Nestlé Nutrition Workshop Series
Volume 28

POLYUNSATURATED FATTY ACIDS IN HUMAN NUTRITION

EDITED BY

UMBERTO BRACCO

RICHARD J. DECKELBAUM

NESTLÉ NUTRITION SERVICES

RAVEN PRESS

Nestlé Nutrition Workshop Series
Volume 28

POLYUNSATURATED FATTY ACIDS IN HUMAN NUTRITION

Editors

Umberto Bracco, Ph.D.
Deputy Director
Nestlé Research Center
Nestec Ltd.
Lausanne, Switzerland

Richard J. Deckelbaum, M.D.
Professor of Pediatrics
Director, Division of Gastroenterology and Nutrition
Department of Pediatrics
College of Physicians and Surgeons of Columbia University
New York, New York, USA

NESTLÉ NUTRITION SERVICES

RAVEN PRESS ■ NEW YORK

Nestec Ltd., 55 Avenue Nestlé, CH-1800 Vevey, Switzerland
Raven Press, Ltd., 1185 Avenue of the Americas, New York, New York 10036

Made in the United States of America

Library of Congress Cataloging-in-Publication Data

Nestlé Nutrition Workshop (28th : 1990 : Mexico City, Mexico)
Polyunsaturated fatty acids in human nutrition / editors, Umberto Bracco, Richard J. Deckelbaum.
p. cm.
Proceedings of the 28th Nestlé Nutrition Workshop on "Polyunsaturated Fatty Acids in Human Nutrition," held in Mexico City Mexico, November 27–30th, 1990.
Includes bibliographical references and index.
ISBN 0-88167-933-X
1. Unsaturated fatty acids in human nutrition—Congresses.
I. Bracco, Umberto. II. Deckelbaum, Richard J. III. Title.
[DNLM: 1. Fatty Acids, Unsaturated—congresses. 2. Nutrition—congresses. W1 NE228 v.88 / QU 90 N468p 1990]
QP752.F35N47 1990
612.3'97—dc20
DNLM/DLC
for Library of Congress 92-11920

9 8 7 6 5 4 3 2 1

POLYUNSATURATED FATTY ACIDS IN HUMAN NUTRITION

The 28th Nestlé Nutrition Workshop, Polyunsaturated Fatty Acids In Human Nutrition, *was held in Mexico City, Mexico, November 27–30th, 1990.*

Workshop participants (*left to right, from front to back row*): D. M. Small, P. R. Guesry, A. A. Spector, U. Bracco, R. J. Deckelbaum, C. Galli, N. G. Bazan, H. Sprecher, H. Okuyama, J. Y. Jeremy, O. Hernell, B. Koletzko, B. Strandvik, E. Eggermont, M. Escobedo, N. Gualde, P. Sarda, D. Spielmann, M. T. Clandinin, W. C. Heird, L. Dufour, K. Widhalm, J.-P. van Biervliet, H. W. Moser, A. H. Merrill Jr., M. A. Crawford, A. Lerdo de Tejada, W. Glinsmann, B. Duvivier, P. Tantibhedhyankul, H. Bourges Rodriguez, P.-J. Lamotte, D. Brasseur, M. Verghote, P.-P. Forget, P. Marien, G. de Bethune, K. de Block, A. Jacob, S. Cunnane, T. Heim, N. S. Bhandari, D. Lauvau, W. Laureys, L. Juhlin, V. Uzcànga Vicarte, E. Vasquez-Garibay, J. A. Garcia-Aranda, J. Timmermans, M. Stedman, L. Emmery, M. Van Eygen, G. van Paemel, F. Aguillen, J. Taveirne, H. Ingelaere, B. de Bont, B. Vanheule, J. Gigot, A. B. Moser.

Preface

Fatty acids in the diet undergo in the body various metabolic processes governing absorption and transport mechanisms, cell membrane activity modulation, and the pool of specific metabolites. The biochemical pathways possess high physiological significance at the tissue level, which has recently produced convincing evidence of the role of dietary fatty acids in human nutrition. In particular, long chain polyunsaturated fatty acids (LCPUFAs), major constituents of cell membrane phospholipids, represent extremely active substrates for metabolic processes mediating physiological activities.

This book presents the papers and discussions of the 28th Nestlé Nutrition Workshop held in Mexico City, November 27–30th, 1990. We address the new perspectives in human nutrition related to polyunsaturated fatty acids (PUFAs), focusing in particular on their biochemistry, metabolism, physiological role and nutritional impact.

We discuss PUFA transport and utilization, its role in phospholipid molecular species, extracellular and intracellular fate, and metabolism to oxidative derivatives. Then we consider the LCPUFAs as mediators—through eicosanoid-dependent and/or independent mechanisms—of physiological processes at the central nervous system level (brain and retinal development), in particular, of diseases such as cystic fibrosis and atopic eczema, as well as in relation to disturbances of lipoprotein metabolism and gynecological disorders. LCPUFAs are also precursors of eicosanoids, produced by a large variety of cells involved in immunoresponse, and they therefore intervene in problems related to immunomodulation.

Evidence of close relationships between dietary LCPUFAs and their structural-metabolic physiological role leads us to consider the problems and opportunities of LCPUFAs in the human diet, with particular emphasis on infant feeding.

The data presented in the chapters and discussion sections should contribute to a better understanding of the role of fatty acids in human biology, while pointing out existing gaps in the area and identifying research needs. The final aim is to contribute to a better knowledge of the role of lipids in human nutrition.

UMBERTO BRACCO, Ph.D.
Lausanne, Switzerland

RICHARD J. DECKELBAUM, M.D.
New York, New York, USA

Contributors

Nicolas G. Bazan
Department of Ophthalmology and Neuroscience Center
Louisiana State University Medical Center
2020 Gravier Street, Suite B
New Orleans, Louisiana 70112-2234, USA

Umberto Bracco
Nestlé Research Centre, Nestec Ltd.
Vers-chez-les-Blanc, P.O. Box 44
1000 Lausanne 26, Switzerland

Michael Thomas Clandinin
Nutrition and Metabolism Research Group
Departments of Foods and Nutrition and Medicine
University of Alberta
Edmonton, Alberta, Canada T6G 2C2

Michael A. Crawford
Institute of Brain Chemistry and Human Nutrition
Hackney Hospital
Homerton High Street
London E9 6BE, England

Richard J. Deckelbaum
Division of Gastroenterology and Nutrition
Department of Pediatrics
College of Physicians and Surgeons of Columbia University
630 West 168th Street
New York, New York 10032, USA

Claudio Galli
Institute of Pharmacological Sciences
School of Pharmacy
University of Milano
Via Balzaretti 9, 20133
Milano, Italy

Norbert Gualde
Université Bordeaux II
URA CNRS 1456 and Fondation Bergonié
180 Rue du Saint-Genès, 33076
Bordeaux Cédex, France

William C. Heird
Baylor College of Medicine
Children's Nutrition Research Center
1100 Bates Street
Houston, Texas 77030, USA

Olle Hernell
Department of Pediatrics
University of Umeå
S-90185 Umeå, Sweden

Jamie Y. Jeremy
Department of Chemical Pathology and Human Metabolism
Royal Free Hospital School of Medicine
University of London Pond Street
London NW3 2QG, England

Lennart Juhlin
Department of Dermatology
University Hospital
S-751 85 Uppsala, Sweden

Berthold Koletzko
Kinderpoliklinik der Ludwig-Maximilians-Universität
Universität, D-W-8000
Munich 2, Germany

Alfred H. Merrill, Jr.
Department of Biochemistry
Emory University School of Medicine
Atlanta, Georgia 30322, USA

Hugo W. Moser
Johns Hopkins University
Kennedy Krieger Institute
707 North Broadway
Baltimore, Maryland 21210, USA

Harumi Okuyama
Faculty of Pharmaceutical Sciences
Nagoya City University
3-1 Tanabedori, Mizuhoku
Nagoya 467, Japan

Donald M. Small
Department of Biophysics
Boston University School of Medicine
80 East Concord Street
Boston, Massachusetts 02118-2394, USA

Arthur A. Spector
Department of Biochemistry
University of Iowa College of Medicine
Iowa City, Iowa 52242, USA

Howard Sprecher
Department of Medical Biochemistry
Ohio State University
1645 Neil Avenue
Columbus, Ohio 43210-1218, USA

Birgitta Strandvik
Department of Pediatrics
University of Göteborg
41685 Göteborg, Sweden

Kurt Widhalm
Department of Pediatrics
University of Vienna
Währinger Guntel 18-20
1090 Vienna, Austria

Invited Attendees

Narendra Singh Bhandari / *Bhopal, India*
Héctor Bourges Rodriguez / *Tlalpan, Mexico*
Daniel Brasseur / *Brussels, Belgium*
Margaret Cheney / *Ontario, Canada*
Stephen Cunnane / *Toronto, Canada*
Guy de Bethune / *Kortrijk, Belgium*
Baudouin de Bont / *Marche lez Ecaussines, Belgium*
Angel Lerdo de Tejada / *Coyoaca D. F., Mexico*
Bernard Duvivier / *Marche, Belgium*
Ephrem Eggermont / *Herent, Belgium*
Luc Emmery / *Opglabeek, Belgium*
Marilyn Escobedo / *San Antonio, Texas, USA*
Samuel Flores Huerta / *Mexico*
Pierre-Philippe Forget / *Embourg, Belgium*
Jose Alberto Garcia-Aranda / *Mexico*
Jean Gigot / *Brussels, Belgium*
Walter Glinsmann / *Washington, D.C., USA*
Tibor Heim / *Toronto, Canada*
Herwig Ingelaere / *Lembeke, Belgium*
Albert Jacob / *Agadir, Morocco*
Paul-Jacques Lamotte / *Embourg, Belgium*
William Laureys / *Halle, Belgium*
Denis Lauvau / *Binche, Belgium*
Paul Marien / *Edegem, Belgium*
Pierre Sarda / *Montpellier, France*
Phienvit Tantibhedhyankul / *Bangkok, Thailand*
Jan Taveirne / *Kortrijk, Belgium*
Jean Timmermans / *Bree, Belgium*
Victor Uzcànga Vicarte / *Monterrey, Mexico*

Jean-Pierre van Biervliet / *Brugge, Belgium*
Maurice van Eygen / *Roeselare, Belgium*
Bea Vanheule / *Roeselare, Belgium*
Gonde van Paemel / *Oostende, Belgium*
Marc Verghote / *Namur, Belgium*

Nestlé Participants

Karel de Block, *Brussels, Belgium*
Pierre R. Guesry, *Vevey, Switzerland*
Laila Khouri-Dufour, *Vevey, Switzerland*
Russell J. Merritt, *Glendale, California, USA*
Cedric de Prelle, *Brussels, Belgium*
Danièle Spielmann, *Lausanne, Switzerland*
Margaret Stedman, *Don Mills, Canada*

Nestlé Nutrition Workshop Series

Volume 29: Nutrition of the Elderly
Hamish N. Munro and Günter Schlierf, Editors; 248 pp., 1992.

Volume 28: Polyunsaturated Fatty Acids in Human Nutrition
Umberto Bracco and Richard J. Deckelbaum, Editors; 256 pp., 1992.

Volume 26: Perinatology
Erich Saling, Editor; 208 pp., 1992.

Volume 25: Sugars in Nutrition
Michael Gracey, Norman Kretchmer, and Ettore Rossi, Editors; 304 pp., 1991.

Volume 24: Inborn Errors of Metabolism
Jürgen Schaub, François Van Hoof, and Henri L. Vis, Editors; 320 pp., 1991.

Volume 23: Trace Elements in Nutrition of Children—II
Ranjit Kumar Chandra, Editor; 248 pp., 1991.

Volume 22: History of Pediatrics 1850–1950
Buford L. Nichols, Jr., Angel Ballabriga, and Norman Kretchmer, Editors; 320 pp., 1991.

Volume 21: Rickets
Francis H. Glorieux, Editor; 304 pp., 1991.

Volume 20: Changing Needs in Pediatric Education
Cipriano A. Canosa, Victor C. Vaughan III, and Hung-Chi Lue, Editors; 336 pp., 1990.

Volume 19: The Malnourished Child
Robert M. Suskind and Leslie Lewinter-Suskind, Editors; 432 pp., 1990.

Volume 18: Intrauterine Growth Retardation
Jacques Senterre, Editor; 336 pp., 1989.

Volume 17: Food Allergy
Dietrich Reinhardt and Eberhard Schmidt, Editors; 320 pp., 1988.

Volume 16: Vitamins and Minerals in Pregnancy and Lactation
Heribert Berger, Editor; 472 pp., 1988.

Volume 15: Biology of Human Milk
Lars Å. Hanson, Editor; 248 pp., 1988.

Contents

Polyunsaturated Fatty Acids in Human Nutrition,
edited by U. Bracco and R. J. Deckelbaum,
Nestlé Nutrition Workshop Series, Vol. 28,
Nestec Ltd., Vevey/Raven Press, Ltd., New York © 1992.

Fatty Acids in Human Biology: Past and Future

Arthur A. Spector

Department of Biochemistry, University of Iowa, College of Medicine, Iowa City, Iowa 52242, USA

This review is a brief summary of the nutritional, metabolic, and functional roles of fatty acids in mammalian systems, with emphasis on processes that play a role in human physiology and diseases. In this context I will point out existing gaps in our knowledge about fatty acids and suggest areas that appear to be most promising for further study.

Table 1 lists the long chain fatty acids that presently are known to have an important role in nutrition and metabolism. There are 20 to 30 additional fatty acids contained in the plasma and tissue lipids, many at a level of less than 1%. It seems likely that some of these also have important functional roles. A case in point is myristic acid (14:0).[1] Since the tissue contain only a very small amount of myristic acid, it was ignored in most studies. Recently, amino-terminal myristoyl groups have been found on several regulatory proteins, including the protein product of the ras oncogene and the catalytic subunit of protein kinase A, and myristic acid has become one of the most intensively studied fatty acids in biological research (1). Specific functions may be found for other fatty acids now classified as minor components, and the list in Table 1 is likely to expand in the future.

FATTY ACID UTILIZATION

Much of the fatty acid transported to the tissues is either in the form of free fatty acid (FFA) or triglycerides contained in very low density lipoproteins and chylomicrons. The lipoprotein triglycerides are hydrolyzed by lipoprotein lipase at the endothelial surface, and the released fatty acid passes into the tissues. As shown in Fig. 1, in both of these major transport systems, it is the fatty acid itself that is presented to the cells. In an attempt to model this physiologic situation, most studies of fatty acid utilization have been done by incubating tissues or cells directly with

[1] The fatty acids are abbreviated as number of carbon atoms: number of double bonds.

TABLE 1. *Biologically important long chain fatty acids*

Fatty acid	Carbons/double bonds	Unsaturated series	Functions
Myristic	14:0	—	Acylation of proteins
Palmitic	16:0	—	Energy storage Oxidative substrate Alkyl/alkenyl ethers Acylation of proteins
Stearic	18:0	—	Phospholipid structure
Oleic	18:1	ω-9	Regulation of bulk membrane fluidity
Linoleic	18:2	ω-6	Arachidonic acid precursor
Arachidonic	20:4	ω-6	Substrate for eicosanoid mediator synthesis
Eicosapentaenoic	20:5	ω-3	Competitor of arachidonic acid and the synthesis of arachidonate-derived eicosanoids
Docosahexaenoic	22:6	ω-3	Membrane structure

fatty acids (2,3). The fatty acid is usually in a physical complex with plasma albumin, the main fatty acid transport protein in the blood and extracellular fluid (4). Most of what we know about the mechanism of fatty acid utilization is based on studies using this experimental design.

Figure 2 summarizes what occurs when fatty acids are presented to cells as a complex with albumin. Although albumin contains multiple binding sites for fatty acid and the binding at several of these sites is strong, some of the fatty acid dissociates and passes to the cell surface in unbound form (4). The higher the molar ratio of fatty acid to albumin, the greater the unbound concentration. As the cells take up the unbound material more fatty acid dissociates from the albumin and

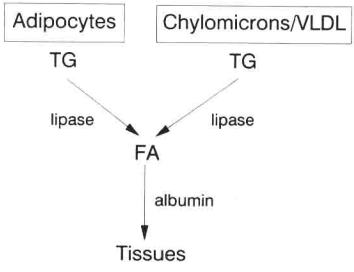

FIG. 1. Supply of circulating fatty acid to the tissues. Abbreviations used: VLDL, very low density lipoproteins; TG, triglycerides; FA, fatty acids. There are two major sources of fatty acid available to the tissues. There are plasma free fatty acid and triglycerides contained in chylomicrons and VLDL, which are large, triglyceride-rich plasma lipoproteins. The plasma-free fatty acid is derived from the hydrolysis of triglycerides stored in adipocytes, mediated by the hormone-sensitive lipase present in the adipocytes. After release into the plasma, the fatty acids bind to albumin, pass through the endothelium, and are transferred through the extracellular fluid to the cells. The circulating triglycerides contained in the chylomicrons and VLDL are hydrolyzed by lipoprotein lipase, an enzyme present on the endothelial surface of the capillaries. The released fatty acids cross the endothelium and pass through the extracellular fluid to the cells. In the extracellular fluid, the fatty acid associates transiently with albumin. Therefore, fatty acid derived from both of these major pathways becomes available to the tissues in the same final form, as free fatty acid bound to albumin.

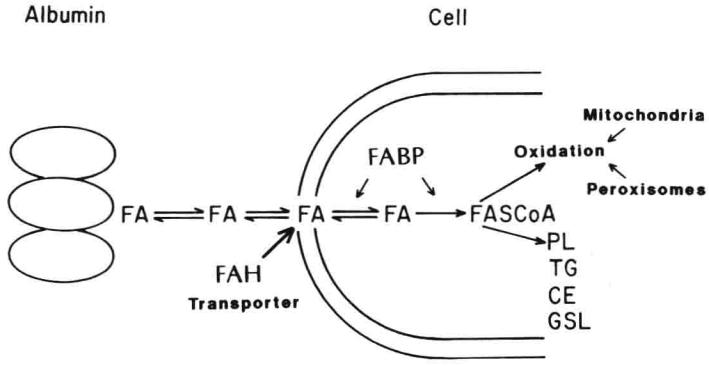

FIG. 2. Mechanism of fatty acid utilization by mammalian cells. Abbreviations used: FA, fatty acid anion; FAH, protonated form of fatty acid; FABP, fatty acid binding protein; FAS-CoA, fatty acyl coenzyme A; PL, phospholipids; TG, triglycerides; CE, cholesteryl esters; GSL, glycosphingolipids. Fatty acid dissociates from the albumin in the uptake process and initially enters a reversibly bound cellular pool. There is uncertainty as to whether the fatty acid crosses the membrane by diffusion of the protonated form, FAH, through the lipid bilayer, or in the anionic form (FA) through the action of a transporter. The cytosolic fatty acid binding protein, FABP, may facilitate uptake by desorbing fatty acid from the membrane, or by directing the fatty acid into specific metabolic pathways.

becomes available for uptake. The amount of fatty acid incorporated by the cells depends on the unbound concentration, which, in turn, depends on the molar ratio (2). Under physiologic conditions, the albumin concentration does not fluctuate much, so the main determinant of the molar ratio is the fatty acid concentration. This is governed by the entry of fatty acid from either the adipose tissue or the plasma lipoproteins.

The cells initially accumulate fatty acid in unesterified form and at this stage much of the uptake is reversible, as indicated by the double arrows in Fig. 2. Most of this fatty acid is probably associated with the plasma membrane, and its accumulation to excessive levels may alter the properties of the membrane and even produce cell injury. At a given molar ratio of fatty acid to albumin, stearic (18:0) and palmitic (16:0) acids accumulate to a greater extent than oleic acid (18:1), and to a much greater extent than linoleic acid (18:2) (5).

Two key questions involving the uptake mechanism remain unresolved. One is whether the fatty acid crosses the plasma membrane by diffusion, probably in the protonated form indicated as FAH (6), or in anionic form through the action of a membrane transporter (7,8). The other is whether the cytoplasmic fatty acid binding protein (FABP) plays a role in the transport process (9), such as by binding the fatty acid at the membrane-cytosol interface and thereby facilitating its desorption.

Oxidative Pathways

Following entry, the fatty acid is activated and the acylcoenzyme A thioester is channeled into the oxidation or esterification pathways. While most of the emphasis

has been on mitochondrial β-oxidation, peroxisomes also are an important site of fatty acid oxidation, particularly for very long chain, highly polyunsaturated, hydroxylated, and branched chain fatty acids (10). As much as 30% of fatty acid oxidation in some cells occurs in the peroxisomes (10). The peroxisomal β-oxidation pathway generates H_2O_2, and this process will probably assume increasing importance as more becomes known about the role of peroxide tone and lipid peroxidation in cellular function and disease processes (11,12). The formation of the alkyl ether bond also occurs in the peroxisomes, and it seems likely that more emphasis will be placed on this organelle in the future because of the increasing number of functions that are becoming attributed to it.

Phospholipid Molecular Species

The incoming fatty acid also is incorporated into phospholipids and glycosphingolipids for membrane biogenesis or replacement, as well as into triglycerides and cholesteryl esters for storage. An aspect of these esterification pathways that is likely to assume increasing importance is the channeling of various fatty acids into different phospholipid molecular species. This is illustrated by the data in Table 2, obtained from human endothelial cultures (13). The diacyl form of phosphatidylcholine in the endothelial cell is composed of 37 different molecular species; those listed in Table 2 account for only 58% of the total in this fraction. The specific activity of labeled arachidonic acid (20:4) incorporated during a 24 hour incubation differs among the five fractions that contain 20:4. In particular, molecular species with two unsaturated fatty acids, the 20:4/20:4 and 16:1/20:4 + 18:2/20:4 fractions, have the highest specific activity. This suggests that they may act as acceptors for incoming arachidonic acid, be more active metabolically, or play a special role in intracellular channeling of arachidonic acid. Detailed information of this type ultimately will be needed for other fatty acids. The complexity of the problem becomes apparent when one considers that there are many different phospholipid classes in a cell besides diacyl

TABLE 2. *Endothelial cell diacyl phosphatidylcholine*

Molecular species[a]	Amount (mol %)	[^{3}H]Arachidonic acid (dpm × 10^{-5}/nmol)
16:0/16:0	11.7	
16:0/18:1	20.8	
18:0/18:1	5.8	
18:1/18:1	5.4	
16:0/20:4	5.0	1.7
18:0:20:4	5.2	1.7
18:1/20:4	3.9	2.3
16:1/20:4 + 18:2/20:4	0.9	4.5
20:4/20:4	0.3	3.1

[a] There are 37 molecular species; those listed total only 58.3 mol %.

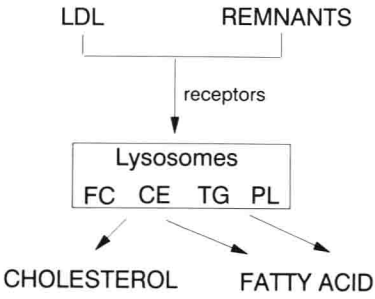

FIG. 3. Supply of fatty acid to the cells as a result of lipoprotein uptake by receptor-mediated endocytosis. Abbreviations used: LDL, low density lipoproteins; FC, cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipids. In addition to cholesterol, fatty acid is released intracellularly when lipoproteins are degraded in the lysosomes. The processing and functional effects of fatty acids made available to cells in this way have not been adequately explored.

phosphatidylcholine and that the types of molecular species may differ in different subcellular compartments, or even in different domains within a single membrane.

Endocytosis of Plasma Lipoproteins

Cells also can obtain fatty acids through receptor-mediated endocytosis of lipoproteins, as illustrated in Fig. 3. The fatty acid aspect of this process has been almost completely ignored because work in this area has concentrated on cholesterol (14). However, when the phospholipids and cholesteryl esters of the low density lipoproteins are hydrolyzed in the lysosomes, fatty acids are released. Furthermore, the remnant lipoproteins taken up in the liver are rich in triglycerides and should provide even more fatty acid. The fundamental question of whether fatty acid released intracellularly during endocytosis is handled in the same way as fatty acid delivered as an albumin complex has not been addressed. Since arachidonic acid provided by low density lipoproteins is available for prostaglandin formation (15,16), the endocytosis route of fatty acid entry may be linked to important metabolic functions.

Phospholipid Fatty Acid Turnover

There is a continuous turnover of fatty acyl groups contained in the cell lipids, including the membrane phospholipids (17,18). This is illustrated schematically in Fig. 4. If fatty acid is present in the extracellular fluid, it will exchange with the intracellular fatty acid that is turning over as a result of mixing in the free fatty acid

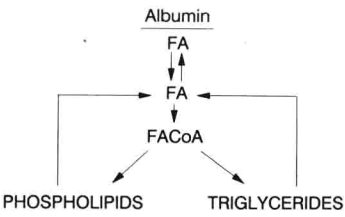

FIG. 4. Turnover of intracellular fatty acids. Abbreviations used: FA, fatty acid; FACoA, fatty acyl coenzyme A. The fatty acid contained in cell lipids, particularly the membrane phospholipids, undergoes continuous turnover. If fatty acids are available in the extracellular fluid, they will mix with the intracellular fatty acid undergoing turnover. Through this process, the intracellular fatty acid composition, including the fatty acid composition of the membrane phospholipids, can change somewhat to reflect the type of fatty acid available in the diet.