylomicrons are very similar, although their protein (PR), phospholipid (PL), and cholesterol content are different (Table 12). This suggests that when lymph chylomicrons enter the plasma compartment they undergo an increase in PR and cholesterol content and a decrease in PL content prior to the TG catabolism. Lymph and plasma chylomicrons TG fatty acid composition differ from VLDL TG fatty acid composition in that VLDL have a lower concentration of medium chain fatty acids and a higher concentration of polyunsaturated fatty acids. This may indicate that the majority of VLDL in the suckling rat are chylomicron remnants, and that during hydrolysis in plasma the polyunsaturated fatty acids are

Table 14. Triacylglycerol Fatty Acid Composition (mol%)[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
LCHY	1.3	8.4	15.6	13.8	22.0	1.9	2.2	14.5	16.5	1.7	1.0	0.3	0.8
	(0.1	0.9	1.2	0.7	0.9	0.2	0.1	1.1	1.4	0.1	0.1	0.1	0.2)
LVLDL	0.8	5.5	11.4	11.6	25.3	1.7	3.1	14.8	17.8	1.8	2.9	0.9	2.3
	(0.0	0.4	1.2	1.2	0.3	0.2	0.3	1.8	1.2	0.2	1.4	0.8	1.6)
LHDL	0.2	3.7	10.8	11.4	25.0	3.0	4.5	15.3	16.2	1.7	3.6	2.2	2.4
	(0.2	2.8	1.8	1.8	1.7	0.1	0.1	0.1	3.2	0.2	0.8	0.1	0.4)
PCHY	1.6	9.6	15.8	13.2	21.0	1.7	2.3	14.3	17.0	1.7	0.9	0.4	0.6
	(0.2	1.5	2.0	1.0	1.2	0.2	0.1	1.9	1.2	0.1	0.2	0.1	0.1)
PF#1	0.8	5.6	10.3	10.4	24.5	1.5	2.4	15.9	20.4	1.6	2.2	1.3	3.3
	(0.4	1.5	2.0	1.0	1.9	0.1	0.1	1.5	2.6	0.1	0.3	0.3	1.0)
#2	0.8	6.2	11.3	11.3	24.3	1.5	2.4	15.4	18.8	1.5	2.1	1.2	3.2
	(0.4	1.4	1.7	0.9	2.0	0.3	0.1	1.7	2.7	0.1	0.3	0.4	1.4)
#3	0.8	1.4	2.7	5.0	22.0	1.0	2.8	15.9	23.4	0.8	7.0	3.9	10.1
	(0.7	0.4	0.8	1.7	1.2	0.3	0.3	2.6	4.7	0.6	1.8	1.5	2.9)
#4	0.3	0.6	1.5	4.0	21.8	0.9	2.8	17.0	25.9	1.0	8.9	3.4	11.9
	(0.5	0.7	1.2	1.5	2.0	0.1	0.2	3.7	2.3	0.1	1.9	0.3	2.3)
#5	ND	TR	0.8	2.8	19.8	0.8	2.7	16.8	25.8	0.9	11.8	3.9	13.7
	(		0.6	0.7	2.2	0.1	0.2	3.3	2.2	0.2	2.0	0.5	1.7)
#6	ND	ND	1.6	3.5	20.1	0.9	2.8	16.7	25.0	1.0	11.6	3.8	12.9
	(		1.1	1.2	2.8	0.2	0.2	3.5	1.4	0.2	1.9	1.1	2.2)
#7	ND	ND	3.1	6.7	21.5	1.2	3.5	15.5	20.8	1.1	11.1	4.7	9.7
	(		2.0	2.2	2.1	0.1	0.4	2.1	2.5	0.4	3.6	1.3	1.5)
#8	ND	ND	5.2	10.9	23.9	1.8	4.1	14.0	19.0	1.3	8.4	4.2	6.7
	(		2.4	3.0	2.3	0.4	0.9	1.4	2.8	0.1	2.2	0.4	0.9)
#9	ND	ND	3.8	7.7	24.4	2.4	4.4	14.6	19.1	1.2	5.8	6.7	7.9
	(		2.9	1.5	2.3	0.8	1.1	2.9	0.9	0.3	0.3	1.1	3.2)
#10	ND	ND	2.8	7.3	29.1	3.8	7.5	16.8	17.2	1.4	6.0	8.5	6.7
	(		3.3	1.6	0.7	1.1	1.0	1.4	1.1	0.5	1.3	0.0	2.4)
#11	ND	ND	2.6	4.2	26.3	3.3	9.8	15.5	12.1	1.5	4.9	13.9	8.4
	(		0.0	0.7	0.5	1.3	0.7	1.2	1.4	1.0	1.2	0.8	3.6)

Table 14.--Continued

[a] Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. TR=fatty acid present at <0.1 mol%, ND=not detected. L=lymph, CHY=chylomicron, P=plasma, and F#'s refer to density gradient fractions.

spared from catabolism compared to others. It is interesting to note that lymph HDL TG carries high amounts of medium chain fatty acids compared to plasma HDL. Another interesting feature is the high concentration of polyunsaturated fatty acids in the plasma LDL-TG fraction. This suggests LDL may be a specific carrier of polyunsaturated fatty acids.

Cholesteryl ester fatty acid composition of lipoproteins

The Table 15 shows the CE fatty acid composition of lipoproteins. The general features of the CE fatty acid composition is the lack of medium chain fatty acids. It is interesting to note that HDL CE carry high concentrations of 20:4 fatty acid (~45%). This may be due to the specificity of the enzyme LCAT which esterifies the HDL CH to CE. All fractions except HDL carry relatively high concentrations of 18:1. In fact there is an inverse relationship between the concentrations of 18:1 and 20:4 fatty acids.

Phospholipid fatty acid composition of lipoproteins

Lipoprotein phosphatidylcholine fatty acid compositions are shown in the Table 16. It can be seen from the Table that the fatty acid composition of PC is almost constant in all the fractions. This may result from PL exchange between the different lipoproteins catalyzed by phospholipid exchange proteins. The only difference between the composition of PC in lymph and plasma, is in the content of 18:2, 22:5 and 22:6. In lymph, the 18:2 content is higher and the 22:5 and 22:6 content is lower when compared to plasma fractions. PC is rich in essential fatty acids 18:2, 20:4, and 22:5.

Table 15. Cholesteryl Ester Fatty Acid Composition (mol%)[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
LCHY	ND	ND	0.8	2.8	21.2	3.1	4.5	32.3	24.6	2.4	6.2	0.9	1.1
	(		0.2	0.8	0.9	0.2	0.0	0.8	2.9	0.4	3.6	0.1	0.2)
LVLDL	ND	ND	0.9	2.3	27.8	3.8	5.9	26.1	21.5	2.9	7.0	0.5	1.3
	(		0.3	0.4	0.6	0.4	1.0	0.6	1.4	1.0	3.7	0.4	0.4)
LHDL	ND	ND	0.9	2.8	14.4	3.5	2.1	5.5	22.0	1.8	44.2	0.2	2.6
	(		0.0	0.0	1.3	0.7	0.8	0.8	0.8	0.3	3.5	0.2	0.1)
PCHY	ND	ND	0.8	4.4	16.8	2.3	3.5	17.0	20.8	1.6	23.6	TR	0.1
	(		0.4	2.0	3.0	1.2	0.5	1.4	3.4	0.6	4.1		0.1)
PF#1	ND	ND	0.3	3.6	21.1	2.4	4.0	30.5	23.4	1.9	12.1	0.4	1.3
	(		0.4	1.2	0.5	0.2	0.2	2.4	3.2	0.2	1.8	0.3	1.1)
#2	ND	ND	0.7	2.8	19.2	2.8	4.6	27.1	24.9	2.0	13.0	0.3	2.8
	(		0.6	0.9	2.2	0.5	0.5	0.8	0.4	0.4	2.9	0.2	0.5)
#3	ND	ND	0.9	2.9	20.1	2.1	4.0	26.9	21.2	2.1	11.5	0.3	2.9
	(		0.4	1.3	0.7	0.6	0.6	0.9	5.8	0.4	2.9	0.2	0.8)
#4	ND	ND	TR	2.1	20.2	2.0	2.4	19.1	27.0	1.1	22.8	0.2	2.3
	(			0.3	1.7	0.1	0.1	2.4	1.7	0.2	0.9	0.1	0.3)
#5	ND	ND	0.1	2.4	21.4	2.1	2.0	15.4	28.1	0.8	25.7	0.2	1.9
	(		0.0	0.3	1.3	0.2	0.0	1.7	1.4	0.2	0.5	0.1	0.3)
#6	ND	ND	0.1	2.8	17.9	2.2	1.1	9.3	28.0	0.5	35.8	0.2	2.0
	(		0.1	0.3	1.5	0.3	0.2	2.0	1.3	0.1	2.5	0.1	0.1)
#7	ND	ND	0.2	3.1	15.5	2.2	0.5	4.9	28.1	0.2	43.4	0.1	1.9
	(		0.0	0.4	0.7	0.3	0.1	1.0	1.1	0.1	1.3	0.0	0.2)
#8	ND	ND	0.2	3.5	17.0	2.3	0.4	3.8	28.7	0.1	45.4	0.2	2.1
	(		0.1	0.8	4.2	0.4	0.0	0.6	2.0	0.1	0.6	0.1	0.1)
#9	ND	ND	0.1	2.8	14.3	2.2	0.4	3.9	28.1	0.2	45.7	0.1	1.7
	(		0.1	0.1	0.3	0.5	0.0	0.9	1.3	0.1	1.0	0.0	0.7)
#10	ND	ND	0.1	2.9	14.1	1.6	0.6	3.5	28.0	0.2	45.3	0.2	3.0
	(		0.0	0.3	0.5	0.2	0.3	0.8	1.3	0.1	0.9	0.1	0.8)
#11	ND	ND	0.8	3.8	14.4	2.2	1.1	3.3	25.8	0.3	43.1	0.3	2.5
	(		0.7	1.9	0.4	0.5	0.5	0.5	0.9	0.3	1.6	0.3	0.5)

Table 15.--Continued

[a] Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. TR=fatty acid present at <0.1 mol%, ND=not detected. L=lymph, CHY=chylomicron, P=plasma, and F#`s refer to density gradient fractions.

Table 16. Phosphatidylcholine Fatty Acid Composition (mol%)[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
LCHY	ND	ND	0.1	1.3	25.4	0.8	17.8	5.2	34.3	0.5	12.5	0.2	2.0
	(		0.0	0.2	0.4	0.1	0.3	0.4	0.5	0.0	0.7	0.0	0.3)
LVLDL	ND	ND	0.1	1.1	25.9	0.8	17.5	4.9	31.6	0.5	15.4	0.2	2.1
	(		0.0	0.1	0.8	0.1	0.4	0.7	1.5	0.1	2.1	0.0	0.0)
LHDL	ND	ND	0.1	1.2	26.4	0.9	16.5	5.4	31.9	0.4	14.8	0.2	2.3
	(		0.0	0.1	1.0	0.2	0.0	0.9	1.2	0.1	1.3	0.0	0.1)
PCHY	ND	ND	0.1	1.3	27.3	0.5	18.9	4.1	28.1	0.2	13.3	0.7	5.4
	(		0.0	0.1	0.9	0.0	0.3	0.1	0.8	0.0	0.9	0.1	0.4)
PF#1	ND	ND	0.1	1.3	28.3	0.5	18.3	4.1	27.0	0.3	14.3	0.5	5.4
	(		0.0	0.3	0.3	0.0	0.6	0.2	0.4	0.0	0.2	0.1	0.1)
#2	ND	ND	TR	1.3	28.3	0.5	18.4	4.0	27.0	0.4	14.0	0.7	5.4
	(			0.5	1.9	0.0	0.5	0.0	1.1	0.1	3.2	0.2	0.2)
#3	ND	ND	TR	1.2	28.6	0.5	18.3	4.2	27.2	0.3	13.3	0.8	5.5
	(			0.2	1.4	0.1	0.5	0.2	1.2	0.1	2.3	0.4	0.2)
#4	ND	ND	TR	1.4	28.8	0.5	18.4	4.3	27.4	0.2	13.0	0.7	5.3
	(			0.3	1.0	0.1	0.4	0.1	0.8	0.1	1.4	0.1	0.3)
#5	ND	ND	TR	1.5	28.8	0.5	18.1	4.2	27.9	0.2	12.8	0.7	5.3
	(			0.2	0.8	0.0	0.4	0.1	0.5	0.0	1.0	0.1	0.1)
#6	ND	ND	TR	1.4	28.5	0.5	18.1	4.2	27.4	0.2	13.4	0.7	5.6
	(			0.2	0.6	0.0	0.4	0.1	0.6	0.1	0.9	0.1	0.3)
#7	ND	ND	0.1	1.4	28.3	0.5	18.0	4.3	27.4	0.3	13.9	0.5	5.5
	(		0.0	0.1	0.8	0.0	0.5	0.1	0.6	0.1	0.9	0.0	0.1)
#8	ND	ND	0.1	1.2	27.8	0.5	18.0	4.3	27.1	0.3	14.6	0.5	5.6
	(		0.0	0.1	0.5	0.0	0.3	0.1	0.5	0.0	0.5	0.1	0.2)
#9	ND	ND	0.1	1.2	27.8	0.5	17.8	4.4	27.0	0.3	14.4	0.7	5.8
	(		0.0	0.1	0.5	0.0	0.3	0.1	0.5	0.1	0.6	0.1	0.1)
#10	ND	ND	0.1	1.1	27.8	0.5	17.7	4.4	27.0	0.3	14.4	0.7	6.1
	(		0.0	0.3	1.3	0.2	0.8	0.1	1.2	0.1	1.4	0.1	0.6)
#11	ND	ND	TR	1.0	27.5	0.5	17.8	4.7	26.5	0.3	14.0	0.8	6.1
	(			0.4	1.3	0.2	1.2	0.3	0.9	0.1	2.0	0.3	0.2)

Table 16.--Continued

[a] Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. TR=fatty acid present at <0.1 mol%, ND=not detected. L=lymph, CHY=chylomicron, P=plasma, and Frn #'s refer to density gradient fractions.

Lipoprotein phosphatidylethanolamine fatty acid compositions are shown in the Table 17. When compared to PC, PE has higher concentrations of 20:4, 22:5 and 22:6, and lower concentrations of 16:0 and 18:2. The fatty acid composition of PE is fairly constant between different fractions, except for 18:0, 18:2, 22:5, and 22:6 fatty acids. The 18:0 fatty acid concentration is comparatively high in lymph fractions as well as in plasma chylomicrons. The 18:2 fatty acid concentration is comparatively high in lymph fractions, as well as in plasma chylomicrons and VLDL. The 22:5 and 22:6 fatty acid concentrations are comparatively low in lymph fractions and in plasma chylomicron fraction. These results indicate there is a slight change in composition of PE fatty acids when lymph lipoproteins enter the plasma compartment.

Lipoprotein sphingomyelin fatty acid compositions are shown in the Table 18. It should be noticed that SP contains some fatty acids different than those present in the other lipids. These fatty acids are 17:0, 20:0, 22:0, 23:0, 24:0, 24:1, and 24:2, which are present in the SP of most animals. Unlike other fractions, SP has almost negligible quantities of polyunsaturated fatty acids. The SP fatty acid composition of all the different fractions are constant. The SP content of lymph is so low that the fatty acid composition cannot be determined with any accuracy.

Lipoprotein lysophosphatidylcholine fatty acid compositions are shown in the Table 19. Lyso PC in general, has a high concentration of 16:0 and 18:0. The fatty acid composition of all the fractions are constant. Lyso PC concentrations were very low in the various

Table 17. Phosphatidylethanolamine Fatty Acid Composition (mol%)[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
LCHY	ND	ND	0.1	0.3	11.4	0.4	30.5	4.6	16.9	0.4	24.4	1.3	9.6
	(		0.1	0.1	0.2	0.0	0.3	0.2	0.3	0.0	0.7	0.3	0.6)
LVLDL	ND	ND	0.2	0.4	11.6	0.5	28.7	4.2	13.4	0.4	26.4	1.2	13.1
	(		0.2	0.0	1.1	0.0	0.9	0.6	1.5	0.1	0.8	0.2	0.3)
LHDL	ND	ND	0.3	0.6	13.8	1.3	25.1	5.9	13.2	0.5	24.0	1.8	13.6
	(		0.1	0.1	0.3	0.5	0.7	1.9	1.2	0.0	2.7	0.5	0.5)
PCHY	ND	ND	0.3	0.8	8.9	0.5	26.8	3.1	13.6	0.3	21.5	3.5	18.8
	(		0.1	0.5	0.9	0.3	2.5	0.4	2.1	0.0	1.7	1.1	2.1)
PF#1	ND	ND	TR	3.9	12.3	0.7	13.3	4.3	12.1	0.6	16.0	6.5	24.3
	(			3.9	6.5	0.5	4.1	4.7	5.6	0.7	6.2	5.4	14.1)
#2	ND	ND	1.0	3.4	10.0	1.1	14.9	4.1	9.0	0.3	18.2	10.3	27.8
	(		0.6	2.5	2.7	0.4	1.0	3.4	3.2	0.4	0.2	6.2	3.1)
#3	ND	ND	TR	TR	10.6	1.1	10.6	3.8	6.5	TR	13.9	19.2	33.8
	(				4.1	0.2	6.6	1.5	1.0		3.3	3.9	6.5)
#4	ND	ND	0.7	2.2	13.1	1.0	10.9	4.9	8.8	0.9	18.3	8.7	30.9
	(		0.8	2.5	6.4	0.6	2.7	1.8	1.7	0.6	4.4	2.1	8.0)
#5	ND	ND	0.1	0.5	8.9	0.4	12.8	2.8	8.2	0.2	20.3	8.3	37.5
	(		0.1	0.2	2.2	0.2	3.4	0.7	1.6	0.1	2.4	1.0	5.4)
#6	ND	ND	0.1	0.9	8.8	0.5	13.5	2.8	8.2	0.2	20.4	7.9	36.6
	(		0.1	0.7	2.8	0.3	3.5	1.3	1.9	0.1	2.5	1.1	7.3)
#7	ND	ND	0.2	0.6	7.5	0.2	13.3	2.2	7.8	0.2	21.5	8.0	38.5
	(		0.1	0.7	1.3	0.0	2.7	0.4	1.4	0.0	1.8	0.7	4.9)
#8	ND	ND	0.1	0.7	8.4	0.3	14.3	2.6	8.6	0.2	21.7	7.5	35.7
	(		0.1	0.5	1.4	0.2	3.8	0.5	1.0	0.0	3.4	0.8	4.2)
#9	ND	ND	TR	0.5	8.0	0.3	15.4	2.6	7.7	0.1	22.6	7.6	35.2
	(			0.4	1.0	0.2	1.9	0.3	1.4	0.1	4.2	1.3	1.1)
#10	ND	ND	TR	1.0	10.2	0.5	15.1	3.9	7.0	TR	21.5	8.2	32.4
	(			1.0	3.1	0.4	3.3	0.3	1.2		4.4	1.1	5.0)

Table 17.--Continued

[a] Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. TR=fatty acid present at <0.1 mol%, ND=not detected. L=lymph, CHY=chylomicron, P=plasma, and F#'s refer to density gradient fractions.

Table 18. Sphingomyelin Fatty Acid Composition (mol%)[a]

	12:0	14:0	16:0	17:0	18:0	18:1	18:2	20:0	22:0	23:0	24:0	24:1	24:2
PCHY	0.6	1.5	29.5	0.9	12.7	2.0	1.4	1.3	3.6	8.4	10.6	17.6	9.3
	(0.2	0.1	1.6	0.2	1.3	0.1	0.2	0.3	0.3	2.3	1.1	1.5	1.5)
PF#1	0.4	2.1	26.3	0.6	12.9	5.0	2.3	0.8	3.4	10.4	9.9	14.8	10.7
	(0.5	0.8	6.7	0.0	5.1	4.2	1.2	0.1	0.2	3.2	0.5	0.7	6.5)
#2	0.8	2.1	26.7	0.7	11.7	4.0	3.0	1.2	4.1	6.7	11.6	18.1	9.3
	(0.5	0.6	1.9	0.3	0.4	0.9	0.8	0.0	0.2	0.7	2.0	1.4	3.2)
#3	1.4	2.3	26.0	0.6	13.1	4.5	3.3	1.3	3.9	7.5	11.4	16.5	7.5
	(1.6	1.6	2.0	0.2	2.3	2.3	2.9	0.3	0.7	3.0	4.5	5.4	0.3)
#4	0.5	1.8	27.7	0.8	14.0	3.2	2.0	1.1	4.2	8.7	12.6	17.1	6.3
	(0.3	0.5	2.4	0.1	4.4	2.0	0.7	0.1	0.6	2.7	2.9	4.6	1.2)
#5	0.3	1.9	28.4	0.7	13.4	1.2	1.3	1.0	4.2	8.9	13.6	18.9	6.2
	(0.2	1.2	0.9	0.0	3.5	0.3	0.6	0.2	0.5	2.4	2.7	3.6	1.2)
#6	0.3	1.8	28.5	0.6	13.4	1.2	1.5	1.0	4.3	8.6	13.3	19.1	6.3
	(0.2	0.8	1.6	0.1	2.1	0.3	0.8	0.1	0.2	1.2	1.8	2.6	0.6)
#7	0.5	2.2	27.7	0.7	12.1	1.3	1.6	1.0	4.7	9.2	13.4	19.2	6.4
	(0.3	1.2	3.8	0.1	4.9	0.5	1.9	0.3	1.3	3.6	3.9	5.0	1.8)
#8	0.4	1.3	29.3	0.8	10.3	1.4	0.7	1.1	4.4	6.5	14.9	21.4	7.4
	(0.3	0.3	2.8	0.2	0.2	0.2	0.1	0.0	0.3	1.1	1.6	1.5	0.7)
#9	0.5	2.0	29.7	0.9	11.4	1.9	1.0	1.1	4.2	6.9	13.4	20.1	7.0
	(0.2	1.0	2.7	0.1	1.5	0.3	0.0	0.0	0.2	1.9	0.5	0.8	0.8)
#10	0.8	1.9	26.6	0.8	11.1	3.1	1.6	1.2	3.5	9.7	9.7	15.5	7.7
	(0.5	1.0	6.3	0.3	0.6	1.3	1.2	0.1	1.1	5.6	3.9	6.1	1.6)
#11	0.9	2.3	28.8	1.6	19.1	5.5	2.2	1.1	3.0	12.1	6.8	11.1	5.4
	(0.4	1.4	9.9	1.5	9.0	2.3	0.5	0.6	0.3	10.4	1.7	2.6	2.0)

[a] Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. TR=fatty acid present at <0.1 mol%, ND=not detected. L=lymph, CHY=chylomicron, P=plasma, and F#`s refer to density gradient fractions.

Table 19. Lysophosphatidylcholine Fatty Acid Composition (mol%)[a]

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
LCHY	ND	ND	0.3	0.6	30.6	0.5	28.3	6.1	16.4	0.4	12.6	0.7	3.4
	(		0.1	0.3	4.6	0.5	1.8	0.3	0.3	0.0	3.4	0.0	0.4)
PCHY	ND	ND	0.3	0.6	32.6	1.0	36.6	5.5	12.2	0.3	6.1	TR	2.6
	(		0.1	0.3	3.0	0.1	1.4	0.4	0.8	0.1	1.6		1.3)
PF#1	ND	ND	0.3	0.5	33.1	1.3	43.2	5.3	8.0	TR	5.3	1.6	1.4
	(		0.1	0.1	1.5	0.7	1.7	2.5	1.4		4.4	0.1	1.2)
#2	ND	ND	TR	1.9	33.1	4.1	40.6	8.4	9.0	TR	2.2	TR	TR
	(			1.7	1.3	0.2	4.1	4.9	1.0		1.8		)
#3	ND	ND	TR	TR	24.9	4.4	31.3	7.8	5.9	TR	11.9	TR	TR
	(				0.1	1.9	2.5	1.5	1.3		2.8		)
#4	ND	ND	TR	0.8	32.7	1.8	39.9	6.6	7.4	TR	5.4	2.6	2.9
	(			0.2	2.2	0.2	3.3	1.3	0.5		2.2	1.7	2.1)
#5	ND	ND	TR	0.5	29.9	0.5	41.7	6.9	10.2	0.6	7.1	1.0	2.1
	(			0.1	6.4	0.4	2.8	3.0	3.3	0.2	3.1	1.1	1.4)
#6	ND	ND	TR	0.7	32.1	0.4	42.4	4.9	8.9	0.4	7.2	1.0	2.0
	(			0.2	2.1	0.3	1.7	1.0	1.9	0.3	1.1	0.4	0.7)
#7	ND	ND	TR	0.8	31.5	0.9	40.8	5.1	9.7	0.3	8.1	0.8	2.1
	(			0.1	2.0	0.7	1.6	0.9	1.4	0.1	2.9	0.4	0.7)
#8	ND	ND	TR	0.7	32.3	0.5	35.2	5.9	11.6	0.5	8.0	1.3	2.6
	(			0.2	4.1	0.1	3.2	0.6	1.5	0.3	2.7	0.9	2.0)
#9	ND	ND	TR	0.6	31.6	0.5	32.7	6.0	11.8	0.5	8.9	1.0	2.8
	(			0.3	5.5	0.1	3.1	0.9	1.1	0.1	3.2	0.7	1.9)
#10	ND	ND	TR	TR	30.2	1.3	28.7	6.9	9.7	0.6	8.7	1.9	3.6
	(				3.6	1.3	4.3	1.2	1.0	0.3	1.7	0.9	0.5)
#11	ND	ND	TR	TR	34.4	3.1	30.1	8.1	7.1	TR	8.4	TR	4.2
	(				2.9	1.1	5.4	1.7	0.2		3.5		2.2)

[a] Mean values for at least three different samples from 10 pups each with standard deviation in parentheses. TR=fatty acid present at <0.1 mol%, ND=not detected. L=lymph, CHY=chylomicron, P=plasma, and F#'s refer to density gradient fractions.

fractions. The difference in fatty acid composition between PC and lyso PC shows an increase of 18:0 fatty acid from about 18% in PC to about 35% in lyso PC, a decrease of 18:2 from about 28% in PC to about 10% in lyso PC, and a decrease of 20:4 from about 14% in PC to about 7% in lyso PC. A similar decrease is also observed for 22:6. These fatty acid compositional changes may reflect the specificity of the enzyme LCAT in the hydrolysis of the PC molecules. This is also seen in the fatty acid composition of CE in lipoproteins.

Apoprotein composition of lipoproteins

The percentage of apoprotein present in different lipoprotein fractions are shown in the Table 20. Apoprotein composition of lymph and plasma chylomicrons are similar. There is a slight increase of apo-C in plasma chylomicrons compared to lymph, indicating the transfer of this apoprotein from HDL to chylomicrons in circulation. Lymph HDL shows a peculiar apoprotein composition. Unlike plasma HDL, lymph HDL contains no detectable amounts of apo-C, and its apo-E content is also very low. Apo-AI is present at a very high concentration in lymph HDL. Apo-B content is highest in the plasma density gradient fractions # 4, 5, and 6, which contain LDL. These three fractions also contain apoproteins other than apo-B indicating the presence of HDL_1 which was observed to be present in the adult rat LDL fraction by Weisgraber, Mahley, and Assmann (1977). Innerarity, Pitas, and Mahley (1980), separated adult rat LDL and HDL using a combination of ultracentrifugation and Geon-Pevikon block electrophoresis. Their results show that pure LDL contains primarily apo-B, whereas HDL_1

contain apo-E and apo-AI as the major apoprotein and lack apo-B. The high concentration of apo-E in fractions 4 to 7 strongly suggests the presence of HDL_1 in these fractions. Fraction #1 which contains VLDL shows a high content of apo-B and apo-E, in accord with the results obtained for the adult rat by Innerarity et al. (1980). Fractions 7 and 8 have low concentrations of apo-B, and in fractions 9 to 11 no apo-B could be detected. Densities of these fractions, their mobilities on the polyacrylamide gels, and their lipid and apoprotein composition indicate fractions 7 to 11 to be HDL. The heterogeneous nature of this HDL fraction is shown by the variation in apoprotein composition of different density gradient fractions. This variation is greatest for the apo-AIV; 8% in fraction 7, to 28% in fraction 10.

Table 20. Apoprotein Percentages of Gradient Fractions[a]

Sample	Apo-B	Apo-AIV	Apo-E	Apo-AI	Apo-C
L Chyl	27	23	13	23	15
L HDL	ND	24	TR	76	ND
P Chyl	22	25	13	17	23
P Frn #1	29	11	38	TR	23
#2	TR	ND	ND	ND	ND
#3	TR	ND	ND	ND	ND
#4	57	22	22	ND	TR
#5	39	15	27	6	13
#6	22	10	39	12	18
#7	12	8	29	24	28
#8	7	13	19	34	26
#9	ND	20	17	38	25
#10	ND	28	19	29	24
#11	ND	23	25	22	31

[a] TR=Apoproteins present at <1%. ND=Not Detected. L=Lymph, P=Plasma, Chyl=Chylomicrons, HDL=High Density Lipoproteins, and Frn#`s refer to density gradient fractions.

DISCUSSION

The purpose of this research was to elucidate lipid absorption and transport in the suckling rat. This has been attempted by the determination of concentrations and fatty acid compositions of the different lipid components involved, and also by characterizing the lipoproteins which are the carriers of lipids in plasma. One of the most interesting problems in suckling rat lipid metabolism is how the animal obtains the necessary polyunsaturated fatty acids for the synthesis of new tissues, while catabolizing fatty acids for much of its energy needs. It may be that the large quantities of medium chain fatty acids present in milk are preferentially oxidized to produce energy, sparing the polyunsaturated fatty acids for biosynthesis. It is also interesting to note that the suckling rat consumes a high fat diet (50% dry wt.), and that it can be used as a model for the adaptation of an organism to a high fat diet.

The results show that rat milk has a high concentration of TG (97% of the lipid), which is rich in medium chain fatty acids (35%) as well as the essential fatty acid 18:2 (13%). In addition, rat milk lipids contain 0.4% PL which is rich in essential fatty acids 18:2, 20:4, and 22:6 (21, 12, and 2% respectively). After digestion of milk, the lipids are transported from intestine to the general circulation mainly via the mesenteric lymph, while part of medium chain fatty acids are transported via the portal vein. The majority of lipid present in lymph is TG (85%), which carries a significant amount of medium chain fatty acids (22%). In contrast, in the adult rat, the medium chain fatty acids are absorbed from the intestine directly into the portal venous system (Playoust and Isselbacher 1964). This difference may be due to the large amount of medium chain fatty acids in the suckling rat diet or to differences in transport of medium chain fatty acids between adult and suckling rats which may be advantageous to the suckling rat. Further experiments are needed to clarify this observation. Another interesting feature of lymph lipid is its high content of PL (9%), which is rich in 18:2 (30%) and 20:4 (13%). The origin of this large amount of lymph PL may be bile PL. Further work is required to determine the origin of this PL, especially its polyunsaturated fatty acids. Comparison of lipids of portal and vena cava samples shows the presence of a comparatively high content of medium chain fatty acids in portal plasma indicating direct absorption and transport of medium chain fatty acids via the portal vein. Vena cava plasma has a low concentration of medium chain fatty acids indicating rapid removal of these fatty acids by the liver. This preferential uptake of medium chain fatty acids by the liver, and the low concentration of these fatty acids in the liver, suggest the liver is using medium chain fatty acids for energy production. The liver PL 18:2 content is less than half that of plasma, whereas the 20:4 and 22:6 content is very much increased. The reason for this difference is not apparent. Plasma and liver PL contain large concentrations of essential fatty acids. Due to its large concentration of the essential fatty acids, plasma PL may be a carrier of these fatty acids. The CE fraction shows differences in fatty acid composition between liver and plasma. The concentrations of 18:2 and 20:4 are higher in plasma compared to liver, and the concentrations of 16:0, 18:0, and 18:1 are much lower. This high amount of 18:2 and 20:4 indicates that plasma CE may also be an important carrier of these essential fatty acids.

The stereochemical analysis of TG shows that the fatty acids are non-randomly distributed among the three positions of the glycerol backbone in all the different samples analyzed. In addition the medium chain fatty acids whenever present, are enriched in position 3 of the TG. It is interesting to note that medium chain fatty acids account for one third of the acids in milk TG and over one half of the position 3 acids. This enrichment of medium chain fatty acids at position 3 may account for the fact that lingual lipase preferentially hydrolyzes medium chain fatty acids from triacylglycerols in the stomach, thereby releasing them to be absorbed and transported via the portal venous system directly to the liver for utilization. There is also some enrichment of 18:3, 20:4, and 22:5 in position 3. The reason for this enrichment is not clear. Lymph TG also have medium chain enriched position 3. The advantage of this arrangement to the suckling rat is not apparent, although it may result in the preferential hydrolysis of medium chain fatty acids from plasma TG by extrahepatic lipoprotein lipases. The major differences between lymph and milk TG reflect a redistribution of long chain fatty acids into position 3 to replace medium chain fatty acids lost during digestion. Plasma TG fatty acid distribution is similar to lymph TG, except for an increased content of PUFA (20:4, 22:5, and 22:6) which are enriched in position 3. Whether this is due to preferential hydrolysis of medium chain fatty acids from the position 3 or some other mechanism remains to be investigated. The fatty acid distribution of liver TG is different from others, especially in position 2 which is enriched in longer chain fatty acids. The origin of these differences in fatty acid distributions in liver TG is not clear at present.

The data presented in this dissertation represent the first attempt to investigate the lipoproteins of suckling rat lymph and plasma. The most striking features of these data are (i) the very high concentration of TG-rich lipoproteins and the absence of LDL in lymph, (ii) The high concentration of all lipoproteins in plasma, especially LDL, (iii) the presence of a class of plasma lipoproteins which have an intermediate density and electrophoretic mobility between LDL and HDL, and which probably represent HDL_1, (iv) plasma VLDL whose composition is different than that of adult plasma VLDL, (v) the presence of HDL in lymph with a peculiar apoprotein composition, and (vi) the marked heterogeneity of all classes of lipoproteins.

Suckling rat lymph contains chylomicrons, VLDL, and HDL but no detectable LDL. Small amounts of LDL have been isolated from adult mesenteric lymph (Riley et al. 1980). If a similar filtration rate of LDL from the plasma compartment to lymph is assumed for both adults and neonates, it would be expected that the neonate would have a higher concentration of LDL in lymph due to the high concentration in plasma. Actually the opposite results were obtained, which would indicate that the permeability of the intestinal blood vessels in the neonate precludes the filtration of LDL into the lymph. Studies using labelled plasma LDL might be used to address this question.

According to the observations of Green at al. (1978) in the adult rat, lymph HDL are secreted as discodial particles lacking a CE core. The action of LCAT on the PC and cholesterol creates the CE core, which gives rise to the globular shape of mature HDL. Adult lymph also contains globular HDL, which is considered to arise from filtered plasma. In fasting lymph the discodial particles predominate, whereas after a fatty meal the rounded particles predominate (Green, et al 1978). The greater proportion of spherical particles in lymph after a fatty meal was assumed to arise from increased filtration from plasma. In the suckling rat the CE content of lymph HDL was slightly higher than that found in plasma HDL. These data may indicate that most, if not all, lymph HDL are spherical rather than discodial. These data may further indicate a high rate of HDL filtration from plasma, which would be associated with the high fat diet of the neonate. The lymphatic HDL of plasma origin could also be the source of the apo E found in the lymph chylomicrons, since intestine is generally thought to be incapable of synthesizing significant amounts of this apoprotein.

Suckling rat plasma contains, in addition to chylomicrons, VLDL, HDL, and a very high level of LDL (about 6-fold higher than adult plasma). This high concentration of LDL in suckling rat plasma may be due to the high rate of influx of lipid into the system. On the other hand one might actually expect the concentration of LDL to be lower than the adult since plasma LDL may be utilized by the rapidly growing tissues in the neonate. The plasma LDL levels may be related to the level of tissue LDL receptors, which may be lower in the neonate than in the adult. Measurement of the LDL receptors would be important in understanding the elevated levels of plasma LDL. The maintenance of high levels of plasma LDL must in part be attributable to an increased rate of production, either directly in plasma from VLDL or via secretion from the liver. Since the factors regulating plasma LDL levels are not well understood, the suckling rat may prove to be a valuable model for further investigation in this area.

Suckling plasma VLDL have a composition different from that of adult plasma VLDL. Because of the lower TG content, the majority of the suckling rat plasma VLDL may actually be chylomicron remnants. Further experiments are required to characterize these lipoproteins. Measurements of size, density, and complete characterization of the apo B components would be valuable in this characterization.

In lymph most of the TG is carried by chylomicrons. Compared to plasma HDL, lymph HDL contain a higher content of TG. The reason for this difference is not clear. In plasma most of the CE and CH is carried by the LDL and HDL fractions. Significant amounts of PL is carried by all the fractions of lymph lipoproteins, as well as plasma LDL and HDL. The major component of PL of all fractions is PC, with PE, SP, and LPC as the minor components. The fatty acid composition of TG of different fractions show that the medium chain fatty acids are confined to chylomicrons and VLDL, while LDL TG carry a high amount of polyunsaturated fatty acids. Whether this fraction is one of the carriers of polyunsaturated fatty acids to the developing tissues remains to be investigated. The fact that medium chain fatty acids are present to a large extent only in chylomicrons indicates that they are not being processed into LDL and HDL as readily as the long chain fatty acids and/or they are being catabolized preferentially to long chAin fatty acids. A general feature of the fatty acid composition of CE in lipoproteins is the lack of medium chain fatty acids and the high content of 18:2. The HDL fractions also show a high concentration of 20:4 (~45%), and its significance is not clear. Another interesting feature of lipoprotein CE is the inverse relationship of its 18:1 and 20:4 concentrations. The reason or the significance of this relationship is also not clear. All phospholipids lack medium chain fatty acids, and all PL except SP, are rich in polyunsaturated fatty acids. The PC fraction is rich in essential fatty acids 18:2, 20:4, and 22:6. It is interesting to note that the fatty acid composition of PC in all lipoprotein fractions is almost constant. The only slight difference is between the lymph and plasma lipoproteins. This constant PC fatty acid composition suggests the presence of phospholipid exchange proteins in plasma. The PE fatty acid composition differs from other phospholipids in that the concentrations of polyunsaturated fatty acids (20:4, 22:5, and 22:6) are extremely high. Unlike the PC fraction, the fatty acid composition of PE shows variations between the different samples. This variation suggests that PE is not as freely exchanged between lipoprotein particles. This may suggest that serum phospholipid exchange proteins have a specificty for PC in the suckling rat. The SP fraction is composed of some different fatty acids (eg. 23:0, 24:0, 24:1, and 24:2) than those found in other PL. The SP fatty acid composition is also constant among all lipoproteins, which indicates the action of PL exchange proteins on SP similar to PC. The lyso PC fraction also shows constant fatty acid composition for the different lipoproteins analyzed. The major fatty acids present in lyso PC fractions are 16:0 and 18:0. The difference between PC and lyso PC fatty acid compositions of HDL, i.e. low levels of the essential fatty acids 18:2, 20:4, and 22:6 in lyso PC, indicates that LCAT may be preferentially transferring these fatty acids to cholesterol. This is also indicated by the fact that HDL CE shows a high concentration of 18:2 and 20:4 fatty acids. However, the exact fatty acid composition of the fractions indicates that there may be factors other than LCAT responsible for the redistribution of fatty acids between PC and lyso PC. The fatty acid composition of the lipids of the different density fractions, as well as their apoprotein composition, show that even within the same class of lipoproteins there is heterogeneity. this heterogeneity may represent subclasses of particles at different stages of metabolism or even particles with entirely different functions. In vivo labelling studies should help to further understand this heterogeniety.

The apoproteins composition of lymph and plasma chylomicrons are similar indicating the origin of plasma chylomicrons to be lymph chylomicrons. Lymph HDL shows an unusual apoprotein composition. Unlike plasma HDL, lymph HDL contains no detectable amounts of apo-C. Lymph HDL has a very high content of apo-AI. The reason for this peculiar apoprotein composition is not known. Fractions 4 to 6 show a high content of apo-B which is typical for LDL. In addition to apo-B, these fractions contain other apoproteins especially apo-E, suggesting the presence of HDL_1. The apoprotein composition of fractions 7 to 11 show variation indicating the heterogenous nature of HDL.

Polyacrylamide gel electrophoresis pattern of the lymph lipoproteins shows the presence of VLDL and HDL in addition to chylomicrons. It also shows that the majority of HDL appears in fraction 8 corresponding to a density of 1.087 mg/ml. The majority of plasma HDL also correspond to the same fraction. The absence of LDL in suckling rat lymph suggests that the intestine is not capable of synthesizing it. However suckling rat plasma showed an increased concentration of LDL compared to the adult, indicating plasma LDL to be an important vehicle of lipid transport during the suckling period. The density intervals in which the plasma LDL and HDL appeared (1.028-1.050 and 1.050-1.173 respectively) differ slightly from those of humans. This fact should be borne in mind when attempting to separate animal lipoproteins, using methods developed for human samples. Polyacrylamide gel electrophoresis shows at least three different HDL species in suckling rat plasma. One of the species which is found predominantly in fractions 6 to 8 and has a reduced mobility on gels may be HDL_1. The apoprotein composition of these fractions strongly support this suggestion. Analytical ultracentrifugal studies of plasma lipoproteins failed to show the presence of chylomicrons and VLDL in plasma suggesting that they are large particles. The LDL and HDL detected show similar properties to those reported for the adult rat.

This research determined the concentrations and fatty acid compositions of different lipid components of the suckling rat, and characterized their lipoproteins. This data was required to understand the basics of lipid metabolism of the suckling rat. The data suggest that lipid absorption and transport in the suckling rat is sufficiently different from that of the adult rat to warrant further investigation.

LIST OF ABBREVIATIONS

ANS	8-Anilino-1-Naphthalene Sulfonic Acid
Apo	Apoprotein
CE	Cholesteryl Ester
CH	Cholesterol
d	density
EDTA	Ethylenediaminetetraacetic Acid
FFA	Free Fatty Acids
GLC	Gas Liquid Chromatography
HDL	High Density Lipoproteins
LCAT	Lecithin Cholesterol Acyl Transferase
LDL	Low Density Lipoproteins
LPC	Lyso Phosphatidylcholine
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PL	Phospholipids
PR	Protein
PUFA	Polyunsaturated Fatty Acids
SDS	Sodium Dodecyl Sulfate
SP	Sphingomyelin
TG	Triacylglycerol
VLDL	Very Low Density Lipoproteins

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