Advances in Lipid Research

Volume 16

Edited by

Rodolfo Paoletti
David Kritchevsky

Advances in Lipid Research

Volume 16

Edited by

Rodolfo Paoletti

Institute of Pharmacology
Milan, Italy

David Kritchevsky

The Wistar Institute
Philadelphia, Pennsylvania

1978

ACADEMIC PRESS
New York San Francisco London
A Subsidiary of Harcourt Brace Jovanovich, Publis

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 63–22330

ISBN 0–12–024916–2

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

EMIL GINTER, *Institute for Human Nutrition Research, Bratislava, Czechoslovakia* (167)

RYSZARD J. GRYGLEWSKI, *Department of Pharmacology, Copernicus Academy of Medicine in Cracow, Cracow 16, Poland* (327)

MICHAEL HELLER, *Department of Biochemistry, The Hebrew University, Hadassah Medical School, Jerusalem, Israel* (267)

B. J. HOLUB,[1] *Department of Biochemistry and Banting and Best Department of Medical Research, University of Toronto, Ontario, Canada M5G 1L6* (1)

H. V. KOTHARI,[2] *The Wistar Institute of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104* (221)

DAVID KRITCHEVSKY, *The Wistar Institute of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104* (221)

A. KUKSIS, *Department of Biochemistry and Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada M5G 1L6* (1)

CHRISTOPHER J. MEADE, *Transplantation Biology Section, Clinical Research Centre, Harrow, Middlesex, England* (127)

JÜRGEN MERTIN, *Transplantation Biology Section, Clinical Research Centre, Harrow, Middlesex, England* (127)

PAUL STARR, *R.I.A. Laboratories, Inc., South Pasadena, California 91106* (345)

[1] Present address: Department of Nutrition, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

[2] Present address: CIBA–Geigy Corporation, Ardsley, New York 10502.

PREFACE

This volume is devoted to a heterogeneous collection of subjects all of which are important to the chemistry or physiology of lipids. The first article contains a discussion of the metabolism of distinct molecular species of phospholipids. The individual members of this class of lipids are shown to possess strikingly different chemical and biological properties. The second article is devoted to a relatively new but potentially important area of lipid metabolism, namely, fatty acids and immunity. In the third article the author considers the influence of vitamin C on cholesterol metabolism, gallstone formation, and atherosclerosis. The possible relationship of latent vitamin C deficiency to lipid disorders is discussed.

The greatest chemical change in aging or atherosclerotic aortas is the large increase in esterified cholesterol. In article four the arterial enzymes of cholesteryl ester metabolism are described and discussed. The discussion covers synthesis and hydrolysis of cholesteryl esters under a variety of conditions. The fifth article is a thorough discussion of phospholipase D, the enzyme that cleaves the base from phospholipids. The occurrence, characteristics, and factors affecting reaction of this enzyme are among the subjects covered. Article six is a brief discussion of certain aspects of prostaglandin metabolism. The last article is a review of the effects of thyroid hormone on atherosclerosis. Its author is one of the pioneers of work with D-thyroxine.

RODOLFO PAOLETTI
DAVID KRITCHEVSKY

CONTENTS

Marginal Vitamin C Deficiency, Lipid Metabolism, and Atherogenesis

Emil Ginter

Arterial Enzymes of Cholesteryl Ester Metabolism

David Kritchevsky and H. V. Kothari

Phospholipase D

Michael Heller

Screening for Inhibitors of Prostaglandin and Thromboxane Biosynthesis

Ryszard J. Gryglewski

Atherosclerosis, Hypothyroidism, and Thyroid Hormone Therapy

Paul Starr

Metabolism of Molecular Species of Diacylglycerophospholipids

B. J. HOLUB [1] AND A. KUKSIS

Department of Biochemistry and Banting and Best Department of Medical Research, University of Toronto Toronto, Ontario, Canada

I. Introduction

Detailed analyses of the structure of individual types of glycerophospholipids from natural sources have revealed that they are comprised of

[1] Present address: Department of Nutrition, University of Guelph, Guelph, Ontario, Canada.

populations of well-defined molecular species which occur in characteristic proportions in different tissues, cell types, and subcellular particles. Metabolic studies have shown that different molecular species of lipids are formed and catabolized at different rates and that these differences are due to the existence of complex enzyme systems which govern the fatty acid composition of the acylglycerols in a given tissue under physiological conditions.

It is well documented that different molecular species of diacylglycerophospholipids exhibit striking differences in their physicochemical properties. Since glycerophospholipids are major components of cellular membranes, membrane structure and function are determined by the physicochemical parameters associated with the complement of phospholipid molecules present. Furthermore, the characteristic metabolic transformations which these lipids undergo are known to be tightly coupled to lipoprotein turnover and function.

The following review summarizes the more recent developments in studies on the metabolism of molecular species of diacylglycerophospholipids along with evidence for their biochemical and physiological individuality. Reference to neutral acylglycerols is made only when these molecules bear direct relationship to acylphosphoglycerol structure or metabolism. The selection of material has been restricted to studies within the animal species. It is hoped that the knowledge summarized herein will help in the further study and better understanding of the role of phospholipids in cell biology and biochemistry.

Extensive reviews on the determination of the structure of the molecular species of diacylglycerophospholipids (Renkonen, 1967) and of diacylglycerols and diacylglycerophospholipids (Kuksis, 1972) are already available as are thoughtful discussions of the enzymes involved in the metabolism of glycerophospholipids (Hill and Lands, 1970; Van Den Bosch *et al.*, 1972) and acylglycerols (Hubscher, 1970). The structure and metabolism of the glyceryl ethers have also been reviewed thoroughly elsewhere (Snyder, 1969, 1972) and will not be specifically considered herein.

II. Chemical Individuality of Molecular Species of Natural Diacylglycerophospholipids

Since the isolation of pure lipid classes, it has been recognized that most natural glycerophospholipids (Smedley-MacLean, 1932) and triacylglycerols (Hilditch, 1940) contain more than one kind of fatty acid per molecule and thus consist of mixtures of homologous molecular species.

Although synthetic work clearly demonstrated that pure molecular species of acylglycerols (Fischer and Baer, 1941) and diacylglycerophospholipids (Baer, 1963; van Deenen and de Haas, 1964) possessed distinctly different chemical and physical properties, little attention was attached to these findings since individual molecular species of natural glycerolipids were difficult to isolate and their metabolism impossible to study. Advances in analytical methodology have permitted more recent workers to overcome these difficulties and have provided evidence of the natural occurrence of a spectacular series of molecular species of diacylglycerophospholipids (Renkonen, 1967; Kuksis, 1972), which play a decisive role in the structure and function of such biological systems as lipoproteins and cell membranes (Van Den Bosch *et al.*, 1972). The main analytical and physicochemical evidence for the chemical individuality of the molecular species of glycerophospholipids is briefly documented in the following discussion.

A. Nonrandom Structure

The preferential association of the saturated fatty acids with the *sn*-1 position and the unsaturated acids with the *sn*-2 position of diacylglycerophospholipids from most mammalian tissues is well established on the basis of hydrolyses with phospholipase A_2 (Tattrie, 1959; Hanahan *et al.*, 1960; de Haas *et al.*, 1962). The introduction of stereospecific analyses (Brockerhoff, 1965, 1975) has led to the demonstration of distinctly different fatty acid populations for the three positions of most natural triacylglycerol molecules. Furthermore, the development of chromatographic techniques which are capable of an effective separation of neutral glyceryl esters on the basis of molecular weight (Kuksis, 1965) and unsaturation (Padley, 1966) has allowed the determination of the molecular association of the fatty acids in acylglycerols. Detailed chromatographic analyses of the molecular association of the fatty acids is also possible for the diacylglycerophospholipids following degradation to diacylglycerols (Renkonen, 1965; Kuksis, 1965; Akino and Shimojo, 1970), phosphatidic acids (Luthra and Sheltawy, 1972a,b), chemical modification of the ethanolamine (Collins 1964; Renkonen, 1967; Sundler and Akesson, 1973; Yeung *et al.*, 1977) and serine (Collins, 1964; Yeung *et al.*, 1977) moieties, or by direct separation of the original choline (Arvidson, 1965), ethanolamine (Arvidson, 1967), inositol (Holub and Kuksis, 1971a) and serine (Salem *et al.*, 1976) phosphatides. These studies have indicated that characteristic association of fatty acids occurs in natural glycerophospholipids, which gives rise to specific molecular species. As a result, the simplified earlier notions

of various random distributions of fatty acids in natural glyceryl esters have been largely dispelled.

1. *Composition of Fatty Acids*

The work of Hilditch (1940) and that of Futter and Shorland (1957) has shown that triacylglycerols and diacylglycerophospholipids from the same tissue exhibit appreciable differences in their fatty acid composition, while Klenk and Bohm (1951) and Hawke (1959) have demonstrated marked differences in the fatty acid composition of individual diacylglycerophospholipids isolated from the same tissue. Table I compares the total fatty acids of triacylglycerols and the choline, ethanolamine, inositol, and serine phosphatides of rat liver as determined by modern methods of analysis. Several consistent differences are found in the fatty acid proportions of these lipids. The triacylglycerols contain much smaller proportions of 18:0 and 20:4 and higher proportions of 18:1 and 18:2 acids than do the glycerophospholipids. The serine and inositol phosphatides contain relatively little 16:0, while the triacylglycerols and the choline and ethanolamine phosphatides are rich in this acid. The composition of the free fatty acid (Muto and Gibson, 1970), phosphatidic acid (Possmayer *et al.*, 1969), and 1,2-diacyl-*sn*-glycerol (Akesson, 1969)

Table I
FATTY ACID COMPOSITION OF RAT LIVER GLYCEROLIPIDS

Fatty acids	Glycerolipids [d] (mole %)				
	TG [a]	PC [a]	PE [a]	PI [b]	PS [c]
16:0	31.1	32.6	23.8	7.1	4.1
16:1	4.4	1.3	—	tr	—
18:0	2.1	19.1	30.2	40.1	47.0
18:1 ($n-9$)	33.4	7.1	5.8	3.1	2.6
18:2 ($n-6$)	22.9	12.8	6.8	3.3	1.9
18:3 ($n-3$)	1.6	—	—	tr	—
20:3 ($n-9$)	—	—	—	3.2	0.7
20:4 ($n-6$)	1.4	23.1	23.8	39.1	24.7
20:5 ($n-3$)	—	—	—	0.2	2.0
22:5 ($n-3$)	0.8	—	—	1.0	1.0
22:6 ($n-3$)	2.7	3.9	9.7	2.2	15.9

[a] Akesson (1969).
[b] Holub and Kuksis (1971a).
[c] Yeung and Kuksis (1976).
[d] Abbreviations: TG, triacylglycerols; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PI, phosphatidylinositols; PS, phosphatidylserines.

fractions also differ from those of the triacylglycerols and diacylglycerophospholipids of rat liver. Comparable differences in the fatty acid composition of the glycerolipids have been shown for various tissues of other animals, although they have not been investigated in as great detail (Kuksis, 1972; White, 1973; Kuksis, 1977). In addition, the ethanolamine phosphatides and to a lesser extent other glycerophospholipids contain, along with the common diacyl esters, alkyl ether and alkenyl ether analogues (Snyder, 1969) which are largely absent from the neutral acylglycerols. Recently, the fatty acid composition of CDP-diacylglycerol from bovine liver and brain has been shown to bear a close similarity to that of phosphatidylinositol from these tissues (Thompson and MacDonald, 1975; Thompson and MacDonald, 1976).

Striking differences, as well as similarities, are evident when comparisons are made among the fatty acids of the acylglycerols and acylglycerophospholipids from different fractions of a given tissue or a population of cells. Table II presents the fatty acid composition of the major diacylglycerophospholipids of the plasma membranes, microsomes, mitochondria, and the Golgi apparatus of rat liver (Keenan and Morré, 1970; Colbeau *et al.*, 1971). Apparently, highly specific compositions of fatty acids are maintained for these membrane fractions despite considerable rearrangement of acyl groups (Hill and Lands, 1970) and exchange of intact phospholipids (Dawson, 1973) known to take place between subcellular membranes. An examination of the fatty acid composition of the glycerophospholipids of the outer and inner membranes of mitochondria and of microsomes of the guinea pig liver has revealed a somewhat lesser specificity, although certain significant differences are present (Parkes and Thompson, 1970). This may indicate that the different membrane proteins preferentially accommodate specific molecular species of glycerophospholipids.

Only limited studies have thus far been made on the individual glycerophospholipids present in membrane subunits and individual lipoproteins. Table III gives the total fatty acid composition of the glycerophospholipids of purified ATPase and the parent sarcoplasmic reticulum of rabbit skeletal muscle (Marai and Kuksis, 1973a). Except for phosphatidylserine and diphosphatidylglycerol there was close similarity in the fatty acid and aldehyde composition among the corresponding phospholipid classes of the enzyme and the total membrane. This is probably due to the fact that the ATPase accounts for over 30% of the total lipid of the sarcoplasmic reticulum (MacLennan *et al.*, 1971). It is less likely, but not impossible, that this similarity in the lipid compositions results from a scrambling of the membrane lipids during the isolation of the enzyme in the presence of deoxycholate since lipoproteins are known to maintain characteristic lipid profiles despite relatively free exchange of lipids with

Table II

FATTY ACID COMPOSITION OF GLYCEROPHOSPHOLIPIDS OF RAT LIVER PLASMA MEMBRANE, MICROSOMES, GOLGI APPARATUS, AND MITOCHONDRIA

Fatty acids	PC [c] (wt %)				PE [c] (wt %)				PI [c] (wt %)				PS [c] (wt %)			DPG [c] (wt %)
	PM [a]	ER [a]	GA [b]	MT [a]	PM [a]	ER [a]	GA [b]	MT [a]	PM [a]	ER [a]	GA [b]	MT [a]	PM [b]	ER [b]	GA [b]	MT [a]
14:0	—	0.8	0.9	0.4	—	—	0.7	0.3	—	2.5	3.9	—	1.5	9.0	7.1	0.2
16:0	32.8	24.5	34.7	27.0	30.6	22.6	33.5	26.6	30.7	19.3	36.3	26.3	38.7	11.1	29.6	7.0
16:1	2.9	3.3	—	3.9	1.2	2.3	0.4	3.2	8.4	1.8	—	5.8	—	1.0	11.8	7.6
18:0	34.9	21.0	22.5	21.6	31.3	23.4	31.8	27.3	36.6	45.0	19.9	38.4	46.1	4.7	8.2	3.6
18:1	10.2	12.3	8.7	13.0	10.1	9.8	5.1	12.0	13.2	7.2	21.9	14.0	8.4	21.8	40.3	19.9
18:2	8.1	17.7	18.1	12.4	6.5	10.3	10.0	5.4	2.9	3.2	1.6	4.2	1.0	52.3	2.9	58.8
20:3	1.1	1.2	—	1.3	0.9	—	—	—	—	—	—	—	—	—	—	1.2
20:4	8.4	15.8	14.5	17.7	16.5	23.1	18.3	22.0	8.0	21.4	10.2	7.6	4.2	—	—	1.8
22:6	1.6	2.9	—	2.9	2.9	7.2	—	3.2	—	—	—	3.2	—	—	—	—

[a] Colbeau *et al.* (1971).

[b] Keenan and Morré (1970).

[c] PM, plasma membrane; ER, endoplasmic reticulum or microsomes; GA, Golgi apparatus; MT, mitochondria; DPG, diphosphatidylglycerols; other abbreviations as explained in text.

Table III

FATTY ACID COMPOSITION OF GLYCEROPHOSPHOLIPIDS OF ATPASE AND SARCOPLASMIC RETICULUM OF RABBIT SKELETAL MUSCLE [a]

Fatty acids	PC (mole %)		PE (mole %)		PS (mole %)		PI (mole %)		DPG (mole %)	
	SR [b]	ATPase	SR	ATPase	SR	ATPase	SR	ATPase	SR	ATPase
14:0	0.2	0.1	—	—	tr	tr	tr	tr	4.5	0.7
16:0A	6.3	5.4	21.0	21.4	—	—	—	—	5.6	2.9
16:0	34.0	33.9	4.6	2.7	8.2	5.5	3.2	2.6	7.1	2.7
16:1	0.8	0.8	1.4	1.0	2.4	1.1	0.5	0.3	2.6	2.3
18:0A	tr	tr	7.0	6.9	—	—	—	—	—	—
18:0	3.7	3.5	7.7	7.6	21.3	30.7	45.2	43.8	8.7	4.2
18:1A	—	—	6.1	6.4	—	—	—	—	—	—
18:1	16.8	18.6	13.6	12.9	9.6	10.0	6.4	5.0	14.3	14.3
18:2	24.7	25.5	6.0	7.0	7.5	11.0	2.5	2.7	41.9	63.1
20:1	0.6	0.5	0.7	0.9	3.0	0.9	1.1	0.4	0.4	1.0
20:2	0.4	0.2	tr	tr	tr	tr	—	—	0.4	2.6
20:3	1.3	1.3	0.3	0.7	3.2	2.3	7.9	8.6	1.3	1.6
20:4	7.5	7.3	16.0	15.8	9.5	10.0	30.3	31.2	4.9	2.4
20:5	0.4	0.4	0.6	0.6	0.2	tr	tr	tr	—	—
22:2	0.2	0.2	0.4	0.5	5.4	4.4	0.6	0.6	tr	1.1
22:3	1.2	1.2	4.9	5.6	8.0	7.9	0.8	2.0	1.3	1.5
22:4	0.6	0.8	2.5	2.9	12.1	5.5	0.4	0.7	3.5	tr
22:5	1.6	1.3	5.6	5.2	6.2	5.8	1.0	1.8	1.0	tr
22:6	0.3	0.3	1.7	1.7	3.2	4.8	tr	0.2	2.5	tr

[a] Recalculated from Marai and Kuksis (1973a).

[b] SR, sarcoplasmic reticulum; ATPase, adenosine triphosphatase; other abbreviations as in Tables I and II.

the medium (Scanu, 1972). The alkyl and alkenyl ether chains are found among the hydrophobic substituents of the glycerol molecule in acylglycerols and glycerophospholipids in other tissues also (Gray and MacFarlane, 1961). Apparently both saturated and unsaturated homologues occur and exhibit extremely high preference for certain phosphatide classes, tissues, and animal species (Snyder, 1969).

2. *Positional Distribution of Fatty Acids*

Further evidence for a nonrandom distribution of fatty acids in natural glycerolipids arises from examination of the positional placement of the fatty acids in these molecules. The stereochemical relationships between natural triacylglycerols and diacylglycerophospholipids were established by Baer and Fischer (1939) and are reflected in the stereospecific numbering system (Anonymous, 1967). On the basis of this reference system, phospholipase A_2 specifically releases the fatty acids from the 2 position of the *sn*-3-phosphatides (van Deenen and de Haas, 1963), while pancreatic lipase attacks the *sn*-1 and *sn*-3 positions of the neutral acylglycerols (Mattson and Beck, 1956; Savary and Desnuelle, 1956). Both of these enzymes have been employed extensively in the analysis of the positional distribution of fatty acids in natural glycerolipids (Kuksis, 1972) and in stereospecific analyses (Brockerhoff, 1975).

Table IV provides some examples of the distribution of fatty acids between the two positions of the more common glycerophospholipids, 1,2-diacyl-*sn*-glycerols and the 1,2-diacyl-*sn*-glycerol moieties of triacylglycerols isolated from rat liver. It is obvious that in both diacylglycerophospholipids and triacylglycerols the *sn*-1 and 2 positions are occupied predominantly by saturated and unsaturated fatty acids, respectively. The latter distributions are consistent with those found in their potential metabolic precursors, phosphatidic acids and free 1,2-diacyl-*sn*-glycerols, although the fatty acid compositions themselves are not congruent with any simple precursor–product relationship. This specific positional placement of fatty acids is maintained in phosphatidylinositol from bovine brain (Holub *et al.*, 1970) as found also for its potential precursor in this tissue, CDP-diacylglycerol (Thompson and MacDonald, 1976). The mono- and diphosphates of brain phosphatidylinositol also show similar positional distributions of saturated and unsaturated fatty acids (Thompson, 1969), as does phosphatidylserine (Wood and Harlow, 1969b). These selective patterns appear to hold for the glycerophospholipids isolated from most total lipid extracts prepared from animal sources. Some exceptions have been noted, however, for triacylglycerols. Thus, analyses of the fatty acid distribution in the glycerolipids of pig kidney and adipose tissue have