Lipid Metabolism in Normoxic and Ischemic Heart

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Reprinted from *Molecular and Cellular Biochemistry* Volume 88:1/2, 1989

Kluwer Academic Publishers
Dordrecht / Boston / London

Distributors

for the United States and Canada: Kluwer Academic Publishers, 101 Philip Drive, Assinippi Park, Norwell, MA 02061, USA
for all other countries: Kluwer Academic Publishers Group, Distribution Center, P.O. Box 322, 3300 AH Dordrecht, The Netherlands

Library of Congress Cataloging-in-Publication Data

Lipid metabolism in normoxic and ischemic heart.

(Developments in molecular and cellular biochemistry;)
"Reprinted from Molecular and cellular biochemistry, v. 88: 1/2, 1989.
Includes bibliographical references.
1. Coronary heart disease–Pathophysiology. 2. Blood lipids–Metabolism. 3. Heart–Metabolism. I. Vusse, G. J. van der. II. Series.
RC685.C6L54 1989 616.1'23071 89–24502
ISBN 0–7923–0479–9

PRINTED IN THE NETHERLANDS

Preface

During recent decades, bewildering progress has occurred in the field of Molecular and Cellular Biochemistry. Progress has been extraordinarily rapid primarily because of the challenge for finding solutions to a wide variety of diseases and the availability of new techniques for monitoring biochemical processes. This has resulted in a voluminous and complex literature in the field of biochemical medicine so that there is a clear need for the synthesis and analysis of the continuing expansion of valuable data. It was thus considered appropriate to initiate a new series of monographs, each dedicated to a specialized area of investigation, encompassing molecular and cellular processes in health and disease.

Most of the biochemical scientists have devoted their energies in understanding the fundamentals of biochemistry and indeed impressive advances have been made in the past. However, the full potential for explanation has been hampered by the concept of universality of biochemical reactions occurring in the cell. In view of the fact that each organ in the body performs a distinct function, it is now beginning to be realized that each cell type is unique in its need to survive and perform its specific function. Accordingly, the aspect of individualty is receiving increased attention for revealing new avenues in the study of pathophysiology of cellular abnormalities. Such an approach in the field of functional biochemistry is expected to accelerate the growth of knowledge in clinical sciences and this can be seen to benefit the development of new strategies to be adopted for the management of organ dysfunction. It is therefore proposed to highlight the functional aspects of molecular biochemistry in this new series of publications.

Although the functions of different cellular components are being revealed and understood at a molecular level, the overriding forces which coordinate and regulate the events in subcellular organelles, remain vague and undetermined. Despite the well known role of lipids, proteins and carbohydrates in determining the cellular structure and function, virtually little is known about the assembly of a given organ and for that matter of a cell with special behavior. Even a simple issue such as the exact utilization of fuel by different types of cells and their components is seen only in a shadowy outline. Similarly, the mechanisms by which membranes maintain the intracellular environment different from that outside the cell are poorly understood. Furthermore, despite the extensive knowledge regarding the role of the genetic apparatus for the synthesis of proteins in the cell, the manner in which these proteins and molecules are put together for their interaction with lipids is obscure. There are numerous other examples which one can cite to emphasize our inadequate knowledge of fundamental processes in the living cell. It is therefore essential to make a multidisciplinary effort in order to further elucidate the secrets of life. This series "Developments in Molecular and Cellular Biochemistry" is an attempt to promote medical research and increase communication in the field of clinical biology in health and disease and it is hoped that these monographs will be received with enthusiasm.

The present volume in this series concerns the analysis of lipid metabolism in normoxic and ischemic heart. It is now well established that free fatty acids are the prime source of energy for the myocardium and phospholipids are the building blocks of cardiac myocytes. Since coronary heart disease, where ischemia and hypoxia are

considered to play a crucial role in the development of heart dysfunction, is a major cause of death in the western world, a symposium was held in Maastricht during September 12–13, 1989 under the chairmanship of Dr. Ger J. van der Vusse to discuss changes in lipid metabolism in the ischemic heart. This volume is thus based on selected papers from this conference. It is our contention that these articles by renowned investigators delineating recent results and discussing significant aspects of their data will provide a unified framework for a multidisciplinary approach to the field of lipid metabolism in the hearth.

Winnipeg, Canada — Naranjan S. Dhalla

CONTENTS

Molecular and Cellular Biochemistry **88**: 1–6, 1989.

Invited Paper

Myocardial fatty acid homeostasis

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Accepted 28 December 1988

Key words: myocardium, lipids, fatty acids

Introduction

Since the pioneering work of Bing and colleagues [1, 2] on cardiac fatty acid metabolism a vast number of scientists have devoted their research time on unraveling the complexity of fatty acid homeostasis in the normal and diseased heart. The broad interests in cardiac lipid metabolism had led to the organization of the 1st International Symposium on lipid Metabolism in the Normoxic and Ischemic Heart in Rotterdam, The Netherlands, in 1986. The proceedings of this symposium have been published in Basic Research in Cardiology, volume 82 (suppl 1), 1987. Prompted by the success of the first meeting the 2nd International Symposium on Lipid Metabolism in the Normoxic and Ischemic Heart has been organized this time in Maastricht, The Netherlands, on September 12 and 13, 1988. Four main aspects of cardiac lipid metabolism have extensively been discussed during this meeting. These four issues were:

a) uptake and transport of lipids in the heart;
b) cardiac phospholipid metabolism and eicosanoid production;
c) the effect of ischemia and reperfusion on myocardial fatty acid homeostasis, and
d) imaging of fatty acid metabolism in the normal and diseased heart with special emphasis on its application in the clinical setting.

In this overview a condensed report will be presented concerning the 'state of the art' of cardiac fatty acid homeostasis highlighting the main issues of the 2nd Cardiac Lipid Symposium.

Uptake and transport of lipids in the heart

Fatty acids (FA) are supplied to the heart via the blood either bound to albumin or as triacylglycerols complexed into hydrophilic lipoproteins [3]. The actual amount of fatty acids extracted by the heart depends on factors such as arterial FA concentration, workload of the heart and the presence of competing energy substrates. The extraction of FA by the heart is very efficient, i.e. up to 70% during one single transit through the cardiac capillary system. Beside FA supplied to the heart as albumin-FA complex, FA can be released from circulating triacylglycerols present in the lipoprotein particles by action of lipoprotein lipase, which is attached to the luminal membrane of the capillary endothelial cells [4]. The origin of lipoprotein lipase is most likely the parenchymal cell, i.e. the muscle cells, of the heart [5]. The enzyme molecule is synthetized in the cardiac muscle cells and undergoes a number of processing steps to activate the enzyme, including glycosylation, prior to transport from the myocyte and binding to the endothelial site of action [6].

The route of FA transport from blood to inside cardiac muscle cells comprises a succession of mechanisms. To achieve the first step of FA extraction, an interaction of the albumin-FA complex with specific sites at the luminal membrane of the endothelium has been proposed [7]. This interaction should accelerate the release of FA from the albumin-FA complex. The FA molecules are sub-

sequently transported through the luminal endothelial cell membrane, the intracellular space (probably by a FABP-mediated process) and the abluminal membrane of the endothelium [8]. FA's travel from the endothelial to the muscular cells through the interstitial space as a complex with albumin.

A protein structure of about 43 kDa localized in the sarcolemma and identified as a membrane fatty acid-binding protein has been suggested to be involved in the flux of fatty acids across the sarcolemma [9, 10]. A smaller intracellular fatty acid-binding protein (FABP) is assumed to facilitate the transport of fatty acids from the sarcolemma to mitochondria and other intracellular sites of fatty acid conversion. FABP is a low molecular weight (15 kDa) protein and is abundantly present in myocardial cells [11], as it accounts for about 5% of all cytosolic proteins. Fournier and Rahim [12] have recently hypothetized that FABP increases its efficacy to transport FA by modulating its affinity for fatty acids by self-aggregation of the protein.

The majority of FA's taken up by the heart is oxidized in the mitochondria to provide ATP for energy-consuming processes. Prior to oxidation the FA molecule is activated by acylCoA synthetase. This enzyme, predominantly located at the mitochondrial outer membrane (Fig. 1) but probably also to some extent at the sarcoplasmic reticulum, condensates FA and Coenzyme A (CoA) to yield acylCoA.

Part of the acylCoA's is directly converted into the intracellular triacylglycerol pool from which it can again be released by endogenous lipases for subsequent mitochondrial fatty acid oxidation. Studies with labeled fatty acids have indicated that part of the extracted fatty acids is also incorporated into the phospholipid pool [14]. The proportion of label that is recovered from the esterified lipid pool depends, among others, on the blood lactate concentration.

Another part is incorporated into acylcarnitine by carnitine-acyl transferase I, localized at the inner site of the mitochondrial outer membrane [13]. Acylcarnitine is transported across the mitochondrial inner membrane by action of carnitine-acylcarnitine translocase. Through this transmembrane protein one molecule of acylcarnitine is exchanged for one molecule of free carnitine present in the mitochondrial matrix. Inside the matrix acylcarnitine is converted into acylCoA. This substance, in turn, is degraded to acetylCoA by the β-oxidation process (Fig. 1). AcetylCoA condensates with oxaloacetate to produce citrate and free Coenzyme A. Citrate is then degraded in the tricarboxylic acid or Krebs cycle.

Recently, attempts have been made to develop sophisticated mathematical models to elucidate the detailed mechanism of cardiac FA transport across the membrane structures and spaces dividing the vascular compartment from the mitochondrial matrix [8].

Cardiac phospholipid metabolism and eicosanoid production

Phospholipids are important constituents of the plasmalemma and intracellular membranes of cardiac cells, creating specific compartments required for adequate cellular function. In addition, membrane phospholipids are indirectly involved in communication processes either between cells or inside the cell. Eicosanoids are produced from arachidonic acid, a fatty acid predominantly stored in the phospholipid pool. Although the precise mechanisms of action of eicosanoids in cardiac tissue have not been elucidated, it is generally believed that these substances are synthetized in the endothelial cells of the myocardial vasculature and exert their biological action on other cell types in the heart [15]. The production of eicosanoids, such as prostacyclin and thromboxane A_2, is enhanced during ischemia and reperfusion of the heart. Whether these compounds have either beneficial or detrimental effects on the functional outcome of the heart after the ischemic insult is still a matter of debate.

Phosphatidylinositol, constituting about 5% of cell membrane lipids, is involved in transducing external signals to the cellular compartment across the sarcolemma [16]. By a delicate process involving phosphorylation and dephosphorylation of phosphatidylinositol substances are produced exerting specific biological activity inside the cell. Among others, modulation of cellular Ca^{2+} ho-

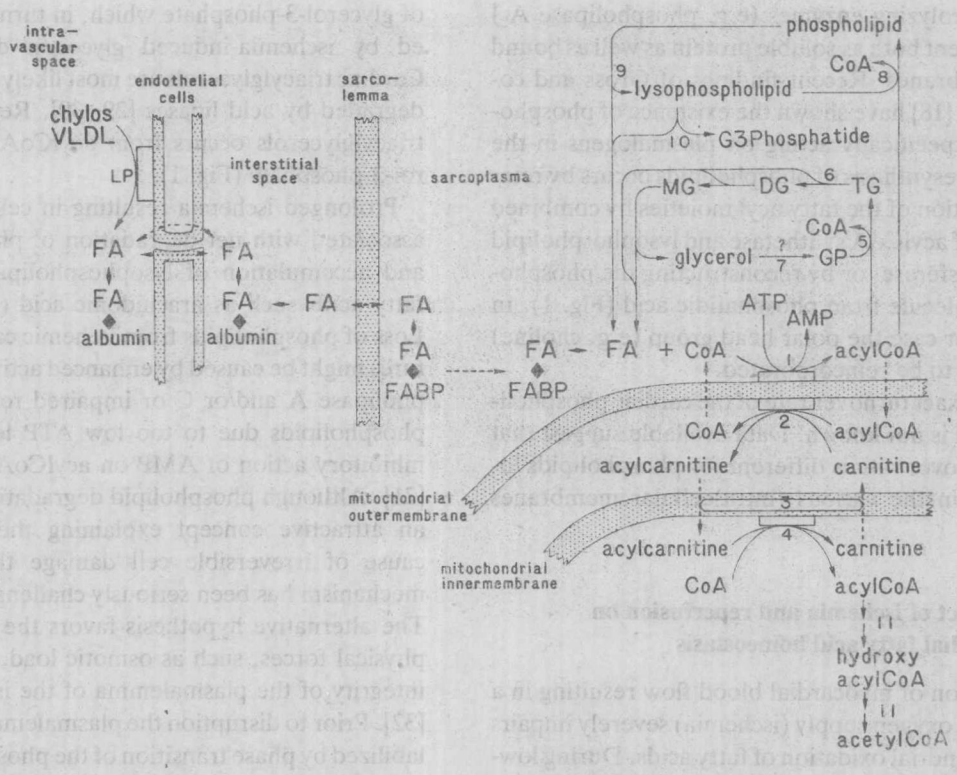

Fig. 1. Schematic representation of uptake and intracellular conversion of long-chain fatty acids (FA) in myocardial tissue. Chylos, chylomicrons; VLDL, very low density lipoproteins; LP, lipoprotein lipase; FABP, fatty acid-binding protein; CoA, Coenzyme A; GP, alfa-glycerolphosphate; TG, triacylglycerols; DG, diacylglycerols; MG, monoacyl-glycerols; 1, fatty acylCoA synthetase; 2, carnitine fatty acyltransferase I; 3, acylcarnitine-carnitine translocase; 4, carnitine fatty acyltransferase II; 5, glycerolphosphate acyltransferase + diacylglycerol-acyltransferase; 6, triacylglycerol lipase; 7, glycerol kinase; 8, lysophospholipid acyltransferase; 9, phospholipase; 10, lysophospholipase; 11, β-oxidation; (Modified from ref. 40, with permission).

meostasis has been described. $Alfa_1$-adrenergic stimulation of the heart is most likely mediated by the metabolic conversion of membrane phosphatidylinositol.

The phospholipid composition of cardiac membranes and the presence of specific phospholipid domains in these membranes determine their physiological function. For instance, cardiolipin is predominantly localized in the mitochondria. With respect to the plasmalemma, sphingomyelin and phosphatidylcholine are the main constituents of the outer leaflet whereas negatively charged phospholipids, such as phosphatidylethanolamine, are almost exclusively localized in the inner leaflet [17]. The asymmetric distribution of phospholipids influences the physico-chemical properties of the plasmalemma. Recent studies by Gross and associates [18] have shown that the majority of the plasmalemmal choline – and ethanolamine – phospholipids are present in the plasmalogen form, i.e. the hydrocarbon chain is connected to the first carbon position of glycerol via an ether linkage. The presence of plasmalogens also affects the physicochemical properties of the plasmalemma.

Membrane phospholipids are continuously subjected to a turnover process. Under steady state conditions degradation keeps pace with resynthesis. A variety of hydrolytic enzymes capable to degrade phospholipids has been identified in the heart. In this respect the activity of phospholipase A_1 and A_2, phospholipase C and lysophospholipase has been reported [19, 20]. Some of the phospholi-

pid hydrolyzing enzymes (e.g. phospholipase A_2) are present both as soluble protein as well as bound to membranes. Recent findings of Gross and coworkers [18] have shown the existence of phospholipases specifically acting on plasmalogens in the heart. Resynthesis of phospholipids occurs by reincorporation of the fatty acyl moieties by combined action of acylCoA synthetase and lysophospholipid acyltransferase, or by reconstructing the phospholipid molecule from phosphatidic acid (Fig. 1). In the latter case the polar head group (e.g. choline) has also to be reincorporated.

The exact turnover rate of the cardiac phospholipid pool is not known. Data available suggest that the turnover rate is different for phospholipids localized in the various (intra)cellular membranes [21].

The effect of ischemia and reperfusion on myocardial fatty acid homeostasis

Reduction of myocardial blood flow resulting in a reduced oxygen supply (ischemia) severely impairs mitochondrial oxidation of fatty acids. During low-flow ischemia with a continuous, albeit reduced supply of exogenous fatty acids, hydroxy fatty acids, acylcarnitine and acylCoA rapidly accumulate in the flow-deprived myocytes [22]. Most of the fatty acids extracted by the heart under low-flow ischemic circumstances is incorporated in the triacylglycerol pool or released in non-metabolized from from the heart into the vascular space [23]. The accumulation of (non-esterified) fatty acids is a relatively slow process. Only after 20 to 30 minutes of ischemia the tissue content of fatty acids significantly rises. A substantial proportion of the accumulated fatty acids is arachidonic acid, which in normoxic tissue is predominantly incorporated in the phospholipid pool [24–26]. This indicates that during ischemia cardiac phospholipid homeostasis is imbalanced.

The turnover of the cardiac triacylglycerol pool is accelerated during the initial period of ischemia [27, 28]. Consequently glycerol accumulates in the ischemic area and is released from the heart when residual blood flow is present. Enhanced triacylglycerol turnover is most likely caused by mass-action of glycerol-3-phosphate which, in turn, is generated by ischemia-induced glycogen degradation. Cardiac triacylglycerols are most likely lysosomally degraded by acid lipases [29, 30]. Resynthesis of triacylglycerols occurs from acylCoA and glycerol-3-phosphate (Fig. 1).

Prolonged ischemia resulting in cell necrosis is associated with net degradation of phospholipids and accumulation of lysophospholipids [31] and fatty acids such as arachidonic acid (see above). Loss of phospholipids from ischemic cardiac structures might be caused by enhanced activity of phospholipase A and/or C or impaired resynthesis of phospholipids due to too low ATP levels or the inhibitory action of AMP on acylCoA synthetase [31]. Although phospholipid degradation provides an attractive concept explaining the proximate cause of irreversible cell damage this putative mechanism has been seriously challenged [31, 32]. The alternative hypothesis favors the notion that physical forces, such as osmotic load, disrupt the integrity of the plasmalemma of the ischemic cell [32]. Prior to disruption the plasmalemma might be labilized by phase transition of the phospholipids in the lipid bilayer of the membrane [33]. If degradation of phospholipids occurs after the loss of cellular integrity, this hydrolytic process must be considered as part of the natural healing process starting with the digestion of cellular debris [31].

In order to rescue the ischemic myocytes from an inevitable death the supply of oxygen has to be installed in due time by restoration of flow. Following reperfusion myocardial fatty acid homeostasis does not immediately normalize. Conflicting results have been reported concerning post-ischemic oxidation of fatty acids. Rosamund and colleagues [23] observed depressed oxidation of fatty acids with a concomitantly enhanced utilization of glucose. In contrast, Huang and Liedtke [34] reported the restoration of fatty acid oxidation during the post-ischemic phase in the previously ischemic heart. In some experimental models a continuous or even increased degradation of phospholipids have been observed after restoration of flow [28]. Pharmacological manipulation of fatty acid oxidation during the ischemic episode has been found to result in changes in the functional outcome of the

reperfused heart. For instance, compounds inhibiting cardiac fatty acid oxidation appear to possess anti-ischemic properties [35]. In addition, L-carnitine and L-carnitine derivatives are able to mitigate the deleterious effect of ischemia in some animal models [36]. The mechanism of action of these compounds remains to be clarified.

Imaging of fatty acid metabolism in the normal and diseased heart

The specific alterations of fatty acid metabolism in the ischemic and reperfused heart have stimulated workers in the field of nuclear medicine to develop techniques to monitor metabolic changes in the diseased human heart with radio-labeled fatty acids.

In principle, two routes for non-invasively studying cardiac lipid metabolism for clinical diagnosis are available. First, with positron emission tomography (PET) the metabolic fate of positron emitting fatty acids, such as ^{11}C-palmitate or other relevant ^{11}C-labeled fatty acids, can be monitored [37, 38]. Second, planar gamma-scintigraphic devices and single photon emission computerized tomography (SPECT) are able to trace gamma-emitting radiolabeled fatty acids [39]. To this end, radiolabeled iodine is complexed to fatty acids or fatty acid derivatives. Synthesis of ^{123}-I fatty acids has been proven to provide applicable tracer molecules.

The advantage of the PET technique is the use of fatty acids undiscernible from the natural fatty acids present in the body and the high spatial and temporal resolution. In contrast, the use of iodinated fatty acids might increase to some extent the uncertainty in the interpretation of the data due to the metabolic fate of the labeled iodine molecule. The disadvantage of PET is the high costs of this sophisticated technique.

Although promising results have been reported using both techniques, conclusions based on imaging or radiolabeled fatty acids are in most cases not unambiguous. The complexity of cardiac lipid metabolism under normoxic and, in particular, ischemic conditions hampers a straight-forward interpretation. Alterations in uptake and back diffusion of the fatty acids, mitochondrial oxidation, and incorporation in the endogenous triacylglycerol and phospholipid pool have to be considered. This notion has prompted Bergmann and associates [37] to investigate the applicability of a relatively simple substrate, i.e. ^{11}C-labeled acetate, for measuring alterations in cardiac metabolism in the diseased heart. This tracer should permit delineation of the relationship between myocardial oxygen consumption, myocardial blood flow and function in patients with cardiac dysfunction of diverse etiologies and their response to therapeutic interventions. However, when acetate is used, characteristic changes in myocardial lipid metabolism will remain invisible so that an incomplete picture is obtained of the metabolic state of the heart under investigation.

Acknowledgements

The authors are greatly endebted to miss Lucienne de Boer in her help to prepare the manuscript.

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Molecular and Cellular Biochemistry 88: 7–15, 1989.

Invited Paper

The role of the endothelium in myocardial lipoprotein dynamics

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Accepted 28 December 1988

Key words: endothelium, lipoproteins, lipoprotein lipase, VLDL, LDL, HDL, cardiomyocytes

Summary

This overview is presented, in the main, to summarize the following areas of myocardial lipoprotein metabolism:

1. The nature and extent of the cardiac endothelium.
2. The interactions between the endothelium and chylomicrons, very low, low and high density lipoproteins in the presence and absence of lipoprotein lipase.
3. The importance of the endothelial lipoprotein lipase and the mechanisms involved in the enzymes' sequestration at that site.
4. The physiological role of lipoprotein lipase in the provision of oxidizable fuel for the heart.

Introduction

To isolate the role of the endothelium in myocardial lipoprotein dynamics, as the title of this paper suggests, is to recognize the central position this single layer of specialized cells holds in relation to controlling the access of various plasma-borne components to the working cardiomyocytes. In doing so certain basic aspects of the morphology and functional behaviour of the endothelium in general must be appreciated. Thus, as defined by Gimbrone in 1979 [1], it is clear that the endothelium is 'important in at least three basic areas; its acts as:
(1) a selective permeability barrier;
(2) a synthetic-metabolic-secretory tissue; and
(3) as a blood compatible 'container'.
Thus, far from being merely a passive cellular lining to the circulation, the endothelium represents a highly organized and active tissue that contributes to many aspects of homeostasis and tissue dynamics.

As more than just a semipermeable barrier between the blood and tissue cells, the endothelial cell system must be regarded as a highly active metabolic and endocrine organ in its own right [2]. Thus, on the one hand, the endothelium is responsible for the inactivation of vasoactive substances like 5-HT and bradykinin while on the other it is intimately involved in the formation of the vasoconstrictor, angiotensin II. Endothelial cells also produce locally active and unstable effectors of adjacent cell function such as prostacyclin and endothelial derived relaxation factor, both of which are potent inhibitors of platelet clumping.

The scale of these and the other interactions occurring at the endothelium can, primarily, be envisaged from a simple consideration of the size ie. area of the endothelial organ. The most often quoted figure for the total surface area of the human endothelium is 700 m^2, of which approximately 270 m^2 is available at rest [3]. The tissue distribution of this total area is of course uneven, depend-

ing on the degree of individual tissue vascularization. Highly vascularized tissues, such as cardiac muscle, having an estimated endothelial area equivalent to 400 $cm^2\ g^{-1}$ [4] or for the heart some 14 m^2 in total. On this basis endothelial cells make up 1–2% of the hearts total cellular mass and accounts for almost 1 kg of human body weight.

Although the endothelium is not a morphologically or functionally homogeneous organ, a few further general statements can be made. Thus, throughout the vascular tree the single layer endothelium is composed, with the exception of the high endothelium of the postcapillary venules in lymph nodes, of flattened and relatively uniform squamous cells separated from the underlying structures by a thin basement membrane [5]. In some tissues, of which adipose tissue is an example, close structural relationships exist between endothelial cells, pericytes, smooth muscle cells and the other parenchymal cells that are normally in close proximity. Thus, pericytic processes protrude into endothelial cells and endothelial invaginations penetrate deeply into pericytes [6]. In the heart however, the interaction between endothelial cells and myocytes is less intimate with no evidence of the intercellular membrane continuities that are found in adipose. If there was a 'typical' endothelial cell it would be from 3 μm thick in the area of the nucleus to between 0.1–1 μm thick at the periphery. The cell would be elongated, aligned with the direction of blood flow and usually between 25 and 50 μm long and 10–15 μm wide [7, 8]. The junctions that are formed between endothelial cells are of two basic types, the tight junction and the communicating junction (gap junction, nexus). The latter being important in all tissues as a low resistance pathway for intercellular communication [9]. Despite the extensive morphological information available on the regional variability in endothelia many questions in relation to the structural and functional correlations that might exist remain unanswered. What is perhaps more clearly defined is the nature of the interactions that occur between various plasma components and the luminal surface of the endothelial cell.

Plasma lipoprotein endothelium interactions

Although the passage of many plasma solutes into tissues is unimpeded by the presence of the endothelium and its underlying basement membrane, in the case of intact lipoprotein particles, like many other molecular species [10], there is restricted passage across the barrier. However, the component lipids of the lipoproteins represent a major proportion of the intermediates that are carried across the vascular surface and provide many of the lipid requirements of the endothelial cells themselves [11]. Indeed endothelial cell metabolism has long been known to be subject to control via the interaction of lipoproteins at the cell surface [12].

The plasma lipoproteins have been the subject of repeated review and the full details of their composition and metabolism will not be reiterated here. Readers are however referred to a number of excellent reviews of the area [eg13].

Interaction of chylomicrons with the endothelium

It is clear that a major functional event at the endothelium is the hydrolysis of exogenously-derived chylomicron triacylglycerol through the action of lipoprotein lipase (see later). However, despite the known functional relationship between the lipoprotein and enzyme at this site it is also clear that chylomicrons can bind in a specific and saturable fashion to the endothelium even in the absence of lipoprotein lipase. As a consequence of such binding, a proportion of the chylomicrons present can be interiorized by the endothelial cells without hydrolysis. The bulk transfer of chylomicron components by such a process being very much slower than when prior hydrolysis by lipoprotein lipase hydrolysis is involved. Thus, although endothelial cells grown *in vitro* do not express any lipoprotein lipase activity they exhibit approximately 2500 high-affinity chylomicron binding sites per cell [14] which show half maximal saturation at 0.1 mM chylomicron triacylglycerol. It can be deduced that, *in vivo*, approximately 1600 such sites exist with an apparent Km for triacylglycerol hydrolysis of 0.06–0.08 mM. By comparison it can be

seen therefore that the number and behaviour of endothelial chylomicron binding sites are similar despite the presence of lipoprotein lipase *in vivo* and its absence *in vitro*. This would of course suggest further that the major factors leading to the sequestration of chylomicrons at the endothelial cell surface must rely on some constitutive endothelial cell surface component and not be the enzyme which nevertheless is the agent whereby the triacylglycerol of the bound complexes are hydrolysed and made available for tissue uptake.

When the known number of endothelial chylomicron binding sites are fully saturated by lipoprotein particles some 5% of the cell surface is occupied. This, in turn, means that, in the presence of lipoprotein lipase *in vivo* and with the much larger number of lipoprotein lipase molecules present far outweighing the number of bound chylomicrons, at any instant some 5–10% of the enzyme molecules are involved in the hydrolysis of the particles present. With such a distribution of chylomicrons, their likely rate of hydrolysis derived from the known turnover rate of the enzyme, the number of enzyme molecules acting on each chylomicron simultaneously and the estimated area of the cardiac endothelium, it is possible to calculate that the potential rate of fatty acid provision from the action of lipoprotein lipase is entirely consistent with the known rates of chylomicron triacylglycerol fatty acid uptake by the heart.

Although triacylglycerol fatty acids from chylomicron hydrolysis can constitute a major substrate for cardiac metabolism they are not the only lipid species obtained from this source by the heart [15]. For example, cholesteryl ester, derived from chylomicra, are also taken up by the heart in a process which is augmented by the presence of active endothelial lipoprotein lipase. Thus, compared with cultured endothelial cells in the absence of lipoprotein lipase, where respectively 6.7 and 0.3 μg/million cells, of triacylglycerol and cholesteryl ester are taken up per day, in the intact heart, in the presence of the enzyme, such uptake may reach 6400 μg of triacylglycerol/10^6 endothelial cells per day and 10.4 μg of cholesteryl ester/10^6 endothelial cells per day. Such a rate of chylomicron-derived cholesterol uptake is able to control endothelial cell cholesterol synthesis effectively and the chylomicron binding sites through which this uptake occurs are not down-regulable. Thus, the chylomicron binding site on endothelial cells must play a major role in the maintenance of the cell's cholesterol balance in addition to any role it may play as an endothelial-surface sequestering site facilitating the lipoprotein lipase catalyzed hydrolysis of chylomicron triacylglycerol [15].

The interaction of very low density lipoproteins with the endothelium

By contrast with chylomicrons, very low density lipoprotein particles carry triacylglycerol of endogenous (hepatic) origin. This source of triacylglycerol, which may account for up to 65 g per day in the human, also makes a quantitatively important contribution to overall energy balance. In the rat, for example, very low density lipoprotein triacylglycerol contributes oxidizable fuel equivalent to 15% of the total oxygen consumption of the body at rest [16]. The rate of direct VLDL triacylglycerol oxidation being between 4 and 5 μmol of fatty acid/kg/min, a value very similar to that seen for albumin bound free fatty acids under identical circumstances.

VLDL are competitive substrates for lipoprotein lipase action at the vascular surface and thus it may be suggested that the chylomicron binding site and the endothelial surface binding site for VLDL could be closely related or identical. Both interactions being, for example, time and temperature dependant, saturable and reversible. Additionally, kinetic experiments indicate that the two lipoproteins can compete for the same binding sites on cultured endothelial cells where lipoprotein lipase is absent. A tenfold excess of VLDL displacing approximately 80% of bound chylomicrons from the cell surface. The binding of VLDL to the endothelium has the properties expected of a high affinity cell surface receptor [17, 18], with half maximal binding being achieved at VLDL protein concentrations of between 5 and 10 μg/ml. The rate of VLDL internalization by endothelial cells is however some 4–5 time faster than is the case for chylomicrons.

The chylomicron-VLDL binding site is distinct from the binding sites for other lipoprotein species that exist on endothelial cells (see later) since:

(a) unlike the other binding phenomena that are observed it is not down-regulable,
(b) Low density lipoprotein (LDL) does not compete for binding or displace bound triacylglycerol-rich lipoproteins and
(c) although interiorization occurs, this is not related to lipoprotein protein breakdown.

In the presence of lipoprotein lipase most of the chylomicrons and VLDL particles sequestered at the endothelial surface are acted upon by the enzyme (see later) to produce so called remnants and intermediate density lipoproteins respectively. There is now reason to believe that although such partly degraded particles will be removed from the plasma effectively by the hepatic remnant receptor they can also bind to the endothelium via a distinct type of binding site which is more often identified with its capacity to act as a high affinity LDL receptor site (see following section).

The interaction of low density lipoproteins with the endothelium

Although the incubation of endothelial cells with lipoprotein deficient serum does not affect the VLDL binding subsequently exhibited by them, the high affinity binding sites for LDL mentioned above are upregulated under the same conditions [19]. When cells, cultured under these conditions, are exposed to LDL it is bound and interiorized via these receptors and the LDL cholesterol that is taken up replenishes the intracellular levels of the sterol to normal levels and, simultaneously, modulates the intracellular pathway of cholesterol biosynthesis [20]. As intracellular cholesterol levels normalize the LDL receptors are down regulated and the overall rate of LDL degradation by the cells declines concomitantly. With endothelial cells cultured under sterol depleting conditions half maximal binding of LDL through the specific LDL receptor is achieved at 10–25 μg LDL protein/ml. Thus at serum concentration of approximately 800 μg/ml these sites will be saturated. However, the numbers of high affinity LDL receptors on endothelial cells is considerably lower than are detected on other cell types. For example, in the up regulated state, the endothelial cell will express some 40,000–85,000 sites per cell compared with 250,000 and 200,000 per cell for skin fibroblasts and smooth muscle cells respectively [15].

In addition to the high affinity binding sites for LDL, endothelial cells like many other cell types also exhibit a high capacity, low affinity, binding and internalization pathway which, at physiological concentrations of LDL, and with upregulated cells can account for a significant proportion of the total cholesterol uptake by the cells [11].

Interaction of high density lipoproteins with the endothelium

As implied above endothelial cells in culture can take up plasma cholesterol via the well-regulated and extensively discussed, LDL-receptor pathway. However, these receptors are progressively suppressed as cells organize and reach confluence [21]. Furthermore, from the forgoing, it may be deduced that endothelial cells may acquire cholesterol from none-LDL sources such as chylomicrons, by a mechanism relatively independent of apoprotein uptake and degradation [22]. This uptake and delivery of cholesteryl esters from chylomicrons, or indeed from artificial lipoprotein-like liposomes, being enhanced by the presence of lipoprotein lipase at the endothelial cell surface [22, 23].

Additionally, endothelial cells can interact with high density lipoproteins (HDL) through a high affinity binding site [24, 25] that is specific and distinct from the rather weak competition that HDL shows for LDL at the LDL receptor. However, although the interaction may be considered as part of the mechanism whereby HDL components are taken up by, for example the parenchymal cells of the liver [26], in the specific case of the receptor-mediated binding of the HDL_3 subfraction, lipoprotein cell interaction leads to the controlled removal of cholesterol from cells. This being achieved via a mechanism that involves promotion of the transport of intracellular cholesterol to