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PHARMACOLOGY OF LIPID TRANSPORT AND ATHEROSCLEROTIC PROCESSES

Section Editor

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Pharmacology of Lipid Transport and Atherosclerotic Processes

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PREFACE

THE role of lipids, in particular plasma lipids, in atherogenesis has been the subject of prodigious research efforts throughout the world during the past 25 years. Although a definitive understanding of the pathogenesis of atherosclerosis has yet to be achieved, this research has yielded a great increase in our knowledge of lipid transport.

By correlating this vast literature, which covers a wide spectrum of areas, this volume of the International Encyclopedia of Pharmacology and Therapeutics aims to provide a concise and coherent picture of current, fundamental information in the following areas: (a) the chemistry of plasma lipids and lipoproteins; (b) the dynamics of the physiology of lipid transport; (c) the interaction of plasma lipids with the arterial wall in relation to atherogenesis in particular; (d) the metabolic characteristics of the arterial wall in relation to atherogenesis; (e) the pharmacologic approach to the control of plasma lipid levels; (f) the pharmacologic approach to the control of atherosclerosis; and (g) the current therapeutic approaches available to physicians for the treatment of hyperlipoproteinemia or atherosclerosis or both.

It is my hope that the goal of this volume has been achieved and that it will be of use not only to researchers in the field of atherogenesis but also to the many physicians who daily must deal with clinical problems related to hyperlipoproteinemia and atherosclerosis.

San Antonio, Texas, U.S.A. EDWARD J. MASORO

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CHAPTER 1

OVERVIEW OF THE PROCESS AND REGULATION OF LIPID TRANSPORT

Edward J. Masoro

San Antonio, Texas, U.S.A.

INTRODUCTION

Man and other mammals are required to transport large quantities of lipid from one part of the body to another. For example, dietary or exogenous lipids are transported from the intestine where they have been absorbed from the lumen of the alimentary tract to various tissue sites for use as structural components or as fuel or to the adipose tissue for storage as an energy reservoir. Endogenous lipid is also transported from one tissue to another and to sites of excretion from the body. Most of this transport is carried out by the cardiovascular system by its circulation of the blood; specifically it is the lipid components of the blood plasma which are either being transported or are serving in a vehicular fashion in this transport or both. The lymphatic system and the interstitial fluid are also involved in lipid transport.

Plasma lipids are of interest not only because of this transport function but also because of their possible role in the genesis of atherosclerosis, a health problem of great proportions. Indeed it is this possible relationship between plasma lipids and atherogenesis that has led physiologists, biochemists, pharmacologists, and clinicians to put forth prodigious effort in the field of lipid transport.

I. PLASMA LIPIDS

Even in postabsorptive man (i.e., 12–18 hr since last eating), the plasma contains a large amount of lipid; data representing those of a typical,

normal man are reported in Table 1. If such a man were to eat a mixed meal containing carbohydrate, fat, and protein, the lipid composition of his plasma would change from that presented in Table 1. The plasma

TABLE 1. LIPID COMPOSITION OF A TYPICAL NORMAL HUMAN PLASMA SAMPLE DRAWN IN THE POSTABSORPTIVE STATE

Lipid class	Concentration (μmoles/100 ml)
Cholesterol	120
Cholesterol ester	310
Lecithin	100
Sphingomyelin	40
Lysolecithin	17
Triglyceride	100
Free fatty acid (FFA)	100

Data derived from Kuksis *et al.* (1969) and Masoro (1968).

concentration of triglyceride would increase to several times that of the postabsorptive level, the extent of increase depending on the nature and amount of food eaten, the genetics of the individual, and the time after ingestion of the meal at which the plasma is sampled (peak triglyceride levels occurring some 3–6 hr after ingestion of food) (Fredrickson *et al.*, 1967). The concentration of plasma free fatty acids usually decreases in response to the ingestion of food (Steinberg, 1966), the extent of the fall relating to the amount and kind of food eaten, the genetics of the individual, and the time after eating chosen to sample the blood. A typical response when eating a mixed meal would lead to a plasma free fatty concentration of about 25 μmoles per 100 ml of plasma some 4–6 hr after eating. Plasma cholesterol, cholesterol ester, and phospholipid levels do increase in response to eating but the extent of increase is slight compared to that of triglyceride (Fredrickson *et al.*, 1967).

A very small amount of lipid probably exists in plasma in aqueous solution as monomers or as micellar aggregates; however, most of the lipid is present in a complex with certain specific proteins (Kritchevsky, 1969). It is this interaction of lipid with protein which permits the large amount of the rather non-polar lipids listed in Table 1 to be soluble or at least dispersible in the aqueous medium of plasma. These lipid–protein com-

plexes are called plasma lipoproteins and on the basis of their physical, chemical, and biological properties, they can be divided into four major classes (Hatch and Lees, 1968) as follows: (1) albumin–free fatty acid, (2) high density lipoproteins (HDL), (3) low density lipoproteins, and (4) very low density lipoproteins. The HDL are further subdivided into three subclasses: HDL_3, HDL_2, and a very high density lipoprotein class (VHDL). The very low density lipoprotein class (VLDL) is also subdivided into three subclasses: very low density lipoprotein proper (VLDL), endogenous particles, and the chylomicrons. Some physical and chemical properties of the various subclasses of lipoproteins are summarized in Table 2. However, the ratios of the lipids to each other and to the protein component vary even within a given subclass of lipoproteins; this is in marked contrast to other plasma proteins where the heterogeneity within a given class of protein (e.g., albumin) is slight. However, the differences in the composition within a given lipoprotein subclass are far less than the differences noted between subclasses.

The physiology of lipid transport can be better understood on the basis of the dynamics of lipoprotein classes than on the basis of simply considering each lipid class (i.e., cholesterol, triglyceride, etc.) as a metabolically homogenous entity. Also, in recent years much of the effort to relate lipids to atherogenesis has involved the consideration of the plasma lipoprotein systems rather than simply lipid constituents (Kannel *et al.*, 1971). However, unequivocal evidence that lipoproteins relate more directly to atherosclerosis than do plasma lipids is not at hand (Kannel *et al.*, 1971).

II. TRANSPORT OF PLASMA LIPIDS

A. TRANSPORT OF FREE FATTY ACIDS (FFA)

The lipoprotein class termed albumin–FFA refers to the complexing of FFA with serum albumin. This is the main transport form of FFA (Fredrickson and Gordon, 1958) although some FFA is found bound to red blood cell membranes and to the other plasma lipoprotein classes. In plasma, under physiological conditions, the molar ratio of FFA to albumin ranges between 0.25 and 2 (Masoro, 1968). The source of the FFA in albumin–FFA is primarily adipose tissue. Adipose tissue contains large quantities of triglyceride stored in its central vacuole as an energy reservoir. To mobilize this triglyceride, enzymes in the cytoplasm of the adipocyte convert it to FFA and glycerol (Steinberg and Vaughan, 1965). The

TABLE 2. PHYSICAL AND CHEMICAL PROPERTIES OF LIPOPROTEIN CLASSES AND SUBCLASSES[a]

Class	Subclass	Hydrated density ($g\ ml^{-1}$)	Molecular weight ($g\ mole^{-1}$)	Composition of lipoprotein (% by weight)				
				Cholesterol	Cholesterol ester	Phospholipid	Triglyceride	Protein
HDL	HDL_2	1.08–1.12	380×10^3	2	20	24	4	50
HDL	HDL_3	1.12–1.16	180×10^3					
HDL	VHDL	1.15–1.20	150×10^3	0.3	3	28	5	62
LDL	—	1.01–1.05	2.2×10^6	10	36	20	12	22
VLDL	VLDL	0.93–1.01	$3–128 \times 10^6$	3–5	10–13	13–20	50–60	5–12
VLDL	Endogenous particles	0.93	$0.02–5 \times 10^9$	4.5	15	10	67	2–5
VLDL	Chylomicrons	0.93	$0.5–430 \times 10^9$	2.1	3.9	4.3	87	2
FFA–albumin	—	1.33	69×10^3	0	0	?	0	99

[a] Modified from Hatch and Lees (1968): HDL, high density lipoprotein; VHDL, very high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

glycerol being water-soluble readily diffuses into the blood. The FFA not being significantly soluble in water must be bound to plasma albumin or some other carrier to be transported in the aqueous blood plasma system. The process of releasing triglyceride in the form of FFA and glycerol, called fat mobilization, is outlined in Fig. 1.

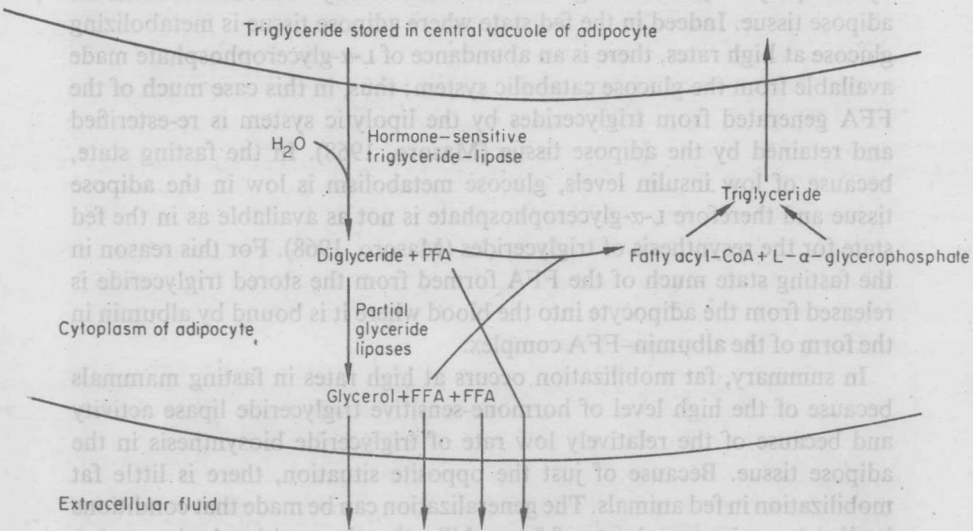

FIG. 1. Schematic presentation of fat mobilization and re-esterification of FFA in adipose tissue.

The hydrolysis of adipose tissue triglyceride is initiated by an enzyme catalyzing the generation of free fatty acid and diglyceride (Vaughan *et al.*, 1964). This triglyceride lipase of adipose tissue is under the control of both the nervous and endocrine systems and is often called the hormone-sensitive triglyceride lipase. Diglyceride does not accumulate in adipose tissue because there are other lipases present which rapidly degrade partial glycerides to glycerol and FFA (Vaughan *et al.*, 1964). The hormone-sensitive triglyceride lipase, which appears to involve the rate-limiting step in the hydrolysis of triglyceride, is less active in the adipose tissue of fed animals than in that of fasting animals. Thus there is a much higher rate of triglyceride to FFA and glycerol in the adipose tissue of the fasting mammal than that of the fed (Hollenberg, 1965).

Since adipose tissue contains only low levels of glycerokinase, it has little ability to metabolize glycerol (Wieland and Suyter, 1957); therefore, most

of the glycerol generated by the lipolytic system outlined in Fig. 1 is mobilized from adipose tissue. However, the adipose tissue is capable of further metabolizing FFA, by first converting it to fatty acyl-CoA and then by reacting the fatty acyl-CoA derivatives with L-α-glycerophosphate to regenerate the triglyceride molecule. Thus FFA released from triglyceride by the lipolysis system of Fig. 1 need not necessarily be mobilized from the adipose tissue. Indeed in the fed state where adipose tissue is metabolizing glucose at high rates, there is an abundance of L-α-glycerophosphate made available from the glucose catabolic system; thus, in this case much of the FFA generated from triglycerides by the lipolytic system is re-esterified and retained by the adipose tissue (Masoro, 1968). In the fasting state, because of low insulin levels, glucose metabolism is low in the adipose tissue and therefore L-α-glycerophosphate is not as available as in the fed state for the resynthesis of triglycerides (Masoro, 1968). For this reason in the fasting state much of the FFA formed from the stored triglyceride is released from the adipocyte into the blood where it is bound by albumin in the form of the albumin–FFA complex.

In summary, fat mobilization occurs at high rates in fasting mammals because of the high level of hormone-sensitive triglyceride lipase activity and because of the relatively low rate of triglyceride biosynthesis in the adipose tissue. Because of just the opposite situation, there is little fat mobilization in fed animals. The generalization can be made that conditions leading to an increased rate of fat mobilization do so either by increasing the activity of the adipose tissue hormone-sensitive triglyceride lipase or by decreasing the rate of triglyceride biosynthesis in adipose tissue or by both mechanisms.

Since the FFA molecule is almost insoluble in water, it can diffuse from the adipose tissue into the plasma only if an acceptor substance is available to solubilize it. Plasma albumin is the primary acceptor substance for this purpose yielding, when interacting with FFA, the water-soluble albumin–FFA (Maşoro, 1968). Therefore the capacity of adipose tissue to release FFA to plasma, which is obviously greatly influenced by the rate of adipose tissue triglyceride lipolysis and biosynthesis as discussed above, may also be influenced by the rate of blood flow through the capillaries of the adipose tissue since the blood flow controls the amount of albumin made available for the binding of the FFA. In man, an increase in fat mobilization induced by norepinephrine, glucagon, and fasting, is in each case accompanied by an increased rate of adipose tissue blood flow (Neilsen *et al.*, 1968).

Usually the concentration of FFA in the plasma is primarily related to

the rate of fat mobilization. For example, in the fed state there is a low rate of fat mobilization and plasma FFA concentrations are usually around 250 μEq.l^{-1} while in the fasting or postabsorptive state in man the concentration of FFA in plasma is approximately 1000 μEq.l^{-1}. The rate of uptake of FFA by the various tissues of the body, such as muscle, heart, liver, etc., is primarily affected by the FFA–albumin molar ratio, the higher this ratio, the greater the rate of uptake (Steinberg, 1966). Since the concentration of albumin in plasma is relatively constant, the FFA–albumin molar ratio is primarily modified by changing levels of plasma FFA. Consequently the ability of tissues to take up FFA increases as the plasma concentration of FFA increases. The FFA probably dissociates from the albumin before it is taken up by tissues and this FFA in free solution is in equilibrium or near equilibrium with that bound to albumin.

Many physiological and pharmacologic agents can influence the rate of fat mobilization from adipose tissue. Both epinephrine and norepinephrine, the catecholamines secreted by the adrenal medulla and the endings of the postganglionic sympathetic fibers, vigorously promote fat mobilization (Steinberg, 1966). They do so by activating the hormone-sensitive triglyceride lipase through a mechanism involving both 3′,5′-cyclic AMP and the protein kinases of the adipose tissue cell (Huttunen *et al.*, 1970a). Indeed it appears to be the phosphorylation of the hormone-sensitive triglyceride lipase by ATP as catalyzed by protein kinases that is responsible for this activation of the lipase (Huttunen *et al.*, 1970b). The very rapid rate of release of FFA and glycerol from adipose tissue during many kinds of stress appears to involve the adrenal–sympathetic system (Bogdonoff and Nichols, 1965).

Many other hormones such as adrenocorticotrophin, thyroid stimulating hormone, luteinizing hormone, melanophore-stimulating hormone, vasopressin, glucagon, and others can stimulate fat mobilization at least in some species. When they do stimulate they apparently do so by changing the levels of adipocyte cyclic-AMP (Butcher, 1968).

Insulin strongly inhibits fat mobilization. It does so partly by promoting glucose metabolism in adipose tissue, thereby making more L-α-glycerophosphate available for the conversion of FFA to triglyceride. Insulin also inhibits the hormone-sensitive triglyceride lipase, but not by lowering the concentration of cyclic-AMP in the adipocyte. It appears that insulin plays an important role in the regulation of fat mobilization during the daily feeding–postabsorptive cycle in man and other mammals (Cahill *et al.*, 1966).

Exercise and cold exposure, conditions which vigorously promote energy

expenditure, markedly increase the rate of fat mobilization and apparently do so primarily by a sympathetic–adrenal response (Himms-Hagen, 1967). Indeed in exercise the increased fat mobilization is only one aspect of a broad response to increased sympathetic activity, e.g., cardioacceleration, splanchnic vasoconstriction, muscle vasodilation, and so forth. Increased fat mobilization as well as these other sympathetic responses play an important role in supplying the exercising muscle with sufficient fuel.

The nervous system is complexly involved with fat mobilization (Bogdonoff and Nichols, 1965). For example, during an interview when a man is made to recall an unpleasant past experience a marked rise in fat mobilization and plasma FFA levels occurs. Similarly, anticipation of physical danger and the arousal of sexual interests both increase plasma FFA levels and do so probably by increasing fat mobilization from adipose tissue.

In summary, FFA–albumin is the transport form that carries the energy stored in adipose tissue as triglyceride to various areas of the body. The level of the FFA in the plasma is primarily regulated by its rate of release from adipose tissue and not by the rate of uptake by other tissues. The rate at which tissues take up FFA from the extracellular fluids directly relates to the concentration of FFA in plasma. From what has been discussed it might be anticipated that the rate of plasma FFA turnover is very rapid; this is indeed the case with half-life of plasma FFA being in the order of a very few minutes (Fredrickson and Gordon, 1958).

B. TRANSPORT OF TRIGLYCERIDES

Triglycerides are transported in plasma primarily in the very low density lipoprotein package, the chylomicron subclass of the VLDL transporting most of the dietary (or exogenous triglyceride) and the VLDL proper and endogenous particles transporting endogenously generated triglyceride (Robinson, 1970). Exogenous and endogenous triglyceride transport can be most easily understood if each is discussed separately.

The digestive system converts the water-insoluble dietary (exogenous) triglyceride into monoglycerides and free fatty acids (FFA) which interact with micelles of bile salts, lecithin, and cholesterol secreted into the lumen of the intestine in the bile to form large mixed micelles (Robinson, 1970). It is pancreatic lipase that catalyzes the hydrolysis of emulsified dietary triglyceride to FFA and monoglyceride. The monoglyceride and FFA, when present in the mixed micelles, interact with the brush border of the

intestinal mucosal epithelial cell and enter it (Johnston, 1968). Within the epithelial cell much of this monoglyceride and FFA is reconverted to triglyceride by a pathway that involves stepwise acylation of monoglyceride by reaction with fatty acyl-CoA (Johnston, 1968). Ultimately the endoplasmic reticulum packages the triglyceride of dietary origin into chylomicrons which also contain protein, phospholipids, cholesterol, and cholesterol ester (Cardell *et al.*, 1967). This chylomicron is delivered to the interstitial fluid at the basal and lateral surfaces of the mucosal epithelial cell by the process of reverse pinocytosis (Dobbins, 1969). From this interstitial fluid the chylomicron particle enters the lacteal with the process of pinocytosis apparently being involved. The lacteals drain into larger lymphatic vessels and ultimately into large veins of the cardiovascular system thus delivering the dietary triglycerides in the package of a chylomicron into the circulating blood plasma.

Since the circulating chylomicrons are very large particles, it is unlikely that they can penetrate the endothelium of most capillaries to reach the cells utilizing triglyceride. Indeed the following process has been suggested as the major way by which the fatty acids contained in chylomicron-triglyceride are made available to tissues of the body. It is proposed that the blood capillaries in many tissues contain on their endothelial surface facing the blood stream the enzyme lipoprotein lipase (Robinson, 1963) and that during blood flow through these capillaries this enzyme contacts the chylomicron-triglyceride causing it to be hydrolyzed to FFA and glycerol. It is believed that much of the FFA penetrates the endothelium to come into contact with the cells of that tissue; however, some of this FFA and most of the glycerol is carried on by the cardiovascular system for use by tissues remote from the site of the triglyceride hydrolysis (Robinson, 1970). In the case of glycerol, it is the liver that primarily utilizes it (Masoro, 1968).

Although tissues, such as heart and skeletal muscle, can utilize chylomicron-triglyceride for energy metabolism, it appears that when absorbing a mixed diet containing appreciable quantities of carbohydrate and protein as well as lipid, it is the adipose tissue that takes up most of the FFA derived from chylomicron-triglyceride (Fredrickson *et al.*, 1967). Upon entering the adipose tissue cell, this FFA is converted to fatty acyl-CoA which reacts with L-α-glycerophosphate (formed by the adipose tissue cells primarily from dietary glucose) to form phosphatidic acid (Masoro, 1968). This phosphatidic acid is primarily converted to triglyceride by the pathway summarized in Fig. 2. In this way the adipose tissue converts ingested triglyceride-fatty acid back to triglyceride for deposition in its central

vacuole. It is the dietary carbohydrate, however, that primarily generates the glyceride-glycerol for this triglyceride formation (Masoro, 1968).

The other transport forms of triglyceride, namely the VLDL proper and the endogenous particle, are primarily derived from endogenous sources and are released into the blood mostly by liver and to a lesser extent by the small intestine (Schumaker and Adams, 1969). Since these processes are

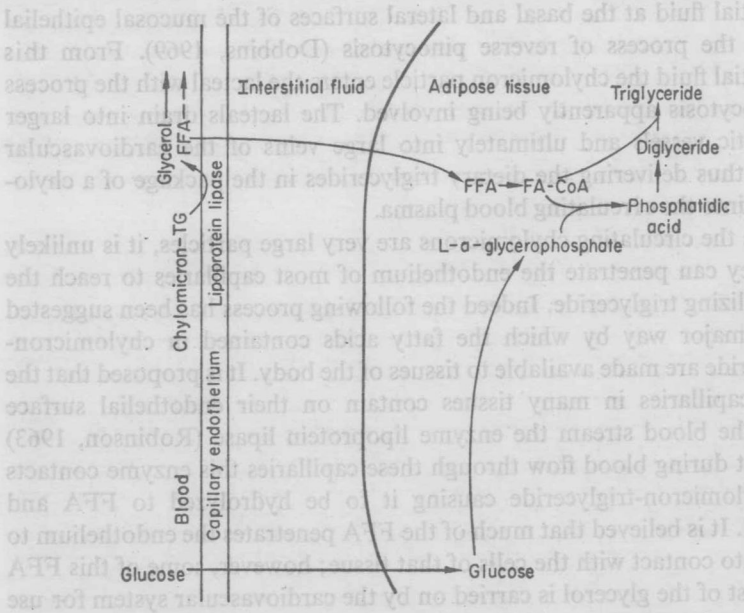

FIG. 2. Schematic summary of processes involved in fat deposition in adipose tissue.

best understood for liver and since what occurs in the intestine is probably similar to that occurring in liver (although the intestine, unlike the liver, can use exogenous precursors too [Ockner *et al.*, 1969a, b]) our discussion will concentrate on the hepatic system.

The liver generates the triglyceride that it secretes in the VLDL proper or endogenous particles packages from three sources. First, the dietary chylomicron-triglyceride appears to serve as a source of this triglyceride (Masoro, 1968). It is not known whether the liver generates some FFA and glycerol directly from chylomicron-triglyceride or whether it solely picks up the FFA and the glycerol carried to the liver by the blood from some extrahepatic tissue where they had been produced by lipoprotein lipase