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I attended this meeting along with several other South African colleagues. I was fortunate to receive unconditional support from AstraZeneca, ensuring that I remain in touch with current developments. Coming from a lipid clinic and laboratory, my interests are largely in severe dyslipidaemias, their therapy and insights into their pathophysiology though I do keep an eye on general developments. Hopefully this report will effectively transmit new information to all interested parties. I certainly have enjoyed the stimulation of this meeting. Hopefully future updates can be undertaken as a service to LASSA members as well as the general membership of SA Heart.

The plenary sessions not only provide up to date information whilst placing a field of pursuit in perspective, but also indicate where current research energy is focussed and is likely to have impact. Special educational symposia are usually placed just ahead of the meeting to bring delegates up to date on clinical issues, especially new pharmacologic management, mostly supported by the pharmaceutical industry in an indirect and unconditional way. Sometimes such meetings are held late in the day during the symposium schedule. Satellite meetings are held around these symposia and they provide opportunities for sharing the most recent data and provide important links for collaboration. The South African research has fallen far behind and there is thus little chance of any of us joining these meetings by virtue of cutting edge research and information. The workshops at these meetings sometimes compete for attention because South African academics frequently cover a broader range of clinical responsibility and have no local expertise to which to turn for developments in the field. It is also interesting to note that there is now more recognition of nutrition in the EAS, not only with the allocation of talks and posters, but also with the sessions that discussed nutritional issues at workshop and plenary level; but also with the bringing of companies providing healthy nutrition, into the supporters of the EAS meetings. Below follows a series of comments with most detail from plenary sessions but also some information on other topics that were of interest to me; in some instances some brief background is also provided. I include what reference I could copy in time and can only hope that they are correct as there has been no time to verify them.

The news from the plenary sessions can be summarised as: (1) Prof Helen Hobbs on triacylglycerol metabolism, [2] Postprandial lipoproteins by Prof Stephen Young, [3] Gut microbiota by Prof Fredrik Bäckhed, [4] the complexity of HDL by Prof John Chapman, a topic shared by many other speakers, [5] pathophysiology of the arterial plaque by Prof Erling Falk, [6] cell biology of the plaque by Prof Ira Tabas, [7]

immunomodulation of the plaque and vaccines by Prof. J Nilsson and [8] the new EAS-ESC guidelines by Prof Alberico Catapano.

Hobbs's lecture began by stressing the role of triglycerides in biology - energy-rich molecules that are not osmotically active as they are insoluble. They ought to be stored in adipose tissue as an increase in triglyceride beyond adipose tissue results in fatty liver. This **hepatic steatosis** is observed in about 50% of obese people and is not always in the setting of insulin resistance. About 10% of people with hepatic steatosis develop non-alcoholic steatohepatitis (NASH) from which about 10% ultimately develop cirrhosis. Non-invasive determination of hepatic triglyceride content by proton magnetic resonance shows that the median triglyceride content of the liver is about 3.6% with a 95th centile value at 5.6% that is now viewed as the upper limit of normal. African Americans and Caucasion Americans had prevalences of steatosis at about 24 and 33% respectively whereas Hispanic American have an higher prevalence at 45%. One Hispanic family was investigated in which there cases of steatosis, NASH and cirrhosis. After intensive genetic investigation a non-synonymous single nucleotide variant was found in adiponutrin, a gene encoded on chromosome 22 and in which Isoleucine was replaced by methionine in this phospholipase also known as PNPLA3. This gene has a patatin-like domain from amino acid 1-276 along a protein of 481 aa. The mutant has low but not absent lipase activity. In this family the steatosis did not correlate with plasma triglyceride, body mass index or insulin resistance. There is a gene-dose effect of the I148M mutation that increases transaminase activity in plasma and raises risk for cirrhosis. Indeed, an investigation found a high prevalence of the MM genotype in cirrhosis. Interestingly, in man this gene is predominantly expressed in the liver whereas in there mouse it predominates in the adipose tissue. It is located in the rim of intracellular lipid droplets. Loss of function leads to lipid accumulation.

Then plenary lecture delivered by Young on the **postprandial lipolysis** of chylomicrons revealed more information on the recently discovered protein, glycosyl phosphoinositol HDL binding protein 1 (GPIHBP1). This protein appears to be an ancient one, resembling proteins in snake venom. It is a 3-fingered protein with 8-10 cysteins holding the structure in place. It appeared originally targeted at acetylcholine receptors and coagulation factors. Humans have 25 similar proteins, most of which have GPI anchor properties; one of which is CD59 that regulates complement activity. Ligands are diverse. The protein has a Ly6 region in which there are 10 cysteines and there is a long acidic domain with many aspartate and glutamate residues. GPIHBP is displaced by heparin which also has negative charges. Note that lipoprotein lipase (LPL) has many negatively charged amino acids. It appears that the GPIHBP1 binds LPL on the endothelial cell to keep it in place at the capillary endothelial bed in adipose and muscle tissue. It is exclusively expressed in endothelial cells in capillaries but not in larger vessels. It appears to recruit LPL from the subendothelial aspect and to transport it to the lumenal aspect of the cell. GPIHBP1 is located in caveolae on the endothelial cells and probably retains the large chylomicron which binds LPL, over this small structure. The implication of this is that the fatty acids liberated by the action of LPL will be retained at this level. Knocking out GPIHBP1 in mice results in severe

hypertriglyceridaemia and in these mice LPL is not found in the lumenal aspect of the capillaries but can be demonstrated in the subendothelial space. Of the 3 fingers in the protein, the middle one appears to be the most important in the function of the protein. Binding is disrupted by C65Y, C65S, C68G, C68Y as well as by the nearby Q115P but Q115A has no effect on the binding to LPL. It is interesting to note that certain mutations in LPL abrogate binding to GPIHBP1: C418Y, C438A and E421K. These mutations mean that LPL will not be transported from the subendothelial space and will not appear on the lumenal aspect of the capillary. There are thus now several reasons, all autosomal recessive, why chylomicronaemia will occur when lipolysis of chylomicrons is defective. The original description was of LPL deficiency but subsequently its activator, apoCii, was discovered to be critical as well. The GPIHBP1 deficiency means LPL cannot be assimilated on the endothelial cell and would also mean no lipolysis of chylomicrons will occur. There is also a lipid maturation factor that is required to mature LPL before its secretion that can induce chylomicronaemia in a recessive fashion. Lastly, apoAv is also required for lipolysis. Whilst all these conditions are recessive, it is possible that the concatenation of these defects can be involved in the milder hypertriglyceridaemias where clearance of chylomicrons could be abnnormal as well as clearance of VLDL,. The latter represents smaller quantities of triglyceride entering the circulation but would be detected in the fasting state.

There is increasing recognition of the interaction between **microbes and man.** Prof Bäckhed's lecture introduced this new field of investigation with definitions relating the microbiome as a parallel to the karyome (all nuclei), the chondriome (mitochondria) and contrasting this with the microbiota where special microbial populations occur in the mouth, skin and gut. It is estimated that there could be 1.5kg of such biomass, with 3 million genes (exceeds human genome) and that these organisms have multiple implications for health. Their growth protects against pathogens, their metabolism provides vitamin K and takes care of many xenobiotics and their fermentation could provide input into human metabolism. Hitherto poorly recognised is their trophic role in gut epithelium and angiogenesis. Histologically, germ-free mice have long thin villi with few vessels whereas mice raised with microbes have shorter, wider villi with more prominent vasculature. It was also noted that the bile acid pool is smaller in germ-free animals. Interestingly, germ-free mice have less fat and upon recolonised gain mass in adipose tissue. Additionally, germ-free mice on a sterile western diet also gain less weight. The gain in adipose tissue is attributed to short chain fatty acids produced by the microbes in the gut and these travel via the portal vein to the liver where they form triglycerides. It appears that angiopoeitin-like factor 4 (AngPtl4, or Fiaf) is involved in this process as Fiaf -/- mice do not resist obesity and have lower triglyceride concentration. Another interesting aspect revealed in this talk is that microbiota may affect atherosclerosis: there is evidence of finding these in plaque. Germ-free apoE -/- male mice have significantly less atherosclerosis than their controls. There also appears to be a correlation between bacterial species and C-reactive protein; and butyrate released by the organisms appears to be anti-inflammatory. There is also a correlation between microbial genes and LDLC concentration. In conclusion,

there is an interplay between the gut microbiota, the diet and the host genotype that requires more elucidation and may on a population basis be quite important in the pathogenesis of metabolic phenotypes that predispose to or protect against disease.

High density lipoprotein metabolism was reviewed by Prof Chapman. The multiple known functions of HDL were briefly summarised but from the lipidology point of view, the most prominent is the role in cholesterol efflux from cells that initiates the reverse cholesterol transport. The most prominent protein in HDL is apolipoprotein Ai; it has 6 amphipathic helices that appear to critically depend on hing areas between them for the adoption of conformations that the changes in HDL size bring about. Amongst these are M86, M148, M112, Y192 and K226. Complete absence of HDL has been described in which patient there were planar xanthomata and arcus cornealis as well as premature coronary disease in a family from Brazil (Santos R, J Lipid Res 2008;49:349-). Mostly the concern is not so much about quantitative changes brought about by these rare mutations but more about qualitative changes that are being demonstrated by modern techniques involving particle structure, its lipidome and its proteome (Khera NEJM 2011, Kompula Chem Phys Lip 2008;155:57-62). The surface area of lipid exposed to metabolic interaction may be affected by the proteins in HDL (Huang Nature Struct Molec Biol 2011). The average appears to be 4 molecules of Ai per HDL particle; but a model of a trefoil of 3 apoAi particles was proposed (Silva, PNASUSA 2008). Generally HDL is divided into 5 classes on electrophoresis, from largest to smallest being HDL2b, HDL2a, HDL3c, HDL3b, HDL3a. The key apoproteins in HDL 3b and 3c appear to be apoD, apoM, PON, apoF and PLTP (Davidson ATVB 2009). It would appear likely that clusters of proteins and lipids are associated with the functionality of HDL. Lipid investigations have revealed variations in phospholipid/unesterified cholesterol ratio, sphingosine-1-P, sphingomyelin, ceramide, cardiolipin, plasmologen, lipid hydroperoxides and oxysterols. Sphingomyelin appears to be very adverse. Therapeutic strategies for modifying HDL are currently not specifically targeted and are judged by their effect on HDLC concentration. Statins raise HDLC by about 5%, fibrates by 1 to 20%, niacin by 5-30%. In conclusion the following needs were indicated in this domain of research: the characterisation of the optimal profile of lipidome and proteome as a gold standard to attain in therapeutics, the identification of key deviations and their temporal and functional effects, humanised animal models to study HDL metabolism and specially targeted therapy.

Prof Falck gave a good overview of **plaque pathophysiology** but had to address some definitions as these may not all conform. A vulnerable plaque is a pro-thrombotic plaque and rupture of such a plaque accounts for about ¾ of obstructive lesions. Plaque erosion accounts for about 25% of the obstructive clots in coronary disease.

From several lines of investigation it is clear that blood enters the rupturing plaque, gruel may be lost from the lesion to cause distal embolisation, and crystals are exposed at the base of the lesion. Factors that predispose to rupture are multiple.: 1. Plaque size. Usually there is expansive remodelling so that the lumen dimensions are unaffected and the lesion is not detected by conventional contrast dyes in

angiography. Alhough current strategies seem successful in curbing the growth of plaques, the trend to obesity may offset this benefit. 2. The necrotic core is more prominent in vulnerable plaques. 3. The fibrous cap is thinner and it appears that 65 microns is a critical thickness for rupture to occur. Macrophage infiltration is prominent and their elaboration of matrix metalloproteinases as well as tissue factor set off further digestion of the cap and more clot formation respectively. Smooth muscle cells under these conditions also produce less collagen, aggravating the risk for rupture. 4. Angiogenic responses may also be adverse. They are set up from the vasa vasora but these immature vessels may exude and haemorrhage, 5. Perivascular inflammation is also a warning. 6. Calcium is deposited in the plaque as a late consequence of inflammation. Intact plaques have more calcium, possibly reflecting quietening of the inflammation but calcium also reflects the extent of plaque formation when used as a coronary score assessment. Stippled calcification may indicate higher risk.

After this presentation, came a fascinating talk from Prof Tabas introduced the term of **efferocytosis**.. The essence is that the necrotic core of a plaque is a macrophage graveyard in which the safe disposal and anti-inflammatory response have failed, resulting in apoptosis and/or necrosis of macrophages. The preferred, possibly physiologic response of efferocytosis lets macrophages handle such a lesion without creating the necrotic core. Anapoptotic cell expresses several proteins, including CD31, CRT, phosphatidyl serine. The latter is recognised by a phagocyte through MerTk with additional proteins S and Gas6 in association. This leads to the engulfment of the apoptotic cell in the process of efferocytosis. MerTK is cleaved by secreted proteolytic enzymes (sheddases), and adding soluble portions of MerTK reduces the uptake of apoptotic cells, interfering with efferocytosis. The process of efferocytosis is limited in MerTK knock-out animals and necrosis is enhanced. A MerTK cleavage resistant mouse had preservation of efferocytosis. It now appears that necrotic macrophages in the plaque are not recognised, i.e. efferocytosis fails. The macrophages from obese mice (ob/ob as well as db/db) have defective efferocytosis - possibly due to higher saturated fatty acid content. It also appears the externalisation of phosphatidylserine that is required for efferocytosis, could be limiting. Interestingly, dendritic cells in the plaque also express efferocytotic machinery and may remove autoantigens. If this process is defective, it may have immune response consequences. It also appears that mature dendritic cells remain in the plaque rather than migrating to nodes. These insights may be taken further to develop additional strategies to combat the development of vulnerable plaques. In a fascinating follow-on, Rudd discussed the finding that imaging with 18-fluoro-2-desoxyglucose finds more glycolytic activity in macrophages and/or endothelial cells in vessels and that vascular uptake correlates with Framingham risk score. It is interesting to note the sodium fluoride can show calcification in plaque.

One educational symposium reflected on the long **history of atorvastatin**. Several trials with this agent established that lower is better for LDL concentration (TNT) and that there is merit in treating early after myocardial infarction (MIRACL), as well as the importance of treating the lipids in diabetes mellitus (CARDS, Colhoun Diabetes Medicine 2002;19:201-) where major cardiovascular events were lowered

by 37% and there was a reduction of stroke by 50% when, simplistically, LDL is lowered from 3.1 to 2.0 mmol/L. Cholesterol ester transfer protein (CETP) inhibition was also frequently discussed along with HDL pathophysiology since the disappointment that torcetrapib, that significantly raised HDLC and lowered LDLC in combination with a statin, resulted in higher mortality - mostly attributed to "off-target" effects on aldosterone that effected hypertension and a change in potassium metabolism. The benefit on stroke reduction (SPARCL) was less than that of coronary event reduction but nevertheless stressed the benefit on lipid lowering treatment to atherosclerosis in general. The fact that in severe renal disease that cardiovascular events roughly equalled renal causes of death (Levey, Am J Kidney Dis 1998;32:853) was stressed but it was suggested that the very late onset of significant lowering of blood cholesterol was not likely to influence atherosclerosis that is usually far advanced by this stage. The SHARP study did recently, however, find that in patients with was advanced renal disease, the introduction of powerful LDL reduction with simvastatin and ezetimibe, did improve coronary mortality. Furthermore, it has been shown that the decline in renal deterioration has been ameliorated with rosuvastatin.

Another educational symposium dealt with the **challenges of implementation**; highlighting the incomplete prescription as well as the poor adherence to instructions with statin therapy. An estimate was made that complete application of current guidelines in Sweden could save an additional 4000 lives per year. There appeared to be significant clinical inertia on the medical side as well as an underestimation of risk (Rapezzi J Cardiovasc Med 2008;9:878-887). Prof Zambon quoted articles (Banegas JR, Eur Heart J 2011**,** van Bruggen, Fam Prac 2009;26:428-36 & Reiner Z Atheroscler 2010;213:598-603) that indicated that about 40% of dyslipidaemia remained untreated in Europe. A survey had shown that, in general, practitioners found guidelines useful but appeared not to know their contents when questioned. Risk tended to be underestimated (Rapezzi J Cardiovasc Med 2008;9:878-887). There were also disappointing aspects in the management of cardiovascular risk attributed to patients (J Manage Care Pharm 2008;15:728-740): adherence to prescription decreased with time. By 6 months, 56% were compliant with statins, by 12 months 43%, by 24 months only 27%. Similar figures pertain to prescriptions for hypertension and diabetes. Prof Wood described a very intensive approach to deal with cardiovascular risk including weekly sessions. This approach resulted in better rates of quitting smoking and exercise by 12 months. In the ensuing discussion the point was made that the lessening of cardiovascular events was negatively impacted by the increase of obesity. The general opinion was also that the polypill was not an appropriate way to deal with risk reduction. Government policies may be needed to address healthcare risk such as campaigns against smoking.

An educational symposium as well as the lecture given by Prof Philip Barter when he received the Anitschkow prize for his contribution to understanding HDL metabolism, dealt with the subject of **cholesterol ester transfer protein (CETP) inhibition** as a strategy to raise HDL cholesterol concentration as an anti-atherogenic treatment. Barter had originally described CETP in 1978. Barter

indicated that HDL is generally viewed as anti-atherogenic and rarely may be atherogenic. There is an inverse relationship between risk and HDLC concentration in recent reviews (including JAMA 2009;302:1993-2000 in the Emerging Risk Factor Collaboration). HDL has the properties of promoting cholesterol efflux from cells and in so doing effects reverse cholesterol transport. HDL also inhibits vascular inflammation and promotes endothelial cell repair involving migration of cells as well as recruitment of progenitor cells. Indeed, apoAi infusions increase endothelial progenitor cell concentration. HDL also has a role in stimulating secretion of insulin from pancreatic islet β-cells. Additionally, HDL has anti-oxidant and antithrombotic activities (and even microbicidal activities). Barter stressed that even in the setting of low LDLC concentrations of (<1.8mmol/L) a low HDLC concentration remains a predictor of cardiovascular risk. The HDL hypothesis as an anti-atherosclerosis lipoprotein is in Barter's view neither negated nor supported in concept by the use of CETP ihibitors even though the raise HDLC, apoAi and decrease LDLC and VLDLC and apoB. The complexity of HDL metabolism was emphasised by indicating that expressing CETP in rodents increased atherosclerosis in animals that would generally be very resistant to atherosclerosis. Additionally, rabbits (which have high CETP activity) are very susceptible to atherosclerosis but this process is effectively limited by CETP inhibitors as well as vaccines against CETP. The disappointing outcome of the ILLUMINATE study (Barter, NEJM 2007;357:2109-2122) with torcetrapib as a CETP inhibitor was discusssed. Off-target effects raising blood pressure and changing electrolyte balance might explain this untoward effect. Two new CETP inhibitors, anacetrapib and dalcetrapib, appear to be free of these effects and are under investigation. Analysis also revealed that torcetrapib did not affect glucose, HbAic or insulin concentration, unlike the atorvastatin only limb in this study that showed the adverse effects of statins at high doses on glucose metabolism. The properties of HDL during CETP inhibition appear not to be adversely affected, including its capacity to promote cholesterol efflux from cells.

Of interest in classical **lipoprotein** pathway was the observation about apoB, the essential protein for forming triglyceride-rich lipoproteins, is present in myocardium of humans and theoretically making it possible that the heart can secrete lipoproteins. Transgenic mice for human apoB have better glucose tolerance. Additionally it appears that apoB and MTP are also found in the pancreas and muscle.

An interesting talk in a workshop was given by Prof Williams who has studied the metabolism of triglyceride-rich lipoprotein remnants that are predictive of atherosclerosis and which are cleared mainly by **heparan sulphate proteoglycans** (HSPG, sulphated or carboxylated to provide negative charges) although high affinity interaction does occur by receptors such as the LDLR, scavenger receptor B1 and others. Syndecan, perlecan and glypican are evolutionary relevant from the stage of worms and flies. Syndecan expression in chinese hamster ovary cells increases clearance of remnants and knocking it out decreases remnant clearance (Stanford J Clin Invest 2009). About 50 genes are required to assemble HSPGs and it appears now that heparan sulphate glucosamine-6-O-sulphatase 2 has implications

for remnant clearance as well as playing a role in the morphogenesis of embryos. Active sulphatase will alter the HSPG properties to have a negative effect on remnant clearance. It is suppressed by insulin at very low concentrations and by adiponectin but enhanced expression occurs in response to advanced glycation endproducts. It is pro-proliferative for hepatomas and its effects are countered by sulphatase 1.

The complexity of **oxysterol metabolism** increases and was the subject of several presentations. Oxysterols are the result of auto-oxidation as well as enzymatic activity, occur at very low concentrations and are already known to relay effects through the nuclear hormone receptor system (LXR). ORP10 is a cytoplasmic oxysterol

binding protein and a single nucleotide variant in it is in linkage with triglyceride concentration and silencing this gene has resulted in increased cholesterol and triglyceride concentrations in the cell and plasma. It appears associated with microtubules in the cell as well as the Golgi apparatus. Desmosterol may also be an important regulator of cholesterol biosynthesis, initiator of reverse transport and stimulant to fatty acid synthesis. It was found to increase under cholesterol loading and oxysterol increase counter to what would be expected when the cholesterol pathway is suppressed; the reason being that there is an alternative pathway from lathosterol to cholesterol which persists after suppression of synthetic enzymes by oxysterols.

Fatty acid transport was discussed in a presentation. The non-esterified fatty acids (NEFA) are poorly soluble in the aquaeous environment (~200μmolar) and some are present on albumin (400μmolar). One of the membrane proteins that is involved in transport of NEFA is CD36, 88kda in size and has an asscociated fatty acid binding protein FABPc of 15kda. There is also a range of fatty acid transport proteins FATP1-6 which are around 63kda in size and which make a link to acylcoA. Most of the protein is intracellular, with 5 transmembrane portions. The interest in intracellular lipid metabolism continues with the finding that some of the lipodystrophies relate to defects in proteins relating to the formation of droplets. Olofson presented work on the formation of lipid droplets; these start near microsomes and involve microtubular attachment. ARAP2 is required for formation of droplets as small inhibitory mRNA to ARAP2 decreases droplet formation and also affects the glucose transporter availability and offers a potential link between glucose and lipid metabolism.

Studies on **endothelium** included the finding that hypoxia results in increased expression of the VLDLR, a receptor related to the LDL receptor and found mainly in heart, adipose tissue and muscle. The accumulation of lipid can then be easily demonstrated in endothelial cells incubated in hypoxia by using oil red O, and the accumulation is blocked by knocking out the VLDLR, and survival of cells to hypoxic stress is enhanced.

There were many highly specialised chemical and cell biologic studies with which it was difficult to identify as such work is not performed locally. Another observation was that, particularly in the case of Denmark, large population surveys and

samplings that were done many years ago are paying off handsomely to answer questions through Mendelian randomisation. Conceptually, this is the use of a large population for studying the impact of a gene of interest as it can reasonably assumed that the rest of the genome is random. Controlled studies can only select a few parameters to control, and nested controlled studies cannot provide the confidence that Mendelian randomisation studies can provide.