Wellcome Film Project

Atherosclerosis: Lipoproteins; Structure and Function

Uptodate

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Black-and-white
Duration: 00:27:35:12

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<Opening titles>

<Opening film of commuters walking along crowded London streets, including men wearing bowler hats, interspersed with scenes of traffic. Brief close-up scenes in sequence of exhaust fumes; mouth smoking cigarette; sausages, egg and bacon frying in pan; callipers measuring thickness of skin fat; blood pressure gauge, rotating molecular diagram.>

<Galton to camera>

In this programme, we're going to discuss the structure and metabolism of lipoproteins. I'm going to start with the role of lipoproteins in fuel metabolism and then <*camera moves to Chait*> Dr Alan Chait will describe the structure and composition of each or the lipoprotein particles that circulate in the bloodstream. <*Camera returns to Galton*> We're then going to take each of the lipoprotein particles in turn and discuss its function for fat transport in the blood.

Well to begin with: the body to maintain its normal activities of muscle contraction, heart beating, maintenance of ionic gradients, requires, of course, a continuous supply of energy to maintain these activities. And the body has evolved a double-fuel supply to meet these needs. There are basically two components to the fuel supply to the body.

<Galton narrates over series of diagrams, using indicator stick, interspersed with talk to camera>

One is a carbohydrate system which is shown on the first chart here, <*diagram*> and the main storage form is glycogen. Glycogen consists of a polymer of glucose residues joined together to form a spiral and branched chain structure. The other fuel store in the body is triglycerides, lipids. This consists of three fatty acids, shown here, here and here, esterified on a glycerol backbone. Now although lipids are more advantageous as a fuel supply than carbohydrates, they suffer from one disadvantage and that is their insolubility. To illustrate this, the part of this molecule that is used for oxidation is the fatty acid component here, and this has to be transported from the depot site to the site for oxidation. And fatty acids are insoluble structures, and because of this they have to be transported on a series of lipoproteins.

<*Galton to camera*> The situation is, of course, different with glycogen, where the storage form consists of this glucose polymer < *previous diagram*> and then for transport, one of these residues gets cleaved from the glucose polymer and secreted into the bloodstream where it is easily soluble; it circulates in the bloodstream to peripheral tissues, such as muscle and heart, where it can be used directly for oxidation purposes.

<*Galton to camera*> Well, now, to transfer fatty acids is a more difficult problem. And the next slide shows how the body does this. <*Next diagram*> The problem is to get fatty acids from the storage site, in adipose tissue and muscle and liver, to transport them in the bloodstream to peripheral tissues for oxidation. Now, as we've said, fatty acids are insoluble and therefore special systems have evolved of lipoprotein

particles whose function is to transport fatty acids from their site of synthesis and storage to peripheral tissues. <*Galton to camera*> And a whole family of lipoproteins have evolved for this. And they're shown on the next chart, please.

<*Next diagram*> Now the important points to note about this chart are that the lipoprotein particles form a family of sizes ranging from 100 angstrom up to 10,000 angstrom. Now the larger particles, represented here, are mainly responsible for carrying triglycerides from tissues such as the gut and the liver to peripheral tissues. There is a smaller range of particles, between about 500 to 700 angstrom, which again are involved in carrying triglycerides from tissues such as the liver to peripheral tissues. And then there are smaller particles further still, particles of around 200 angstrom which are probably breakdown products of these other larger particles. Breakdown products in the sense that the triglyceride particles have been removed <*to camera*> from these particles, so that they produce a smaller one for circulation. <*Back to diagram*> They've now fulfilled their role as a carrier of triglycerides from storage and synthetic depots to peripheral tissues for utilisation.

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<*To camera***>** So, what we want to do now is to consider the structure and composition of each of these particles in turn. And I think Dr Chait now is going to deal with these in more detail.

<Chait to camera>

Well, as their name implies, lipoproteins consist of both lipid and of protein.

<Chait narrates over series of slides, using indicator stick, interspersed with talk to camera>

The lipoproteins are cholesterol and its esters, triglyceride and phospholipid. These three occur in all the lipoproteins but occur in different proportions in the different

lipoprotein classes. They're also all bound to protein. The protein component is called apolipoprotein, or sometimes simply referred to as apoprotein.

<*To camera, holding 3-dimensional model of molecule*> How then do the proteins combine to form a lipoprotein molecule? Well, the exact structure is not known with certainty, but we do know that in essence the lipid portion, the hydrophobic or the portion which has a low affinity for water, is on the inside of the molecule, whereas the hydrophilic portion, or the portion for good affinity for water, is on the outside.

<*Prising model apart to show illustrated centre*> Now the phospholipids are lipids with both hydrophobic and hydrophilic portions, and they are called antipathic. This model over here shows one way in which the lipids may combine to form the lipoprotein molecule. The hydrophilic or polar heads of the phospholipid will be on the outside, facing outwards towards water, whereas the hydrophobic, or water-repelling portions of the phospholipid, will be pointing inwards. This model fails to show the cholesterol esters and the triglycerides, but they would be in this inside portion sheltered from water. Surrounding it, one has a coat of hydrophilic substance which will be largely protein and the polar heads of the phospholipids.

Another model is as follows <*showing illustrated centre of another model*>. In this model, we again just show the phospholipid which is seen here as a lipid bilayer with polar heads facing both outwards and inwards, and in this model, it is really a spherical representation of many currently accepted models of membrane structure. There's also uncertainty to the exact location of the protein. One possibility is that it may be combined by hydrophilic interactions on the outside of the molecule. Another possibility is that it may be associated by hydrophobic interaction, in which case it appears as such, almost like icebergs floating in a sea of lipid.

<*To camera*> Before considering the various lipoprotein classes, I would like to briefly mention something about the apoproteins. Could I have next card please? This card will just show you some of the <*next chart*> currently used nomenclature for apoproteins, in which we have three main groups: one has apo A, apo B and apo C – apolipoproteins. Apo A is divided into two non-identical peptides called A-I and

A-II. Both of these have glutamine as their common carboxy-terminal amino acid. Apo B has serine as its carboxy-terminal acid.

There are three components of the apo Cs. They are called apo C-I, II and III, and have serine, glutamic acid and alanine as their carboxy-terminal amino acids respectively.

<*To camera*> Let us now consider the individual lipoprotein classes, bearing in mind that within each class, cholesterol, triglyceride and phospholipids bear a relatively constant relationship to each other, and also to protein. However, between the different lipoprotein classes, the relationship of these lipids to each other is sufficiently different to allow this separation by physicochemical means.

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<*Next diagram*> The four classes are named according to separation by electrophoresis or on their density class by ultracentrifugation. Fortunately, the nomenclature derived from these two operational procedures < *to camera*> are more or less interchangeable. <*Back to diagram*> Let us first consider chylomicrons. Under normal circumstances, they're present in plasma only after the ingestion of a fat containing meal. They are the largest and least dense of the lipoprotein molecules, and remain at the origin on electrophoresis. They consist largely of triglyceride – about 90% by weight are accounted for by this lipid. There is a very small protein component.

Whereas chylomicrons carry exogenous triglyceride, endogenous triglyceride is transported mainly as pre-β-lipoprotein. <*Next diagram of pre-β-lipoprotein*> This has α-2 mobility on electrophoresis and is also known as very low density lipoprotein, abbreviated as VLDL. Once again, you will see that triglyceride is the major lipid, accounting for about 60% of the molecule by weight. Now I would like to emphasise that both chylomicrons and pre-β-lipoproteins are rather heterogeneous substances and there can be quite a lot of variation in the composition.

<*Next diagram of β-lipoprotein*> β-lipoprotein is the chief cholesterol-bearing lipoprotein in plasma. 25% of this molecule is protein, 75% lipid – most of which is cholesterol. It has β mobility on electrophoresis and is also known as low density lipoprotein or LDL.

<*Next diagram of α-lipoprotein*> α-lipoprotein, the smallest and most dense lipoprotein has α-1 electrophoretic mobility, and consists of about half of protein and half of lipid, which is largely cholesterol and phospholipid. This is also known as high density lipoprotein or HDL.

Apo B is the chief lipoprotein of β-lipoprotein. Apo B, however, also occurs as a major apoprotein of pre-β-lipoprotein. This lipoprotein <*indicating pre-β-lipoprotein*> also contains significant amounts of apo A-I and Apo-A-II, which are the chief lipoproteins of α-lipoprotein. The apo C apoproteins occur in both α- and in β-apoproteins.

<*To camera*> It may well be that in future we will consider lipoprotein classification in chemical terms, thinking in terms of apoprotein composition. However, to date we still rely on separation by electrophoresis and ultracentrifugation. And because of this, I think we should actually see how these things are done in practice.

<Galton narration over film of electrophoresis and ultracentrifugation procedures>

The cellulose acetate strip is placed on the electrodes and electrophoretic tank and the electrodes are placed in position. A coverslip is placed over the strip, and the sample to be electrophoresed is placed on a glass slide to be taken up with the applicator. This is being done now. Sample going on to applicator. And then the serum sample is placed on the cellulose acetate strip. Several samples can be run at the same time on this strip, but we're just showing one for the sake of example.

The tank is covered now and the power switched on to apply the electric voltage between the electrodes. The voltage being applied across the electrodes can be

seen on this voltmeter, and the system is run for between 20 to 30 minutes to allow adequate separation of the lipoprotein classes on the cellulose acetate strip.

At the end of the run, the power is switched off and the strip is removed from the electrophoretic tank, and then placed in the staining solution. It's left in the lipid stain for about 20 to 30 minutes. And rather than wait this time, we already have a strip in the tank that has been stained and we'll take this out and show you what the appearances are like. The strip is placed in a washing solution and the lipoprotein classes are now stained up and are visualised as bands on the strip. And, for example, on the right-hand track, you can see two clearly demarcated lipoprotein classes that have separated and stained.

The other major technique for separating lipoproteins is by ultracentrifugation, and here you see a zonal rotor in place in a centrifugal well at the end of a 24 hour run. The lipoproteins are being pumped out through those tubes to the fraction collector, and whilst that is being done, they are passed through a spectrophotometer for a record of the proteins that are appearing. And these are recorded on a strip chart to give us an idea of the types of lipoproteins that have emerged, and it's also quantitative from measurements of the peak heights. So this allows separation and measurement of the different lipoprotein species in blood.

Here you see assimilation of how the lipoprotein bands may appear after centrifugation. They separate as bands which are distinct from each other and can be collected from the tube for measurement and assay.

<Galton to camera>

Now let's examine each lipoprotein particle in turn to see how it functions as a fat transporting system within the body. And to help us do this, we've set up an organ framework to view this on.

<Galton narrates over diagram>

Now here you see various tissues: the intestine, the liver and two tissues that utilise triglyceride fatty acids, namely muscle and adipose tissue. And let's start with the first particle, chylomicron. The chylomicra are synthesised by the gut from dietary fatty acids. Dietary fat is first of all digested, hydrolysed and the fatty acids enter the intestinal wall, where they're converted back to triglycerides and then associated with apoprotein to form chylomicra. The chylomicra are secreted into the lymph where they circulate to various tissues, triglycerides and muscle. So, here we can put in the chylomicra secreted by the gut, mainly in carrying triglycerides – 90% of the particle is composed of triglycerides – and they circulate to adipose tissue and to muscle, where they are metabolised. So that is one form of particle transporting triglyceride, fatty acid from intestine to peripheral tissue. Now another particle is, of course, the pre-β-lipoprotein. Alan.

<Chait to camera and then narrating over a diagram, interspersed with talk to camera again>

Right well, as I mentioned earlier, the chief function of pre-β-lipoprotein is the transport of endogenous triglyceride. This occurs mainly in the liver, < *diagram*> where the source of the triglyceride is free fatty acid. The actual source of the free fatty acid depends to a large extent on the dietary state. For instance, in the fasted state <*to camera*> free fatty acid derives almost entirely from adipose tissue stores where they are released by lipolysis of stored triglyceride. On the other hand, during the fed state, the fatty acid derives both from dietary fat and also from fats synthesised in situ in the liver. At the smooth endoplasmic reticulum, the free fatty acids combine with α-glycerone phosphate to form triglyceride <*back to diagram*>. Apoprotein is synthesised in the rough endoplasmic reticulum, and the triglyceride and apoprotein come together, together with cholesterol and phospholipid to form the pre-β-lipoprotein molecule. This is then secreted from the liver into plasma, and is then taken up by adipose tissue and by muscle.

It is now also realised that during the fasting state, not only does pre-β-lipoprotein enter plasma from the liver, <*to camera*> but the gut also plays some part in

lipoprotein secretion. <back to diagram> So, in addition, we have pre-β-lipoprotein synthesised in the intestinal wall reaching the circulation.

We've also heard so far that chylomicrons transport dietary fat, pre-β-lipoproteins transport endogenous fat. This is not the entire picture, because it is now also appreciated <*to camera*> that during fat absorption, not only do chylomicrons appear in blood, but one also gets the appearance of pre-β-lipoproteins as well. David, will you now say a few words about the removal of these various lipoproteins from the plasma.

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<Galton to camera>

Yes, well, the lipoproteins having been secreted by the gut and liver reach peripheral tissues and there they're broken down by a set of enzymes which hydrolyse triglyceride fatty acids to free fatty acids

<Galton narrates over diagram, using indicator stick>

The free fatty acids transfer across the endothelial lining and enter the extravascular space where they are then taken up by peripheral tissue such as adipose tissue or muscle for utilisation. Now the situation is more complex than this because there's certainly more than just one lipoprotein lipase on the endothelial lining, there are several including tri-, di- and monoglyceride lipases that are responsible for the hydrolysis of triglyceride carrier lipoproteins. In addition, there are probably other enzymes which aid in the degradation of lipoproteins in the periphery. And one such enzyme that we've listed here is lecithin-cholesterol acyltransferase, LCAT for short. And this enzyme is responsible for the esterification of cholesterol in the bloodstream of lipoproteins using lecithin as a substrate.

<*To camera*> And this reaction is shown in more detail in the next chart because it is a somewhat newer reaction, <*next diagram*> and shows lecithin-cholesterol

acyltransferase catalysing the transfer of a fatty acid from lecithin to cholesterol, thereby producing cholesterol ester and lysolecithin as a product. And although this enzyme <*indicating shows lecithin-cholesterol acyltransferase*> is not strictly speaking a degradative enzyme, it is probably involved in the tailoring and modelling of lipoproteins during their breakdown and hydrolysis in the periphery to produce free fatty acids for uptake by other tissues.

<*To camera*> So that now leaves us with the other two lipoproteins to consider, the β- and α-lipoproteins and, Alan, I think you were going to say about that.

<Chait to camera>

Well one of the major advances in the understanding of lipoprotein metabolism over the last few years has been the realisation that β-lipoprotein is, in fact, the end product of pre-β-lipoprotein catabolism. You've just heard from David […]

<Chait narrates over diagram>

[…] that during the hydrolysis of triglyceride from pre-β-lipoprotein, lipid is lost. In addition, one gets a loss of the A and the C peptides, leaving the residual lipid and B peptides which transfer through an intermediate density lipoprotein to β-lipoprotein. Experiments using iodine-labelled protein have shown, without doubt, that this transfer from pre-β to β via intermediate density lipoprotein is uni-directional. The reverse transfer of peptides does not occur, and at the same time as the β moves in that direction, the apo A and apo C seem to be lost in the direction of α-lipoprotein. This transfer is not uni-directional and at a later stage you can get transfer back of your apo A and apo Cs to pre-β-lipoprotein.

<*To camera*> It would thus appear that your α-lipoprotein may be a mobile reservoir of these peptides, apo A and apo Cs which are important in the degradation of your pre-β-lipoproteins. Now, I think I should say a few words on the function of the apoproteins. To date, we don't understand them fully, but gradually things are being learnt about them. Firstly, I'd like to say a word or two about the C-II peptide <*next*

diagram>. This peptide is an activator of the enzyme lipoprotein lipase, and hence facilitates the catabolism of pre-β-lipoprotein.

<*To camera*> It is also believed that some of the apoproteins are activators of the other enzyme which you recently heard about, LCAT. To date, it is not quite sure which proteins are involved. Some authorities believe it to be the apo A-I peptide, others believe it to be a new peptide which I have not mentioned thus far called apolipoprotein D.

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Apo B is another important peptide which seems to be necessary for the secretion of both pre-β-lipoprotein and chylomicrons. We know this from the rare disease where you have a hereditary deficiency of this B peptide. In this condition, not only is β-lipoprotein absent from plasma but one also gets a total lack of chylomicrons and pre-β-lipoprotein as well.

Some of the other apoproteins may actually have a structural rather than a direct functional role, and I'm sure we'll be hearing a lot more about them in the years to come. David, I think that at this stage you'd like to perhaps summarise what we've said about the interconversions and the dynamic properties.

<Galton to camera>

Well, it seems therefore that the lipoprotein system is a very complex one for the transport of synthesised triglycerides from the liver and the gut, primarily, to peripheral tissues such as muscle and adipose tissue […]

<Galton narrates over diagram>

[…] and it's summarised on the board here. And this, I think, illustrates quite suitably the complexity of the system. Chylomicrons are synthesised and secreted by the gut into the circulation, carrying triglycerides, primarily from the intestine to peripheral

tissues – adipose tissue and muscle. Pre-β-lipoproteins are synthesising triglyceride, secreted as pre-β-lipoproteins into the circulation, again where they are taken up by the periphery muscle and adipose tissue, broken down into fatty acids which enter muscle for utilisation, leaving a β-lipoprotein which circulates. In addition, there is another particle, the α-lipoprotein, whose function we're not clear about yet but may well act as a reservoir of apoprotein.

< Galton to camera>

So this is a complex dynamic system responsible for the movement of triglyceride fatty acids between the various tissues of the body. Thank you.

<End credits>