The Hyperlipidaemias

Clinical and Laboratory Practice

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BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD LONDON EDINBURGH MELBOURNE

Osney Mead, Oxford OX2 OEL
8 John Street, London WC1N 2ES
9 Forrest Road, Edinburgh EH1 2QH
P.O. Box 9, North Balwyn, Victoria, Australia

ISBN 0 632 00148 8

First published 1976

British Library Cataloguing in Publication Data
Lewis, Barry
The hyperlipidaemias: clinical and laboratory practice.
Bibl.–Index.
ISBN 0–632–00148–8
1. Title
616.3'99 RC 632.H87
Hyperlipemia

Distributed in the U.S.A. by
J.B. Lippincott Company, Philadelphia
and in Canada by
J.B. Lippincott Company of Canada Ltd, Toronto

Set in Monophoto Imprint

Printed and bound in Great Britain by
William Clowes & Sons, Limited,
London, Beccles and Colchester

Preface

The hyperlipidaemias are of concern to physicians interested in metabolic diseases, to cardiologists, dermatologists, paediatricians, rheumatologists and vascular surgeons; and they are of growing importance to the chemical pathologist. Increasing numbers of general practitioners are becoming involved with the detection and management of these disorders which are amongst the commonest diseases of industrialized populations. There is also a trend towards the view that investigation and treatment of disorders of lipid metabolism is a clinical speciality in its own right: lipid clinics are being set up in many hospitals, dealing by secondary or tertiary referral with patients selected in various ways (Chapter 16).

My aim in writing this book has been to present a cohesive account of the clinical and laboratory aspects of these disorders, together with a condensed description of normal lipid metabolism in man. The clinical chapters largely reflect my experience in running a lipid clinic at Hammersmith Hospital. I have made no attempt to review the literature comprehensively; nor would this have been feasible in a field in which the numbers of papers is growing almost logarithmically. Some overlap between the chapters will be evident if the book is read through, and this is intentional: to meet the needs of many who will wish to refer directly to a particular section I have attempted to write a series of relatively self-contained essays only partially interdependent on other chapters.

If control of hyperlipidaemia is to contribute to the prevention of ischaemic heart disease in the community, as opposed to the individual patient, it is clear that the major target will be the common, mild to moderate abnormalities of lipid metabolism. I have argued in this volume and elsewhere that the investigation and management of patients in this category are well within the reach of the general physician, and that adequate laboratory investigation of these disorders does not demand expensive, time-consuming laboratory tests.

My interest in disorders of lipid metabolism was initiated some 20 years ago by Dr A. Keys during his peripatetic researches as he defined the epidemiology of hypercholesterolaemia and its relationship to diet. I owe a debt of gratitude also to Professor T.R.E. Pilkington and to the late Dr A. Antonis from whom I acquired the fundamentals of lipoprotein and lipid biochemistry; Dr H. Gainsborough also stimulated my interest in cholesterol metabolism, prompting a familial predisposition to this field of

research. I warmly thank Dr N.B. Myant, with whom I worked more recently, for his ongoing stimulus. It was he, a decade ago, who first suggested that I write this book: the task would have been easier had I promptly taken his advice! I am grateful to Professor I.D.P. Wootton who has provided me with guidance, encouragement and generous research facilities. Among my present and former colleagues whose work is cited extensively in this volume, to whom I owe deep appreciation, I must mention Professors L.A. Carlson and M. Mancini who initiated me to the pleasures of international collaborative work, Dr A. Chait, Dr G. Sigurdsson and Miss A. Nicoll. I am grateful to Professor C.C. Booth for making possible the clinical study of hyperlipidaemia over many years, and to many colleagues at Hammersmith Hospital who have welcomed me to the ambience of clinical research. Mrs B. Salvage has given me patient and efficient secretarial help; my wife, and the library staff at the Royal Postgraduate Medical School have borne the brunt of surveying the literature in this prolific field.

I appreciate the encouragement of my publishers, Blackwell Scientific Publications. It is a pleasure to acknowledge the help of Mr A. February who prepared the line drawings, and that of the staff of the Photographic Department at the Postgraduate School.

I gratefully acknowledge the generosity of the following for permitting me to include their published or unpublished material, or for helpful discussions: Dr M. Armstrong (Figure 9.4), Dr H. Blackburn, Dr J. Borensztajn, Dr P. Borrie (Figure 11.6), Dr B. Brewer, Professor L.A. Carlson (Figure 9.6), the Department of Health and Social Security (Figure 8.3), Dr R.J. Havel, Dr E.D. Janus (Plate II, C,), Dr J.P. Kane, Dr A. Keys, Dr P. Laggner, Dr R.I. Levy, Professor M. Mancini (Figure 9.1), Professor T.R.E. Pilkington, Dr J. Stamler (Figure 9.5), Dr D. Steinberg and Dr R.W. Wissler (Figure 9.5). I am grateful to the Editors of the *Lancet* and the *European Journal for Clinical Investigation* and to Williams and Wilkins Co. for permission to reproduce published figures. Professor A.S. Truswell, Professor I.D.P. Wootton, Dr D. Gompertz, Miss A. Nicoll, Dr M.C. Philips and Dr N.B. Myant have kindly read portions of the manuscript; this does not absolve me from responsibility for the views expressed.

Lastly, I would like to record my warm appreciation to the many patients and normal subjects who generously agreed to participate in our studies described in this volume.

London 1976 *Barry Lewis*

Abbreviations

apo-A	apolipoprotein A, chiefly present in high density lipoproteins
apo-B	apolipoprotein B, the major apoprotein of low density lipoproteins
apo-C	apolipoprotein C, chiefly present in very-low density lipoprotein
CEFA	cholesteryl ester fatty acids
EFA	essential ester fatty acids
FA	Fatty acids
FFA	free fatty acids
HDL	high density lipoproteins
IDL	intermediate density lipoprotein, of density 1·006–1·019 g/ml ($=LDL_1$)
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoproteins
LPL	lipoprotein lipase
VHDL	very-high density lipoprotein
VLDL	very-low density lipoprotein

Contents

1

Chemistry of the Lipids of Plasma

The lipids constitute a wide range of substances of biological origin; they have in common a predominantly hydrocarbon structure with few charged groups. The resulting apolar composition of lipid molecules allows free solubility in such organic solvents as chloroform and ether, but poor solubility in water. Lipids have in common physical rather than structural properties. Although no purely chemical definition is possible because of the heterogeneous composition of this group of substances, the structure of all lipids includes long hydrocarbon chains [1]; in various lipids these chains may be straight or cyclic or branched. Metabolically, the lipids are more closely interrelated, being transported together in the plasma lipoproteins, and sharing certain regulatory mechanisms.

This chapter is intended to supply definitions, and to outline briefly some aspects of lipid chemistry.

The major lipids in mammalian plasma and tissues are:

Group 1 Fatty acids
Glycerides (Mono-, di- and triglyceride)
Cholesterol and cholesteryl esters

Group 2 Glycerophospholipids
Sphingolipids: phosphosphingolipids; glycosphingolipids

With qualification, the following may be added: bile acids; prostaglandins and steroid hormones.

Group 1 has received various names—'neutral lipids', 'simple lipids', 'non-polar lipids'—all of which are somewhat unsatisfactory; the lipids in this group have in common very low polarity. Group 2 has as synonyms 'complex lipids' and 'polar lipids'; the preferable term, 'amphipathic lipids' [2], indicates a measure of solubility in both non-polar and polar solvents. Rules for lipid nomenclature have recently been proposed by a commission set up by the International Union of Pure and Applied Chemistry and the International Union of Biochemists [2].

FATTY ACIDS

The fatty acids are present, as such, in minute concentrations in plasma and cells; they will be discussed first as they are constituents of most lipid classes. They comprise a

hydrocarbon chain and one carboxylic acid group and are thus aliphatic monocarboxylic acids (Figure 1.1). They vary in the number of carbon atoms (4–24), in the number and position of double bonds (0–6) and in the configuration about the double bonds. In most fatty acids the hydrocarbon chain is straight, but trace quantities of branched-chain fatty acids are found, and also of hydroxy fatty acids particularly in the sphingolipids. Although fatty acids with even numbers of carbon atoms predominate, small amounts of acids with odd carbon numbers occur.

Fatty acid composition is commonly abbreviated to a term giving the carbon number followed by the number of double bonds: 18:2 indicates a fatty acid with eighteen carbon atoms and two double bonds.

The wide range of chain lengths is arbitrarily divided into a long-chain group (twelve or more carbon atoms), medium-chain fatty acids (eight to eleven carbon atoms) and a short-chain series.

The orientation of the hydrocarbon chains about a carbon–carbon double bond is fixed (in contrast to single bonds which are free to rotate). Most natural fatty acids of

$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CQOH$

PALMITIC ACID, 16:0 M.P. 63°C

$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH=CH.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH$

$\omega 9$

OLEIC ACID, 18:1, *cis* M.P. 13°C

$CH_3.CH_2.CH_2.CH_2.CH_2.CH=CH.CH_2.CH=CH.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH$

$\omega 6$

LINOLEIC ACID, 18:2, *cis-cis* M.P. −5°C

Figure 1.1 Common long-chain fatty acids.

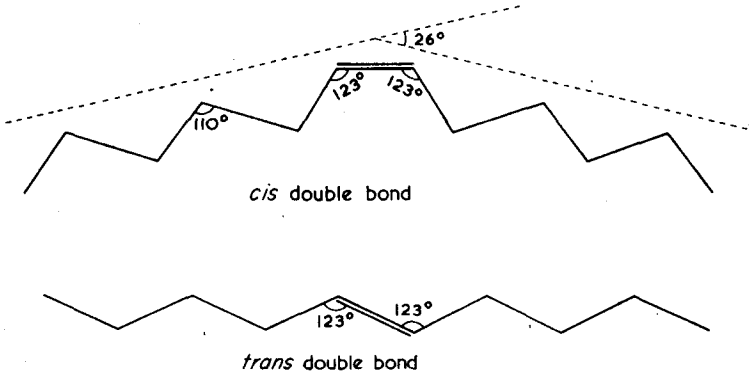

Figure 1.2

mammalian origin are *cis*-orientated, producing an angle in the hydrocarbon chain (Figure 1.2); *trans*-acids may appear during hydrogenation and so arise during margarine manufacture.

A biologically important classification distinguishes fatty acids on the basis of the number of double bonds. The term saturation describes the capacity of a fatty acid to add pairs of hydrogen atoms at its double bonds, reducing them to single bonds; saturated fatty acids have no double bonds, monosaturated acids have one, polyunsaturated fatty acids (polyenes, PUFA) have two or more. Double bonds are always separated by a methylene group, i.e. $-CH=CH-CH_2-CH=CH-$.

Some fatty acids of biological importance are listed in Table 1.1.

Table 1.1 Fatty acids of biological importance.

Numbering	*Systematic name*	*Common name*
12:0	Dodecanoic	Lauric
14:0	Tetradecanoic	Myristic
16:0	Hexadecanoic	Palmitic
16:1	*cis*-9-Hexadecenoic	Palmitoleic
17:0	Heptadecanoic	Margaric
18:0	Octadecanoic	Stearic
18:1	*cis*-9-Octadecenoic	Oleic
18:1	*trans*-9-Octadecenoic	Elaidic
18:2	*cis*-9, *cis*-12-Octadecadienoic	Linoleic
18:3	*cis*-9, *cis*-12, *cis*-15-Octadecatrienoic	α-Linolenic
18:3	*cis*-6, *cis*-9, *cis*-12-Octadecatrienoic	γ-linolenic
20:0	Eicosanoic	Arachidic
20:4	*cis*-5, *cis*-8, *cis*-11, *cis*-14-Eicosatetraenoic	Arachidonic
22:1	*cis*-13-Docosenoic	Erucic
Branched 20:0	3,7,11,15-Tetramethylhexadecanoic	Phytanic

Positional isomerism occurs in unsaturated fatty acids, and to describe the position of the double bond or bonds two alternative terminologies may be used. Counting the carboxylic acid carbon as 1, the site of a double bond is given by the number of the first carbon atom at the bond, e.g. octadeca-9-enoic acid. The other numbering procedure is especially useful in describing biogenetically related groups of polyunsaturated fatty acids. In the interconversion of fatty acids, chain elongation takes place at the carboxylic acid end, hence the number of carbon atoms between an existing double bond and the methyl carbon remains constant; any new double bonds are added at 3-carbon intervals. To describe the position of the double bonds in an unsaturated fatty acid and its elongated or further desaturated derivatives, it is merely necessary to specify the number of carbon atoms from the methyl carbon to the first double bond. By convention, the letter omega precedes this number, e.g. ω9. Linoleic acid, an 18:2 fatty acid with its double bonds at carbons 9 and 12 counting from the carboxylic acid group is converted to a 20:3 with double bonds at 8, 11 and 14 when numbered in this manner. Using the alternative system, the relationship between linoleic acid and its derivates is evident, for they are both of the ω6 series (18:2, ω6 and 20:3, ω6) (Figure 1.1).

Essential fatty acids [3, 4] **(EFA)**

Certain polyunsaturated fatty acids are essential nutrients in that they cannot be synthesized by mammals [5] and in that a specific deficiency state results in animals deprived of an adequate dietary intake. Although they cannot be synthesized, they are to some extent interconverted, and undergo many chemical transformations [6]. Apart from a few recent additions, the EFA deficiency syndrome was well described in 1929 by Burr and Burr [5]; it includes failure to gain weight, polydipsia, skin lesions, infertility, susceptibility to infection—particularly of the lung, electrocardiographic changes in rats, anaemia, fatty liver, diarrhoea, haematuria and poor wound healing.

Assays for EFA activity include resumption of weight gain, healing of skin lesions in rats, ability to act as a precursor for prostaglandin biosynthesis, and other *in vitro* tests. Certain polyunsaturated fatty acids do not perform equally well in the various tests, so that their inclusion as EFA is a matter of controversy and depends on the definition chosen [4].

The structural characteristics of EFA are, therefore, not entirely definable, but several features have been delineated. One is *cis*-orientation at all double bonds. Most have double bonds at C9–10 and C12–13 numbering from the carboxylic acid end. Until recently, all known EFA were $\omega 6$ or $\omega 3$, but it is now clear that certain $\omega 5$ and $\omega 7$ fatty acids with odd carbon numbers have some of the characteristics of EFA, and are prostaglandin precursors [7]. Beerthuis *et al.* [7] thereupon proposed a structure (Figure 1.3) to which EFA either conformed or could be converted by chain elongation and desaturation. In the Beerthuis model, $n = 3$, 4 or 5; Guarneri and Johnson [3], reviewing several reports that a measure of EFA activity was present in some $\omega 2$, 3 and 4 acids, suggested extending the model to include $n = 2$ or 1.

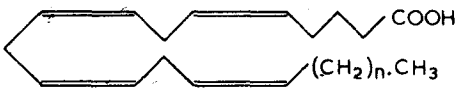

Figure 1.3 Beerthuis model of essential fatty acid.

Numerous fatty acids compete with each other for desaturating and chain-elongating systems. Competitive inhibition concerns particularly the dehydrogenation (desaturation) systems, which are microsomal. But microsomal chain-elongating enzymes may also be competitively inhibited [8]. This competitive inhibition, investigated extensively by Holman and his colleagues [9–11] can lead to the induction of EFA deficiency by feeding a diet rich in saturated or other nonessential fatty acids [8, 10]. In dietary deficiency of the EFA linoleic acid (18:2, $\omega 6$), the nonessential oleic acid (18:1, $\omega 9$) undergoes desaturation and elongation to 20:3, $\omega 9$, and the nonessential palmitoleic acid (16:1, $\omega 7$) is similarly converted to 18:1, $\omega 7$. In EFA deficiency, this 20:3, $\omega 9$ fatty acid (eicosa-5,8,11-trienoic acid) accumulates in tissues, and in serum phospholipid and cholesteryl ester. Holman [13] has therefore suggested that the ratio of trienoic to tetraenoic acids indicates EFA deficiency when it exceeds 0·4. Tinsley [14] has noted exceptions to this; but the presence of 20:3, $\omega 9$, carefully characterized, is an important index of EFA deficiency.

The desaturation–elongation mechanisms produce derivatives which, as stated, re-

tain the double bond positions identified by numbering from the methyl end of the molecule. If the parent acid is an EFA, the derivatives often retain EFA activity; the products of a non-EFA do not acquire EFA activity (as assessed by most tests). The concept of unsaturated fatty acid families is based upon these relationships; the $\omega 6$ family, including linoleic acid and its 20:3 $\omega 6$ and 20:4 $\omega 6$ derivatives, all possess activity. The $\omega 3$ family, with linoleic acid as the parent substance and 20:5 $\omega 3$ and 22:6 $\omega 3$ as derivatives, are less active by the same tests of EFA function. The $\omega 9$ family, based on oleic acid and including 20:3 $\omega 9$, and the $\omega 7$ family of palmitoleic acid and its 18:1 $\omega 7$ derivative, are not only non-essential but increase in tissues and in some serum lipid fractions in EFA deficiency.

Polyunsaturated fatty acids also play an important structural role; they are present in phospholipids of the membrane lipoproteins e.g. of mitochondria, lysosomes and red cells. Mitochondrial swelling and red cell lysis are readily induced in EFA deficient rats. Metabolically, they are rapidly oxidized, and there is evidence that unsaturated fatty acids are oxidized even more readily than saturated fatty acids [15]. Certain polyunsaturated fatty acids are the direct precursors of the prostaglandins (see p. 6). Polyunsaturated fatty acids are characteristically present in lecithin (in the 2-position) and in the cholesteryl esters of plasma.

Fatty acids are present in low concentration (0·5 millimolar, about 10–15 mg/100ml) in plasma, free in the sense that they are not present in the form of more complex lipids. The free fatty acid (synonyms: non-esterified or unesterified fatty acid) is not present in simple solution but is reversibly bound to albumin which has two high-affinity binding sites per molecule. This small pool of free fatty acid has an extremely rapid turnover, and its oxidation is a major source of metabolic energy in the fasted state.

Free fatty acid is released from adipose tissue, by hydrolysis of stored triglyceride; its composition resembles that of adipose tissue triglyceride. A representative analysis of plasma free fatty acid composition [16] is given in Table 1.2.

Table 1.2 Plasma free fatty acid composition

Fatty acid	%
14:0	2·1
16:0	30·0
16:1	7·5
18:0	9·3
18:1	44·0
18:2	7·0

Although certain EFA are substrates for prostaglandin synthesis, the syndrome of EFA deficiency is not corrected by prostaglandin administration. The effects of EFA deficiency on membrane structure are no doubt direct, and intracellular organelles are thereby rendered more susceptible to chemical insults or ageing; mitochondria of EFA deficient rats swell and show uncoupling of oxidative phosphorylation.

The significance of polyunsaturated fatty acids in human nutrition is undoubted, but most evidence is relatively recent [39]. Case reports, mostly concerning infants on low fat diets or with malabsorption, e.g. by Hansen and Wiese [17], describe skin lesions

and susceptibility to pulmonary infection. An extensive literature review has been provided by Söderhjelm [18]; there is now abundant evidence of impaired growth and scaly skin changes with keratinization in infants on formula diets low in linoleic acid [19] and the minimum requirement for linoleic acid by infants appears to be about 2% of calories, or 1–2% [20]. Fetal linoleate content is increased by supplementing the mother's dietary intake [21]. EFA deficiency in patients receiving parenteral nutrition are mentioned in Söderhjelm's review, and three cases resulting from intestinal malabsorption have been published by Press and Thompson [22]. There have also been suggestions that multiple sclerosis is associated with deficiency of certain essential fatty acids in nervous tissue [23].

Prostaglandins

The prostaglandins are a group of lipids with potent biological activities on smooth muscle, cardiac muscle, adipose and other tissues. Few areas of investigation have progressed as rapidly in the few years since Bergström's isolation and identification of these compounds [24]. They are derived from polyunsaturated fatty acids, as shown in Figure 1.4.

The parent substance may be regarded as the C20 prostanoic acid. Polyunsaturated fatty acids of the $\omega 6$ series are oxygenated by molecular oxygen and undergo ring

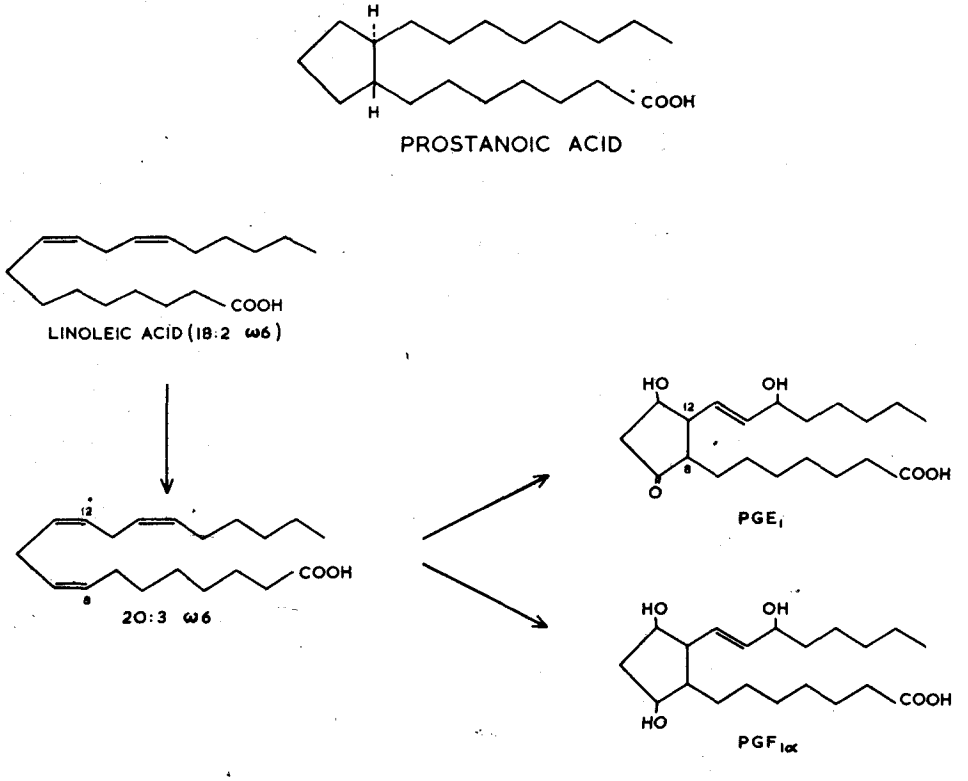

Figure 1.4 Relationship between linoleic acid and prostaglandins PGE_1 and $PGF_{1\alpha}$.

closure between carbons 8 and 12. The prostaglandins are C20 acids, with 1 to 3 double bonds, and with hydroxy and carbonyl functional groups. The actions of the several prostaglandins vary and some are mutually antagonistic.

Glycerides (Figure 1.5)

One, two or all three of the hydroxyl groups of glycerol may be esterified with fatty acids, forming respectively monoglyceride, diglyceride ('partial glycerides'), and triglyceride. Of these, triglyceride is by far the most abundant, and is synonymous with neutral fat. Partial glycerides, particularly monoglyceride, are present in substantial amounts in intestinal mucosa during absorption of the digestion products of dietary triglyceride.

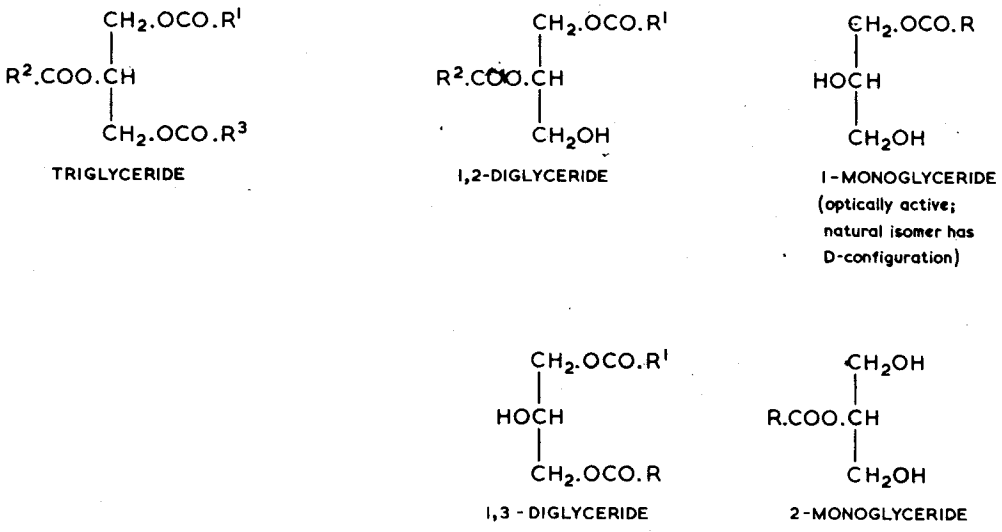

Figure 1.5

Natural triglycerides contain mixtures of fatty acids; these are not, as was once believed, randomly distributed, but there is a tendency (varying in degree between different fats) for unsaturated acids to occupy the 2 or beta position of the glycerol molecule, and for saturated acids to occupy the 1 and 3 (α and α^1) positions.

The consistency and melting point of triglyceride is determined largely by the chain length and saturation of its constituent fatty acids. Hardness and higher melting points are characteristic of fats containing long-chain saturated fatty acids; fats containing predominantly unsaturated or short-chain fatty acids have low melting points (Table 1.3).

The melting point of mixed triglycerides is also influenced by the position of the fatty acids within the molecule. In common usage fats are triglycerides which are solid at room temperature while oils are liquid at room temperature.

Triglyceride of both dietary and endogenous origin is transported in plasma lipoproteins; it is stored mainly in adipose tissue (of which it comprises about 60–85% of wet weight) and to a small extent in most other tissues.

Table 1.3 Melting points of triglycerides.

Triglyceride	°C
Glyceryl tristearate	72
Glyceryl tripalmitate	65
Glyceryl trioleate	5
Glyceryl trilinoleate	−10

The fatty acid composition of adipose tissue is certainly under genetic influence, and varies from species to species [25]. The effect of the composition of dietary fat upon that of adipose tissue is substantial, a finding which was inapparent until the long-term feeding experiments of Hirsch [26]. On altering the composition of food fat, several months may elapse before adipose tissue biopsies reflect the dietary pattern, and 1–2 years are required for the effect to become maximal. Crawford [27] has drawn attention to the role of diet in explaining species differences in the triglyceride fatty acids of meats; techniques in animal husbandry, such as high calorie feeding with carbohydrate-rich fodder may increase the proportion of saturated fatty acids; wild-herbivores have a substantially higher percentage of polyunsaturated fatty acid in muscle than do domestic beef.

Plasma triglyceride contains mainly long-chain fatty acids, the composition of which is rapidly influenced by that of dietary fat. It includes both dietary and endogenous fatty acids; the latter are derived chiefly from circulating free fatty acids (released from

Table 1.4 Endogenous triglyceride fatty acids in plasma.

Fatty acid	%
14:0	2·2–4·2
16:0	35–46
16:1	7·6–9·5
18:0	7·0–11
18:1	34–40
18:2	1·5–4·3

Table 1.5 Fatty acid composition of human adipose tissue [28].

Fatty acid	%
12:0	0·35
14:0	2·4
16:0	24·6
16:1	5·6
18:0	6
18:1	50
18:2	9·5