

Mathematics in Medicine Study Group

September 2005

Hepatic Lipoprotein Metabolism

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The major fate of circulating low density lipoprotein (LDL) is LDL receptor (LDLR)-mediated uptake by the liver (Figure 1). LDLRs are situated in coated pits, which are specialised structures within the plasma membrane. LDLRs mediate the cell surface binding and endocytosis (uptake into the cell) of plasma lipoproteins containing either apolipoprotein (apo)B or apoE. LDL contains apoB which acts as a ligand to mediate the binding of LDL to the LDLR. Upon binding to the receptor, LDL is internalized and incorporated into intracellular vesicles, in which dissociation of LDL from the LDLR occurs followed by degradation of its components into their constituent parts (amino acids, fatty acids and cholesterol). The empty receptors are recycled to the cell surface. The number of LDLRs at the cell surface is regulated by the intracellular cholesterol content, such that it is increased in response to a drop in intracellular cholesterol levels and decreased in response to a rise in cholesterol.

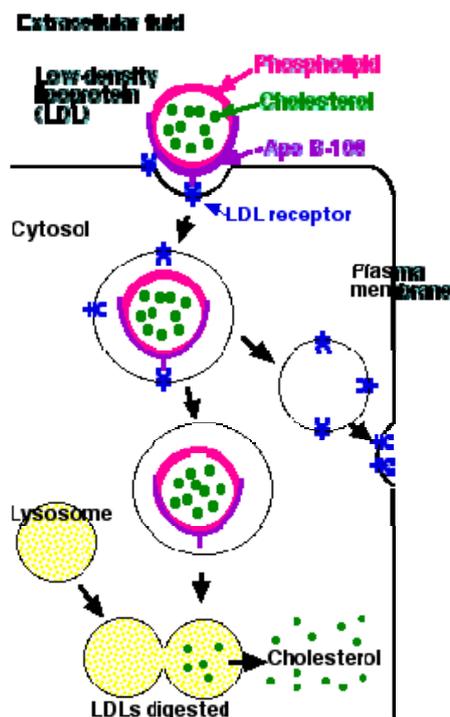


Figure 1 Hepatic endocytosis of LDL and LDLR recycling.

ApoE containing triglyceride-rich lipoproteins (TRL) can also bind to the LDLR, using apoE as a ligand for the receptor. LDL uptake by liver cells *in vitro* has been shown to be reduced in the presence of TRL in a dose dependent manner (Figure 2). Changes in the expression of genes encoding proteins involved in cholesterol metabolism indicate that there is a drop in cholesterol levels in cells incubated with TRL and LDL, compared to those incubated with LDL alone. Two alternate hypotheses, outlined in figure 3, have been put forward to explain this observation:

Hypothesis 1

TRL bind to the cell surface proteoglycans via apoE. TRL are not taken up into the cell, but block receptor sites leading to a decrease in LDL uptake, resulting in a drop in intracellular cholesterol levels.

Hypothesis 2

TRL bind directly to LDLRs via apoE. TRL are taken up into the cell in replacement for LDL. Due to their multiple apoE molecules and larger size, one TRL particle blocks the entry of several LDL particles, resulting in a net decrease in the amount of cholesterol entering the cell.

Question 1

From in-vitro data on LDL uptake in the absence and presence of TRL, can we distinguish between the two possible mechanisms of TRL inhibition of LDL uptake outlined above? Could a mixture of the two mechanisms explain the observations?

TRL containing fatty acids of different compositions show different abilities to inhibit LDL uptake in this in-vitro system. TRL containing saturated fatty acids (TRL-SAFA) more effectively compete with LDL than TRL containing mono- or polyunsaturated fatty acids (TRL-MUFA and TRL-PUFA respectively). This effect is independent of particle number (TRL contain one apoB molecule per particle, therefore apoB concentration can be used as a measure of particle number).

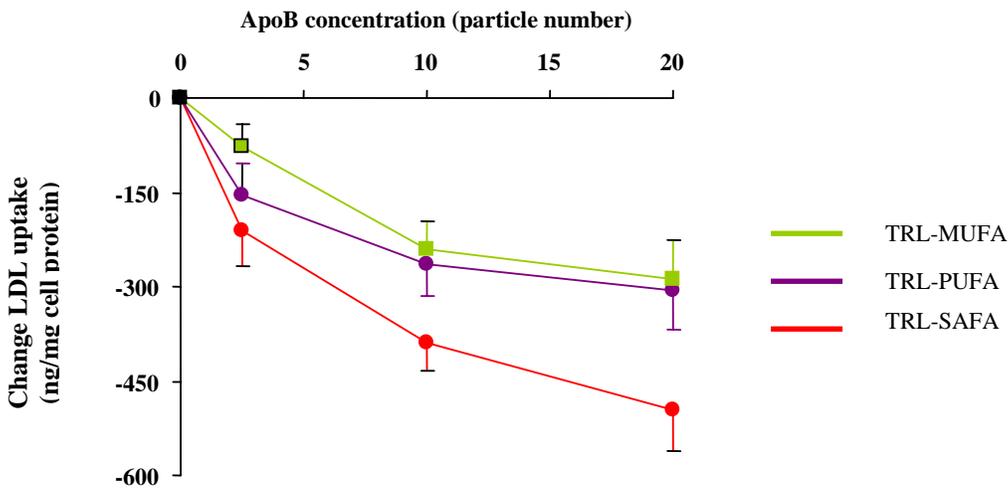


Figure 2. Change in LDL uptake with increasing numbers of TRL-MUFA, PUFA or SAFA particles.

TRL-SAFA particles are larger than TRL-PUFA and TRL-MUFA and they contain an average of 3 molecules of apoE per particle compared with an average of 2 molecules on TRL-PUFA and TRL-MUFA. Upon standardisation of the number of apoE molecules per experiment, the differential competition is lost and all 3 TRL types exhibit similar inhibition of LDL uptake (figure 4B), indicating a key role for apoE concentration in TRL binding.

Question 2

Assuming TRL inhibit LDL uptake by a combination of the two mechanisms described in Figure 3, can we work out differences in the relative contribution of each of the two mechanisms to the decrease in cholesterol observed in the presence of TRL-SAFA, TRL-MUFA or TRL-PUFA?

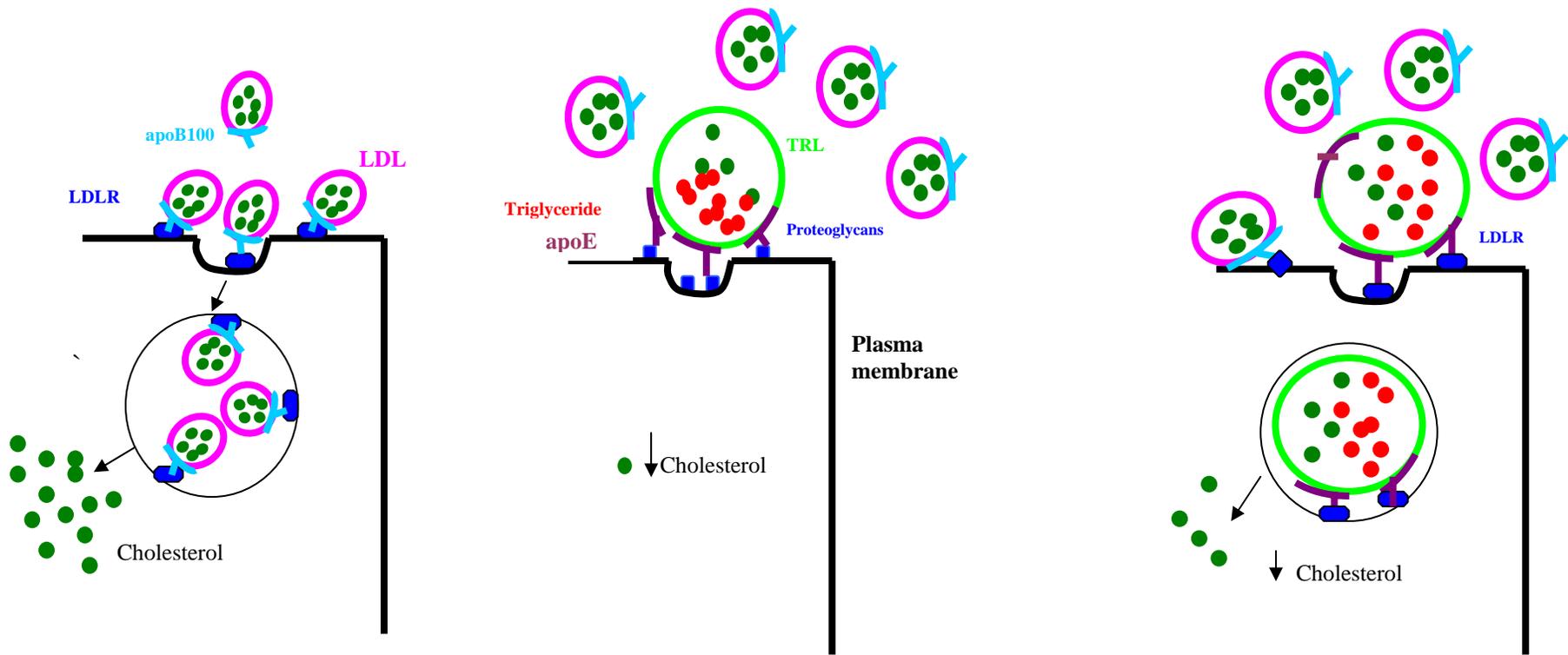


Figure 3. Possible mechanisms by which TRL may influence LDL uptake.

Control

In the absence of TRL, cholesterol-rich LDL particles bind to the LDL receptor on cells via apoB100. Cellular uptake of receptor bound LDL and subsequent degradation to its constituent parts results in an increase in the flux of cholesterol into the cell.

In response to this genes involved in both cholesterol synthesis and uptake of plasma cholesterol (contained within lipoproteins) are down-regulated.

Hypothesis 1

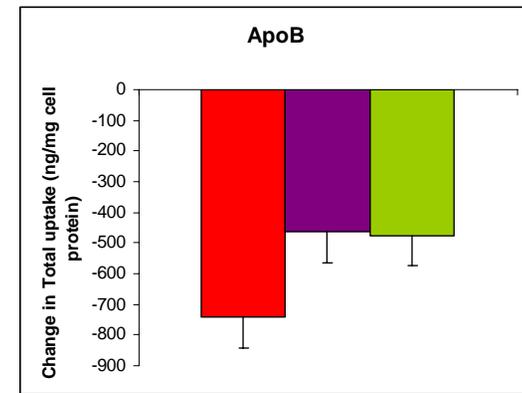
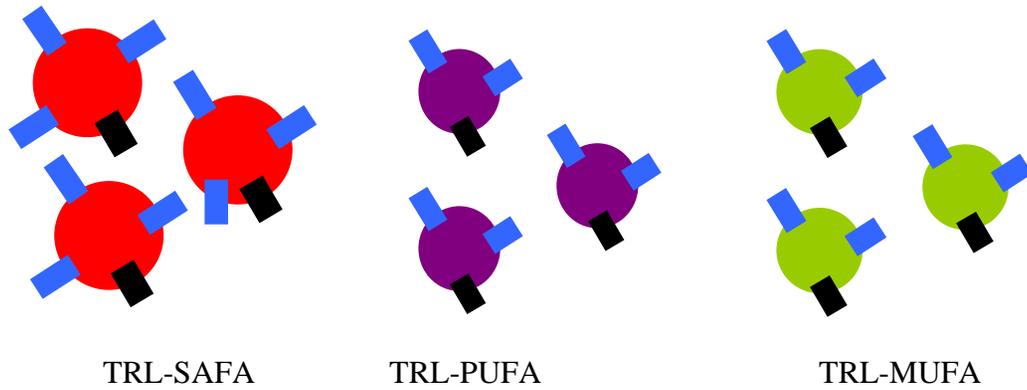
TRL bind to proteoglycans via apoE preventing LDL from accessing the LDLR. TRL are not internalized but simply act as a barrier preventing LDL uptake. Intracellular cholesterol levels fall as a result and genes involved in increasing cholesterol synthesis and uptake are up-regulated.

Hypothesis 2

TRL bind via a single or multiple apoE ligands to the LDLR. Bound TRL enter the cell by endocytosis. Competition for LDLR binding by the cholesterol-poor TRL results in a decrease in cholesterol-rich LDL uptake. Intracellular cholesterol levels therefore drop in comparison to cells incubated with LDL alone and genes involved in increasing cholesterol synthesis and uptake are up-regulated.

N.B. Not to scale. Clathrin pits are large and can accommodate many lipoproteins simultaneously.

A)



B)

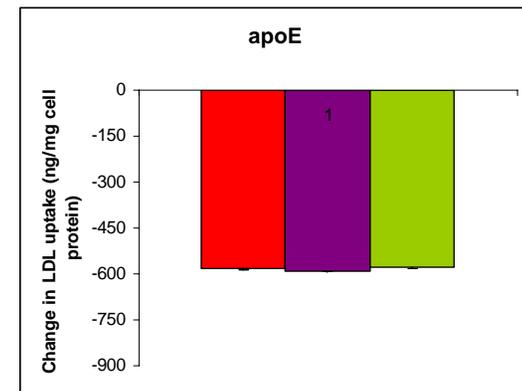
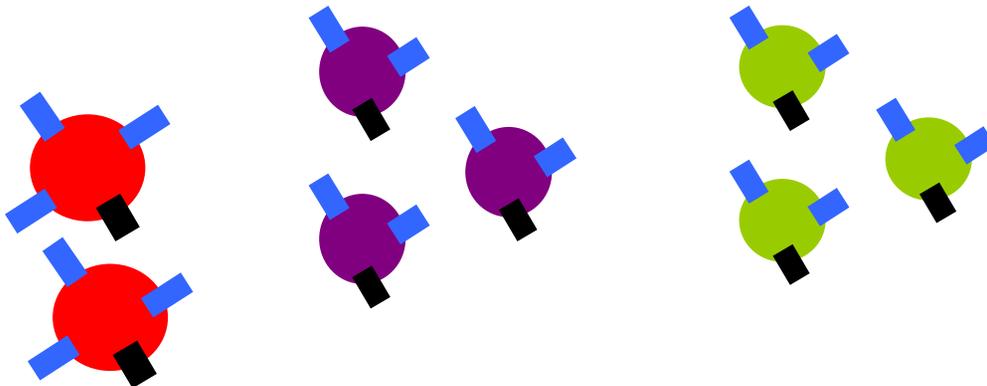


Figure 4. ApoE concentration, but not lipoprotein particle number, determines the ability of TRL to inhibit LDL uptake.

A) TRL-SAFA, TRL-MUFA and TRL-PUFA exhibit differential rates of inhibition of LDL uptake when standardised for particle number (apoB concentration).

B) Differential inhibition of LDL uptake by TRL-SAFA, TRL-MUFA and TRL-PUFA is lost when standardized for apoE concentration.