

2 Macromolecules

2.1 A new method for the rapid separation of plasma lipoproteins

Graham, J.M., Higgins, J.A. and Gillot, T.
Atherosclerosis, **115 (Suppl)**, S123 (1995)

Variations in the relative amounts of plasma lipoprotein classes and /or molecular changes in their lipid components and apoproteins, are important factors in the development of atherosclerosis. A new equilibrium centrifugation method, which avoids high salt gradients, provides high resolution and concentration of lipoproteins for electrophoretic, compositional and structural analysis. The method uses a new non-ionic medium, **iodixanol**, available as a 60% (w/v) solution called OptiPrep (Axis-Shield PoC, Oslo). Plasma is adjusted to 12.5% **iodixanol**; overlaid with 0.1-0.2x its volume of saline and centrifuged at approx. 350,000g for 2.5-3 h in a vertical or near-vertical rotor. Using 3.9 ml tubes, unloaded dense end first; soluble proteins are contained in the first 1.0 ml, HDL in the 1.2-2.4 ml fraction; LDL in the 2.6-3.4 ml fraction and VLDL in the 3.6-3.9 ml fraction. There is little overlap between the three classes. Fractions can be analysed directly, by gel electrophoresis and for lipid content. The method may resolve different sub-classes of these lipoproteins: this is currently being investigated. Because of the concentration effect achieved by the gradient, Lp(a), which is recovered in a single 0.25 ml fraction, can be easily demonstrated on gels when levels in the whole plasma are below the limits of detection. The technique thus provides a means of fractionating and concentrating all plasma lipoproteins in a single step; it should prove to be a valuable diagnostic and analytical tool in studies of lipoprotein metabolism.

2.2 A novel method for the rapid separation of plasma lipoproteins using self-generated gradients of iodixanol.

Graham, J. et al
Atherosclerosis, **124**, 125-135 (1996)

We describe a new method for the rapid fractionation of plasma lipoproteins, which makes use of a new simple: plasma or serum is mixed with iodixanol followed by centrifugation in a vertical or near vertical rotor. Separation of VLDL, LDL and HDL can be achieved in 3 h and the lipoprotein fractions are comparable in density and composition with those prepared using conventional salt based gradients. Each class of lipoprotein can be removed in a single fraction, or a profile of lipoprotein distribution can be obtained using a gradient fractionation. Because the medium is inert, fractions from the gradient can be analyzed by agarose gel electrophoresis or assayed for lipid content or apolipoprotein composition by SDS-PAGE without removing the **iodixanol**. Small differences in electrophoretic mobility of HDL and LDL across several gradient fractions suggest that subfractionation of these classes may occur. The new method is simple, rapid and versatile with potential application for preparation of lipoproteins and for analysis of lipoprotein profiles in the research and clinical laboratory.

2.3 Isolation of plasmid DNA.

Rickwood, D. and Patel, N.
Mol. Biol. Cell, **7**, 162a (1996)

Centrifugation of DNA in CsCl-ethidium bromide gradients remains a widely used technique for the isolation of high purity plasmid DNA. There are two major problems with separations using this technique. The first is that because ethidium bromide intercalates into the DNA helix, it is a powerful mutagen, leading to dangers in working with it and difficulties in disposing of the ethidium bromide after use. The other problem is that ethanol precipitation of the plasmid can cause the precipitation of CsCl thus complicating recovery of the DNA. We have devised a new method for the purification of plasmid DNA using self-generating **OptiPrep** gradients containing a fluorescent dye, DAPI, to mark the position of the DNA in the gradient. Crude plasmid DNA is prepared using the standard alkaline lysis method and then purified by centrifugation in a gradient of 28% **OptiPrep** containing 0.005% DAPI at 150,000g for 40 hours at 5°C. The native DNA is separated from the denatured chromosomal DNA, RNA and proteins. As judged by electrophoresis, the plasmid DNA obtained after centrifugation is free of contaminating chromosomal DNA and RNA and can be ethanol precipitated directly from the gradient solution.

2.4 Rate zonal sedimentation of proteins in one hour or less.

Basi, N.S. and Rebois, V.

Anal. Biochem., **251**, 103-109 (1997)

Rate zonal sedimentation gives information about the shape and size of proteins, and is useful for investigating protein-protein interactions. However, rate zonal sedimentation experiments typically last approximately 1 day. In contrast, this report describes a rate zonal sedimentation method requiring 1 h or less. This was accomplished by centrifuging small density gradients (200 μ l) prepared with sucrose or **OptiPrep** in a fixed-angle rotor at high relative centrifugal force. By using small gradient volumes, the sample dilution that occurs with larger gradients and with many chromatographic techniques was also avoided. For a variety of proteins, plots of $s_{20,w}$ versus distance sedimented during centrifugation in a TLA 120.2 rotor were linear. As a practical application, sedimentation of the heterotrimeric stimulatory G protein and its dissociated α -subunits were determined. The results were similar to those obtained in an SW 50.1 rotor and agreed with previously published values. Long periods of centrifugation might preclude the study of some unstable proteins or the investigation of protein-protein interactions whose affinities are too low to survive the lengthy centrifugations required to carry out traditional rate zonal sedimentation experiments. A rate zonal sedimentation technique that rivals many chromatographic methods in celerity will help to circumvent these problems.

2.5 Effect of dietary fish oil or sunflower oil on plasma lipoproteins and hepatic gene expression in the hamster

Bennett, A.J., Kendrick, J.S., Anderton, K.L., Higgins, J.A. and White, D.A.

Atherosclerosis, **130 (Suppl. 1)**, S24 (1997)

An increased dietary consumption of fish oils reduces the occurrence of coronary artery disease. Although this probably involves several mechanisms, one effect of increased consumption of fish oil is a fall in plasma triacylglycerol (TAG) in humans and experimental animals. This has led to the idea that fish oil fatty acids lower VLDL secretion by the liver and hence reduce the risk of atherosclerosis. To investigate this, we have supplemented the diets of hamsters with 10% fish-oil or 10% sunflower oil and examined blood lipoproteins, VLDL secretion and the mRNA levels of genes involved in apolipoprotein B metabolism. Hamsters were fed 1.5 ml of either fish oil (Maxepa) or sunflower oil by daily gavage. Blood samples were taken at weekly intervals. The lipids were analysed and the lipoprotein classes separated on **iodixanol** gradients and by agarose electrophoresis. In fish oil fed animals, plasma TAG fell by 25% over a three-week period. There was no significant change in the TAG levels of either chow fed animals or sunflower oil fed animals or in the cholesterol levels of the three groups of animals. Plasma VLDL were considerably reduced and there was a small increase in LDL and HDL in the fish-oil fed animals. In the sunflower oil fed and chow fed animals, there was no significant change in the plasma lipoproteins. Consistent with these results from *in vivo* experiments, secretion of VLDL by hepatocytes isolated from fish-oil hamsters were considerably reduced compared with hepatocytes from chow-fed and sunflower-oil fed animals. The fall in plasma VLDL in fish-oil fed hamsters was not accompanied by a fall in LDL. To determine whether this is due decreased LDL uptake we determined the mRNA levels for the LDL receptor (LDL-R) in liver from hamsters fed fish-oils, sunflower oil or chow for three weeks using the mRNA protection assay. The mRNA for apo-B, HMG-CoA reductase and microsomal triacylglycerol transfer protein (MTP) were also measured in the same liver samples to examine possible effects on other candidate proteins involved in apo-B metabolism (Table 1). Dietary fish-oils reduced the levels of mRNA for the LDL-R by approximately 60% while dietary sunflower oil had no significant effect, mRNA for HMG-CoA reductase was reduced 60% by feeding fish-oils and 20% by feeding sunflower oils. These results suggest that there may be a complex metabolic relationship between apo-B synthesis and uptake by the liver which is perturbed by feeding fish-oils.

2.6 **Dietary fish oils modify the assembly of VLDL and expression of the LDL receptor in rabbit liver**

Wilkinson J., Higgins, J.A., Fitzsimmons, C. and Bowyer, D.E.
Arterioscler. Thromb. Vasc. Biol., **18**: 1490-1497 (1998)

Supplementation of the diet of rabbits with fish oil or sunflower oil resulted in significant changes in the lipoproteins and lipids in serum. Compared with chow-fed rabbits, dietary fish oils decreased very low-density lipoprotein (VLDL), increased low-density lipoprotein (LDL), and shifted the peak of the LDL to denser fractions, whereas sunflower oil increased high density lipoprotein and shifted LDL to the lighter fractions. The amount of LDL receptors in fish oil-fed rabbit liver decreased by >70% while there was only a small fall in these levels in sunflower oil-fed rabbit liver. The concentrations of apolipoprotein (apo) B in the subcellular organelles of the secretory compartment (rough and smooth endoplasmic reticula and Golgi fractions) were also changed by dietary lipids. In both sunflower oil- and fish oil-fed liver, apo B was increased in the lumen of the rough endoplasmic reticulum compared with fractions from chow-fed rabbit liver. The apo B in the trans-Golgi lumen from fish oil-fed livers was reduced and occurred in particles of $\rho \sim 1.21$ g/mL. In contrast, apo B in the trans-Golgi lumen from livers of sunflower oil-fed rabbits was increased and occurred in particles of $\rho < 1.21$ g/mL. These results suggest that feeding of fish oils causes an interruption in the intracellular transfer of apo B and hence assembly of VLDL. This leads to an enrichment of the rough endoplasmic reticulum membranes with cholesterol, thus down-regulating the expression of the LDL receptor.

2.7 **Carotenoid composition and antioxidant potential in subfractions of human low-density lipoprotein.**

Lowe, G.M. et al
Ann. Clin. Biochem., **36**, 323-332 (1999)

Carotenoids and vitamin E are transported in human plasma complexed with lipoproteins. The bulk of them are associated with low-density lipoprotein (LDL) where they may act as antioxidants and thus delay the onset of atherosclerosis. In this study we have used a simple, rapid ultracentrifugation technique in which plasma lipoproteins are fractionated in self-generated gradients of **iodixanol**, a non-ionic iodinated density gradient medium. The carotenoid content and composition of a number of LDL subfractions was determined by reversed-phase HPLC. Lycopene, β -carotene and β -cryptoxanthin were mainly located in the larger, less-dense, LDL particles whereas lutein and zeaxanthin were found preferentially in the smaller, more-dense, LDL particles. When the antioxidant content of these fractions was expressed per mg of LDL protein, a different distribution was found with significantly lower concentrations of carotenoid and vitamin E associated with the smaller protein-rich, fractions of LDL. Strong positive correlations were found for total carotenoid and vitamin E concentrations with the lag-time of Cu^{2+} -mediated oxidation of LDL subfractions. The more dense LDL subfractions, which had lower levels of these antioxidants, were more readily oxidised, highlighting their possible role in atherosclerotic events.

2.8 Blood appearance, metabolic transformation and plasma transport proteins of ¹⁴C- astaxanthin in Atlantic salmon (*Salmo salar* L.)

Aas, G.H., Bjerkgeng, B., Storebakken, T. And Ruyter, B.
Fish Physiol. Biochem., **21**, 325-334 (1999)

The time of appearance in blood, and transport of astaxanthin, and catabolic transformation of astaxanthin to idoxanthin were investigated in Atlantic salmon (*Salmo salar*) that had been force-fed a single dose ¹⁴C-astaxanthin. In addition to the LPs, a major protein, associated with radiolabeled astaxanthin was detected. The maximum level of radiolabeled carotenoids in blood was attained 30 h after administration of ¹⁴C-astaxanthin. Radioactive idoxanthin (combined 3'4'-*cis* and 3'4'-*trans* glycolic isomers of idoxanthin) appeared after 6 h and a stable level was obtained after 18 h. LPDP and LP, separated by ultracentrifugation, contained on average 89 and 11% of the total radioactivity in plasma, respectively. During the 168 h experiment, maximum radioactivity in LP appeared after 22h. Separation of plasma by ultracentrifugation on a discontinuous NaCl/KBr-gradient and an **iodixanol**-gradient confirmed that most of the radiolabel carotenoids were present in the HDPF that did not contain LPs (58%), whereas HDL and LDL contained 36 and 6% of the radioactivity, respectively. Of the recovered radioactivity, astaxanthin in the HDPF comprised 82%, idoxanthin 5% and unidentified compounds 12%, whereas HDL contained 78% astaxanthin, 22% idoxanthin and no unidentified compounds. Protein from the fractions with high density and high radioactivity (iodixanol-gradient) were separated by PAGE under non-denaturing conditions and showed a radioactive band with parallel migration length to BSA and salmon albumin. These results show that astaxanthin is rapidly converted to idoxanthin and that the majority of astaxanthin in the plasma is associated with a protein other than LPs, presumably albumin. The identity of this protein requires verification.

2.9 In healthy adults, postprandial lipaemia results in triglyceride enrichment of very low-density lipoprotein, enhanced oxidative stress and deterioration in endothelial function

Anderson, R.A. et al
Atherosclerosis Suppl **154**(2), 434 (1999)

There is a significant relationship between postprandial lipaemia (PPL) and coronary artery disease in patients with both normal and abnormal carbohydrate metabolism. PPL has recently been shown to cause endothelial dysfunction in normal individuals: attenuated by antioxidants. We therefore studied the effects of PPL on endothelial function (EF), free radicals (FR) and triglyceride (TG) metabolism in healthy subjects. **Methods:** Twelve subjects with no history of vascular disease or diabetes were studied, (five male, seven female; mean age 43 years). After a 12-h overnight fast, EF, lipid profiles and venous FR levels were measured in the fasting and peak postprandial phase (at 4 h) subsequent to ingestion of a standard fat meal. Flow-mediated dilatation (FMD) was assessed, as a measure of EF in the brachial artery, and expressed as the percentage change in brachial artery diameter. Lipoprotein subfractions were assessed using a density gradient medium, **iodixanol**. Lipid-derived FR levels were measured in venous blood by electron paramagnetic resonance spectroscopy; results expressed in arbitrary units. **Results:** There was an increase in venous FR postprandially (mean \pm SD), 2.4 ± 0.1 to 3.3 ± 0.2 ($P < 0.05$), and a decrease in FMD, $6.26 \pm 1.3\%$ to $4.8 \pm 1.3\%$ ($P < 0.05$). TG levels (in mmol/l) were also significantly elevated at 4 h., 1.3 ± 0.5 to 2.2 ± 0.7 ($P < 0.05$). There were no changes in TG content of high- and low-density lipoprotein cholesterol but there was an increase in TG content of very low-density lipoprotein (VLDL), $54 \pm 6.5\%$ to $59.3 \pm 5.7\%$. **Conclusion:** We show for the first time that PPL in healthy individuals is associated with increased lipid-derived free radicals and deterioration of endothelial function. This was associated with relative TG-enrichment of VLDL particles.

2.10 Ciprofibrate reduces the postprandial generation of triglyceride-rich lipoproteins and attenuates the associated endothelial dysfunction and oxidative stress in non-insulin dependent diabetes mellitus

Evans, L.M. et al

Atherosclerosis Suppl 154(2), 434 (1999)

Introduction: Triglyceride-rich lipoproteins (TGRL) may be involved in atherogenesis by mechanisms including endothelial dysfunction and enhanced oxidative stress. Non-insulin-dependent diabetes mellitus (NIDDM) results in excess vascular disease and exaggerated excursions of postprandial TGRL. We studied the effect of fibrate therapy on the relationship between postprandial lipaemia (PPL), endothelial function (EF) and oxidative stress in NIDDM. **Methods:** Twenty NIDDM patients were studied following an overnight fast and 4 h after a fatty meal. Lipoproteins were separated using an iodixanol density gradient medium. Free radicals (FR) were directly measured using electron paramagnetic resonance spectroscopy. EF was assessed by measuring flow-mediated brachial artery dilatation (FMD). Subjects were randomised in a double-blind manner to 3 months of placebo (P) or ciprofibrate (C) (100 mg OD).

Results (mean \pm SD): Seventeen subjects completed the study. At base line, both groups exhibited similar changes in FMD [(% change) $3.8 \pm 1.8\%$ to $1.8 \pm 1.3\%$ (C) vs. 3.3 ± 1.7 to $1.7 \pm 1.1\%$ (P)]. Increase in FR [(arbitrary units) 2.9 ± 1.3 (C) vs. 3.1 ± 1.5 (P)]. Rise in plasma TG [(mmol/l) 2.8 ± 2.1 to 6.7 ± 6 (C) vs. 2.8 ± 1.7 to 7 ± 7.3 (P)] and TG enrichment of very low-density lipoprotein (VLDL) [(% TG content) $59.6 \pm 4.6\%$ to $73.4 \pm 6.9\%$ (C) vs. $61.2 \pm 5.9\%$ to $76.1 \pm 9.8\%$ (P)]. Following treatment fasting and PP, FMD improved in the treatment group with reductions in fasting and PP TG, VLDL-TG content and FR. FMD, 4.8 ± 1.1 to $3.4 \pm 1.1\%$ (C) vs. 3.4 ± 1.2 to $1.8 \pm 1.1\%$ (P) ($P < 0.05$). TG, 1.5 ± 0.8 to 2.8 ± 1.3 mmol/l (C) vs. 3.1 ± 2.1 to 6.6 ± 4.1 (P) mmol/l ($P < 0.05$). VLDL-TG (%), 50.1 ± 6.2 to $59.5 \pm 4.3\%$ (C) vs. 60.6 ± 3.9 to $72.9 \pm 6.9\%$ (P) ($P < 0.05$). FR, 0.3 ± 0.6 (C) vs. 1.1 ± 0.9 (P) ($P < 0.05$). **Conclusions:** This study demonstrates that ciprofibrate may reduce the risk of vascular disease in NIDDM by mechanisms involving improved EF, attenuated PPL, enhanced catabolism of TGRL and reduced FR release.

2.11 Consequences of interaction of a lipophilic endotoxin antagonist with plasma lipoproteins

Rose, J.R. et al

Antimicrobial Agents and Chemotherapy, 44(3), 504-510 (2000)

E5531, a novel synthetic lipid A analogue, antagonizes the toxic effects of lipopolysaccharide, making it a potential intravenously administered therapeutic agent for the treatment of sepsis. This report describes the distribution of E5531 in human blood and its activity when it is associated with different lipoprotein subclasses. After in vitro incubation of (14 C) E5531 with blood, the great majority (>92%) of material was found in the plasma fraction. Analysis by size-exclusion and affinity chromatography and density gradient centrifugation indicates that (14 C)E5531 binds to lipoproteins, primarily high-density lipoproteins (HDLs), with distribution into low-density lipoproteins (LDLs) and very low density lipoproteins (VLDLs) being dependent on the plasma LDL or VLDL cholesterol concentration. Similar results were also seen in a limited study of (14 C)E5531 administration to human volunteers. The potency of E5531 in freshly drawn human blood directly correlates to increasing LDL cholesterol levels. Finally, preincubation of E5531 with plasma or purified lipoproteins indicated that binding to HDL resulted in a time-dependent loss of drug activity. This loss in activity was not observed with drug binding to LDLs or to VLDLs or chylomicrons. Taken together, these results indicate that E5531 binds to plasma lipoproteins, making its long-term antagonistic potency dependent on the plasma lipoprotein composition.

2.12 Ciprofibrate therapy improves endothelial function and reduces postprandial lipemia and oxidative stress in type 2 diabetes mellitus

Evans, M. et al

Circulation, **101**, 1773-1779 (2000)

Background-Exaggerated postprandial lipemia (PPL) is a factor in atherogenesis, involving endothelial dysfunction and enhanced oxidative stress. We examined the effect of ciprofibrate therapy on these parameters in type 2 diabetes mellitus.

Methods and Results-Twenty patients entered a 3-month, double blind, placebo-controlled study. Each subject was studied fasting and after a fatty meal, at baseline, and after 3 months of treatment. Glucose and lipid profiles were measured over an 8-hour postprandial period. Endothelial function (flow-mediated endothelium-dependent vasodilatation [FMDI] and oxidative stress (electron paramagnetic resonance spectroscopy) were measured after fasting and 4 hours postprandially. At baseline, both groups exhibited similar PPL and deterioration in endothelial function. After ciprofibrate, fasting and postprandial FMD values were significantly higher (from $3.8 \pm 1.8\%$ and $1.8 \pm 1.3\%$ to $4.8 \pm 1.1\%$ and $3.4 \pm 1.1\%$; $P < 0.05$). This was mirrored by a fall in fasting and postprandial triglycerides (3.1 ± 2.1 and 6.6 ± 4.1 mmol/L to 1.5 ± 0.8 and 2.8 ± 1.3 mmol/L, $P < 0.05$). Fasting and postprandial HDL cholesterol was also elevated (0.9 ± 0.1 and 0.8 ± 0.1 mmol/L and 1.2 ± 0.2 and 1.2 ± 0.1 mmol/L, $P < 0.05$). There were no changes in total or LDL cholesterol. Fasting and postprandial triglyceride enrichment of all lipoproteins was attenuated, with cholesterol depletion of VLDL and enrichment of HDL. There were similar postprandial increases in oxidative stress in both groups at baseline, which was significantly attenuated by ciprofibrate (0.3 ± 0.6 versus 1.5 ± 1.1 U, $P < 0.05$).

Conclusions-This study demonstrates that fibrate therapy improves fasting and postprandial endothelial function in type 2 diabetes. Attenuation of PPL and the associated oxidative stress, with increased HDL cholesterol levels, may be important.

2.13 Collagen-bound von Willebrand factor has reduced affinity for factor VIII

Bendetowicz, A.V., Wise, R.J. and Gilbert, G.E.

J. Biol. Chem., **274**(18), 12300-12307 (2000)

von Willebrand factor (vWf) is a multimeric adhesive glycoprotein that serves as a carrier for factor VIII in plasma. Although each vWf subunit displays a high affinity binding site for factor VIII *in vitro*, in plasma, only 2% of the vWf sites for factor VIII are occupied. We investigated whether interaction of plasma proteins with vWf or adhesion of vWf to collagen may alter the affinity or availability of factor VIII-binding sites on vWf. When vWf was immobilized on agarose-linked monoclonal antibody, factor VIII bound to vWf with high affinity, and neither the affinity nor binding site availability was influenced by the presence of 50% plasma. Therefore, plasma proteins do not alter the affinity or availability of factor VIII-binding sites. In contrast, when vWf was immobilized on agarose-linked collagen, its affinity for factor VIII was reduced 4-fold, with K_d increasing from 0.9 to 3.8 nM. However, one factor VIII-binding site remained available on each vWf subunit. A comparable reduction in affinity for factor VIII was observed when vWf was a constituent of the subendothelial cell matrix and when it was bound to purified type VI collagen. In parallel with the decreased affinity for factor VIII, collagen-bound vWf displayed a 6-fold lower affinity for monoclonal antibody W5-6A, with an epitope composed of residues 78-96 within the factor VIII-binding motif of vWf. We conclude that Collagen induces a conformational change within the factor VIII-binding motif of vWf that lowers the affinity for factor VIII.

2.14 Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin

Osterlie, M., Bjerken, B. And Liaaen-Jensen, S.
J. Nutr. Biochem., **11**, 482-490 (2000)

Appearance, pharmacokinetics, and distribution of astaxanthin E/Z and R/S isomers in plasma and lipoprotein fractions were studied in 3 middle-aged male volunteers (37-43 years) after ingestion of a single meal containing a 100 mg dose of astaxanthin. The astaxanthin source consisted of 74% all-E-, 9% 9Z-, 17% 13Z-astaxanthin (3R, 3'R, 3R, 3'S: meso-, and 3S, 3'S-astaxanthin in a 1:2:1 ratio). The plasma astaxanthin concentration – time curves were measured during 72 hr. Maximum levels of astaxanthin (1.3 ± 0.1 mg/L) were reached 6.7 ± 1.2 hr after administration, and the plasma astaxanthin elimination half-life was 21 ± 11 hr. 13Z-astaxanthin accumulated selectively, whereas the 3 and 3'R/S astaxanthin distribution was similar to that of the experimental meal. Astaxanthin was present mainly in very low-density lipoproteins containing chylomicrons (VLDL/CM); 36-64% of total astaxanthin), whereas low-density lipoprotein (LDL) and high-density lipoprotein (HDL) contained 29% and 24% of total astaxanthin, respectively. The astaxanthin isomer distribution in plasma, VLDL/CM, LDL and HDL was not affected by time. The results indicate that a selective process increases the relative proportion of astaxanthin Z-isomers compared to the all-E-astaxanthin during blood uptake and that astaxanthin E/Z isomers have similar pharmacokinetics.

2.15 Fractionation and characterization of oligomeric, protofibrillar and fibrillar forms of β -amyloid peptide

Ward, R.V. et al
Biochem. J., **348**, 137-144 (2000)

The β -amyloid (A β) peptide, a major component of senile plaques in Alzheimer disease brain, has been shown previously to undergo a process of polymerisation to produce neurotoxic forms of amyloid. Recent literature has attempted to define precisely the form of A β responsible for its neurodegenerative properties. In the present study we describe a novel density-gradient centrifugation method for the isolation and characterization of structurally distinct polymerized forms of A β peptide. Fractions containing protofibrils, fibrils, sheet structures and low molecular mass oligomers were prepared. The fractionated forms of A β were characterized structurally by transmission electron microscopy. The effects on cell viability of these fractions were determined in the B12 neuronal cell line and hippocampal neurons. Marked effects on cell viability in the cells were found to correspond to the presence of protofibrillar and fibrillary structures, but not to monomeric peptide or sheet-like structures of polymerized A β . Biological Activity correlated with a positive reaction in an immunoassay that specifically detects protofibrillar and fibrillary A β ; those fractions that were immunoassay negative had no effect on cell viability. These data suggest that the effect of A β on cell viability is not confined to a single conformational form but that both fibrillary and protofibrillar species have the potential to be active in this assay.

2.16 Capillary isotachopheric analysis of serum lipoprotein using a carrier ampholyte as spacer ion

Inano, K., Tezuka, S., Miida, T. and Okada, M.
Ann. Clin. Biochem., **37**, 708-716 (2000)

We have developed a novel analytical method for serum lipoproteins using a commercially available capillary electrophoresis apparatus, BioFocus 3000 (Bio-Rad Laboratories Co Ltd, USA). The analytical principle is isotachopheresis (ITP), using a carrier ampholyte, BioLyte 7/9, as a spacer ion. The method allows a much higher resolution of lipoproteins than of amino acid mixtures. Serum lipoproteins are normally separated into 13-15 peaks, including some shoulder peaks. The reproducibility of repeated analysis within a day was relatively good with the coefficient of variation within the range 0.9-1.1%. VLDL, LDL and HDL prepared by discontinuous density ultracentrifugation could be further separated by capillary ITP. This high-resolving ability of our method enabled detection of small amounts of abnormal lipoprotein species. For example, small dense LDL, which is thought to be an atherogenic lipoprotein, could be detected within the LDL group peak. Moreover, an abnormal HDL, apolipoprotein E-rich HDL, was also detected by a single analysis. These findings suggest that our capillary ITP method is a useful means for detailed analysis of lipoproteins and thus for clinical diagnosis of hyperlipoproteinaemic subjects.

2.17 A randomized trial of the effects of garlic oil upon coronary heart disease risk factors in trained male runners

Zhang, X-H., Lowe, D., Giles, P., Fell, S., Board, A. R., Baughan, J. A., Connock, M. J. and Maslin, D. J. *Blood Coagulat. Fibrinolysis*, **11**, 67-74 (2000)

Most trials of bulb garlic and garlic powder tablets indicate reduced coronary heart disease (CHD) risk in elevated-risk subjects. Most persons taking garlic supplements lack overt risk of CI-ED. However, no trials have tested steam-distilled garlic oil (GO) capsules with healthy subjects. The objectives of the present randomized, double-blind, placebo-controlled study were to determine whether GO capsules reduce CHD risk in trained male runners. Twenty-seven volunteers (mean age, 28.8 years) completed the study. Each took 12.3 mg/day GO (or placebo) capsules for 16 weeks. Main outcome measures were 95% confidence intervals (CIs) between GO and placebo groups for differences in changes of blood pressure (BP), plasma lipids, total antioxidant status (TAS), low-density lipoprotein (LDL) composition and blood clotting factors. Principal results as mean differences (95% CI) between GO and placebo are: pulse, 2.9 beats/mm (-0.8 to 6.7), $P = 0.12$; systolic BP, -4.5 mmHg (-10.8 to 1.9), $P = 0.16$; plasma total cholesterol, 0.01 mmol/l (-0.34 to 0.37), $P = 0.95$; plasma triglycerides, -0.20 mmol/l (-0.43 to 0.03), $P = 0.09$; plasma TAS, 45 pmol Trolox equivalent/l (-35 to 124), $P = 0.26$; LDL density, 0.0019 g/ml (-0.0005 to -0.0043), $P = 0.12$; LDL triglycerides/protein, -0.078 mg/mg (-0.149 to -0.007), $P = 0.03$; LDL cholesterol/protein, 0.24 mg/mg (-0.69 to 0.22), $P = 0.3$; LDL TAS/triglycerides, 29 nmol/mg (11 to 68), $P = 0.15$; prothrombin time, 0.99s (-0.36 to 2.35), $P = 0.14$; partial thromboplastin time, 3.0s (-1.0 to 7.1), $P = 0.13$. Results were null statistically. Trends with GO were mostly towards lower CHD risk, and a larger study (-150 subjects) is required to test their validity.

2.18 Putative fusogenic activity of NSF is restricted to a lipid mixture whose coalescence is also triggered by other factors

Brügger, B. et al
EMBO J., **19**(6), 1272-1278 (2000)

It has recently been reported that N-ethylmaleimide-sensitive fusion ATPase (NSF) can fuse protein-free liposomes containing substantial amounts of 1,2-dioleoyl-phosphatidylserine (DOPS) and 1,2-dioleoyl-phosphatidyl-ethanolamine (DOPE) (Otter-Nilsson *et al.*, 1999). The authors impart physiological significance to this observation and propose to re-conceptualize the general role of NSF in fusion processes. We can confirm that isolated NSF can fuse liposomes of the specified composition. However, this activity of NSF is resistant to inactivation of N-ethylmaleimide and does not depend on the presence of α -SNAP (soluble NSF-attachment protein). Moreover, under the same conditions, either α -SNAP, other proteins apparently unrelated to vesicular transport (glyceraldehyde-3-phosphate dehydrogenase or lactic dehydrogenase or even 3 mM magnesium ions can also cause lipid mixing. In contrast, neither NSF nor the other proteins nor magnesium had any significant fusogenic activity with liposomes composed of a biologically occurring mixture of lipids. A straightforward explanation is that the lipid composition chosen as optimal for NSF favors non-specific fusion because it is physically unstable when formed into liposomes. A variety of minor perturbations could then trigger coalescence.

2.19 The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes

Anderson, R.A. et al
Atherosclerosis, **154**, 475-483 (2001)

Post-prandial lipaemia (PPL) is a factor in atherosclerosis and results in reversible endothelial dysfunction in healthy individuals. Oxidative stress and triglyceride (TG)-rich lipoproteins have been implicated. Type 2 diabetes (NIDDM) results in exaggerated PPL. We attempted to delineate the mechanisms of PPL induced, endothelial dysfunction (EF) and oxidative stress in 12 NIDDM and 12 matched healthy subjects. Subjects underwent a fat tolerance test, with endothelial function assessed by flow-mediated vasodilatation and oxidative stress measured by venous lipid-derived free radicals *ex vivo* and lipid peroxidation products over the postprandial phase. Fasting TG, post-prandial hypertriglyceridaemia and the TG enrichment of all lipoproteins was significantly greater in NIDDM. Post-prandial endothelial function inversely correlated with fasting HDL-C ($r = -0.84$, $P = 0.001$) in both the control and NIDDM groups. The deterioration in EF in the NIDDM group also correlated with TG enrichment of VLDL and LDL. PPL in both groups also resulted in increased oxidative stress. The increment in free radicals correlated with TG enrichment of VLDL in both groups and was, therefore, greater in NIDDM. Thus, PPL – with the production of TG-

enrichment of VLDL – results in endothelial dysfunction by an oxidative stress mechanism in both groups. The magnitude is greater in NIDDM. Fasting HDL-C appears to contribute to the protection of the endothelium against this phenomenon. Hence, exaggerated PPL associated with reduced HDL-C may be important in the pathogenesis of vascular disease, particularly in NIDDM

2.20 **Rapid identification of LDL subclass phenotypes by iodixanol gradient centrifugation**

Davis, I.G. and Griffin, B.A.

Atherosclerosis, **159**, 247-252 (2001)

A rapid and simple method was developed to identify LDL subclass phenotypes for the classification of an atherogenic lipoprotein phenotype. Plasma (3 ml adjusted to 12% **iodixanol**) was pre-stained (Coomassie Blue R250), under-layered beneath 9% iodixanol and subject to ultracentrifugation (3 h, 65,000 rpm 16°C, (341,000 g)) in a near vertical rotor (Beckman NVT65). A digital photograph of separated LDL bands was downloaded onto a PC and analysed using Total Lab ID gel-scan software (Pharmacia, UK). LDL subclass phenotypes were characterised by Rf values, density intervals and by cross-reference to LDL subclass profiles obtained by our established salt density gradient technique. LDL profiles corresponded to a predominance of light LDL-I, LDL-II or small dense LDL-III. This new method provides a more rapid separation of LDL subclasses than existing salt gradients, with multiple runs and larger rotors (Beckman NVT 65.2 or VTi 65.2) increasing throughput to 48 samples in 24 h.

2.21 **Direct evidence for a two-step assembly of ApoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum of rabbit enterocytes**

Cartwright, I.J. and Higgins, J.A.

J. Biol. Chem, **276**(51), 48048-48057(2001)

The aim of this study was to investigate the types and characteristics of chylomicrons precursor in the lumen of the secretory compartment of rabbit enterocytes. Luminal contents were separated into density subfractions in two continuous self-generating gradients of different density profiles. In enterocytes from rabbits fed a low fat diet, newly synthesized and immunodetectable apoB48 was only in the subfraction of density similar to high density lipoprotein (dense particles); the luminal triacylglycerol (TAG) content was low and only in the subfraction of density similar to that of chylomicrons/very low density lipoproteins (light particles). After feeding fat, newly synthesized and immunodetectable apoB48 was in both dense (phospholipid-rich) and light (TAG-rich) particles. Luminal TAG mass and synthesis increased after fat feeding and was only in light particles. Pulse-chase experiments showed that the luminal-radiolabeled apoB48 lost from the dense particles was recovered in the light particles and the secreted chylomicrons. All of the light particle lipids (mass and newly synthesized) co-immunoprecipitated with apoB48. However, in the dense particles, there was a preferential co-precipitation of the preexisting rather than newly synthesized phospholipid. Assembly of apoB48-containing TAG-enriched lipoproteins is therefore a two-step process. The first step produces dense apoB48 phospholipid-rich particles, which accumulate in the smooth endoplasmic reticulum lumen. In the second step, these dense particles rapidly acquire the bulk of the TAG and additional phospholipid in a single and rapid step.

2.22 **Superior role of apolipoprotein B48 over apolipoprotein B100 in chylomicron assembly and fat absorption: an investigation of apobec-1 knock-out and wild-type mice.**

Kendrick, J.S., Chan, L., and Higgins, J.A.

Biochem. J., **356**, 821-827 (2001)

Editing of apolipoprotein (apo)-B100 mRNA to yield apo-B48 is a specific and developmentally regulated step in enterocytes of mammals. However, the functional significance of this step is not known. Since mice containing only apo-B100 have not been documented to exhibit any difference in intestinal fat absorption from wild-type mice, the evolutionary advantage of apoB mRNA editing has been questioned. In the present study, we have compared fat absorption and chylomicron assembly in apobec-1 knock-out (KO) or wild-type (WT) mice subjected to different dietary manipulations: low-fat chow, a fat-enriched “Western” diet and overnight fasting. Experiments in vivo and in vitro revealed differences in the ability of KO and WT enterocytes to assemble and secrete chylomicrons under different dietary conditions. After overnight fasting, chylomicrons secretion is reduced considerably in KO compared with WT enterocytes. This is not due to reduced synthesis of apo-B or triacylglycerol (TAG), but appears to be a result of impaired assembly of chylomicrons, so that triacylglycerol accumulates in the enterocytes. After feeding with fat, secretion of chylomicrons enriched in pre-existing TAG is stimulated in KO compared with WT mice. IN the present study, we have documented for the first time that apo-B100 is considerably less efficient that

apo-B48 in exerting its role in the early stage of chylomicron assembly, which is rate-limiting in the fat-fed state. Apo-B mRNA editing may result in more efficient fat absorption, specifically under conditions of food shortage or low-fat content, and thus provide an evolutionary advantage.

2.23 Vesicle permeabilization by protofibrillar α -synuclein: Implications for the pathogenesis and treatment of Parkinson's disease

Volles, M.J. et al

Biochemistry, **40**, 7812-7819 (2001)

Fibrillar alpha-synuclein is a component of the Lewy body, the characteristic neuronal inclusion of the Parkinson's disease (PD) brain. Both alpha-synuclein mutations linked to autosomal dominant early-onset forms of PD promote the in vitro conversion of the natively unfolded protein into ordered prefibrillar oligomers, suggesting that these protofibrils, rather than the fibril itself, may induce cell death. We report here that protofibrils differ markedly from fibrils with respect to their interactions with synthetic membranes. Protofibrillar alpha-synuclein, in contrast to the monomeric and the fibrillar forms, binds synthetic vesicles very tightly via a beta-sheet-rich structure and transiently permeabilizes these vesicles. The destruction of vesicular membranes by protofibrillar alpha-synuclein was directly observed by atomic force microscopy. The possibility that the toxicity of alpha-synuclein fibrillization may derive from an oligomeric intermediate, rather than the fibril, has implications regarding the design of therapeutics for PD.

2.24 A rapid single-step centrifugation method for determination of HDL, LDL, and VLDL cholesterol, and TG, and identification of predominant LDL subclass

Sawle, A., Higgins, M.K., Olivant, M.P., and Higgins, J.A.

J. Lipid Res., **43**, 335-343 (2002)

Determination of the circulating levels of plasma lipoprotein HDL, LDL, and VLDL is critical in the assessment of risk of coronary heart disease. More recently it has become apparent that the LDL subclass pattern is a further important diagnostic parameter. The reference method for separation of plasma lipoproteins is ultracentrifugation. However, current methods often involve prolonged centrifugation steps and use high salt concentrations, which can modify the lipoproteins structure and must be removed before further analysis. To overcome these problems we have now investigated the use of rapid self-generating gradients of **iodixanol** for separation and analysis of plasma lipoproteins. A protocol is presented in which HDL, LDL, and VLDL, characterized by electron microscopy and agarose gel electrophoresis, separate in three bands in a 2.5 h centrifugation step. Recoveries of cholesterol and TG from the gradient were close to 100%. The distribution profiles of cholesterol and TG in the gradient were used to calculate the concentrations of individual lipoprotein classes. The values correlated with those obtained using commercial kits for HDL and LDL cholesterol. The position of the LDL peak in the gradient and its shape varied between plasma samples and was indicative of the density of the predominant LDL class. The novel protocol offers a rapid, reproducible and accurate single-step centrifugation method for the determination of HDL, LDL, and VLDL, cholesterol, and TG, and identification of LDL subclass pattern.

2.25 Persistent and transient replication of full-length hepatitis C virus genomes in cell culture

Pietschmann, T. et al

J. Virol., **76**, 4008-4021 (2002)

The recently developed subgenomic hepatitis C virus (HCV) replicons were limited by the fact that the sequence encoding the structural proteins was missing. Therefore, important information about a possible influence of these proteins on replication and pathogenesis and about the mechanism of virus formation could not be obtained. Taking advantage of three cell culture-adaptive mutations that enhance RNA replication synergistically, we generated selectable full-length HCV genomes that amplify to high levels in the human hepatoma cell line Huh-7 and can be stably propagated for more than 6 months. The structural proteins are efficiently expressed, with the viral glycoproteins E1 and E2 forming heterodimers which are stable under nondenaturing conditions. No disulfide-linked glycoprotein aggregates were observed, suggesting that the envelope proteins fold productively. Electron microscopy studies indicate that cell lines harboring these full-length HCV RNAs contain lipid droplets. The majority of the core protein was found on the surfaces of these structures, whereas the glycoproteins appear to localize to the endoplasmic reticulum and cis-Golgi compartments. In agreement with this distribution, no endoglycosidase H-resistant forms of these proteins were detectable. In a search for the production of viral particles, we noticed that these cells release substantial amounts of nuclease-resistant HCV RNA-containing structures with a buoyant density of 1.04 to 1.1 g/ml in iodixanol gradients. The same observation was made in transient-

replication assays using an authentic highly adapted full-length HCV genome that lacks heterologous sequences. However, the fact that comparable amounts of such RNA-containing structures were found in the supernatant of cells carrying subgenomic replicons demonstrates a nonspecific release independent of the presence of the structural proteins. These results suggest that Huh-7 cells lack host cell factors that are important for virus particle assembly and/or release.

2.26 Protein interactions with myocilin

Wentz-Hunter, K., Ueda, J. and Yue, B.Y.J.T.
Invest. Ophthalmol. Vis. Sci., 43(1), 176-182 (2002)

PURPOSE. To identify factors that interact in vivo with myocilin, a glaucoma gene product.

METHODS. The yeast two-hybrid system with myocilin as the bait and a human skeletal muscle cDNA library as the prey was used to identify potential factors that interact with myocilin. Interactions were also examined in bovine trabecular meshwork (TM) cells through a mammalian two-hybrid system. Biochemical coimmunoprecipitation from both human TM cell lysate and in vitro translated proteins was also used to confirm results obtained from yeast analysis.

RESULTS. Twenty positive clones isolated through yeast two-hybrid screening were deemed potential myocilin partners. Sequence analysis determined that two of them encoded for myocilin from amino acids 64 to 268. Myocilin was also found to interact with a component of the myosin motor protein, myosin regulatory light chain (RLC). The myocilin–myocilin and myocilin–RLC interactions revealed by the yeast system were further confirmed and demonstrated in cultured TM cells, by means of a mammalian two-hybrid system, and through biochemical coimmunoprecipitation, subcellular fractionation, immunofluorescence, and immunogold double labeling.

CONCLUSIONS. These results indicate that myocilin can form homomultimers in vivo, independent of the olfactomedin-like domain. Further analysis established that the leucine zipper motif of myocilin may be necessary for the myocilin–RLC interaction. The interaction of myocilin with RLC, a component of the myosin motor protein complex, implies a role for myocilin in the actomyosin system, linking in turn this novel protein to functional status of the TM.

2.27 Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation

Ceriello, A.C. et al
Circulation, 106, 1211-1218 (2002)

Background— Postprandial hypertriglyceridemia and hyperglycemia are considered risk factors for cardiovascular disease. Evidence suggests that postprandial hypertriglyceridemia and hyperglycemia induce endothelial dysfunction through oxidative stress; however, the distinct role of these two factors is a matter of debate.

Methods and Results— Thirty type 2 diabetic patients and 20 normal subjects ate 3 different meals: a high-fat meal; 75 g glucose alone; and high-fat meal plus glucose. Glycemia, triglyceridemia, nitrotyrosine, and endothelial function were assayed during the tests. Subsequently, diabetics took 40 mg/d simvastatin or placebo for 12 weeks. The 3 tests were performed again at baseline, between 3 to 6 days after the start, and at the end of each study. High-fat load and glucose alone produced a decrease of endothelial function and an increase of nitrotyrosine in normal and diabetic subjects. These effects were more pronounced when high fat and glucose were combined. Short-term simvastatin treatment had no effect on lipid parameters but reduced the effect on endothelial function and nitrotyrosine observed during each different test. Long-term simvastatin treatment was accompanied by a lower increase in postprandial triglycerides, which was followed by smaller variations of endothelial function and nitrotyrosine during the tests.

Conclusions— This study shows an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial function, suggesting oxidative stress as common mediator of such effect. Simvastatin shows a beneficial effect on oxidative stress and endothelial dysfunction, which may be ascribed to a direct effect as well as the lipid-lowering action of the drug.

- 2.28 **Characterization of HCV RNA particles from the serum of a patient with common variable immunodeficiency on isotonic iodixanol (Optiprep) gradients. Association with apolipoprotein-B100**
Nielsen, S. et al
J. Hepatol., 36, Suppl. 1, 87 (2002)

Objective: To investigate the association of HCV RNA and lipoprotein in the serum of an antibody negative patient with common variable immunodeficiency.

Methods: The serum collected six weeks post-orthotopic liver transplant from a patient with common variable immunodeficiency and chronic HCV infection was fractionated by sequential density centrifugation on sodium bromide and by isopycnic centrifugation on isotonic **iodixanol (Optiprep)**.

Results: Whilst sequential density centrifugation yielded HCV RNA fractions of high (> 1.2 g/ml), intermediate (1.063-1.21 g/ml) and low (< 1.063 g/ml) density of approximately equal titre, on iodixanol gradients all the RNA was recovered at the top of the gradient with a density below 1.13 g/ml. Following precipitation with anti-apoB100 or manganese chloride and heparin the majority of the HCV RNA was removed from the serum leaving only a minor peak with a density of 1.13 g/ml on iodixanol gradients. Treatment of sera with sodium desoxycholate released further particles with a density of 1.13 g/ml in iodixanol gradients, suggesting that this is the density of the virus particle stripped of serum lipoproteins. Treatment with 0.18% NP40 produced a single peak of HCV RNA in a fraction with a density of 1.23 g/ml and a sedimentation coefficient of 150S in iodixanol gradients, taken to be naked viral cores.

Conclusions: Fractionation of HCV RNA on iodixanol in isotonic conditions indicates that essentially all RNA is present in lower density fractions and is associated with low density or very low density lipoproteins.

- 2.29 **Characterization of the structural proteins of HCV isolated from human liver**
Nielsen, S. et al
J. Hepatol., 36, Suppl. 1, 87 (2002)

Objective: To determine the molecular weights of the structural protein of HCV recovered from infected human liver.

Methods: Macerates of a six week post-orthotopic liver transplant from a patient with common variable immunodeficiency and chronic HCV infection were shown to contain 9 LogIU of HCV RNA/g. Macerates were analyzed by isopycnic centrifugation on **iodixanol** density gradients. HCV RNA was precipitated from crude macerates with manganese chloride and heparin and the solubilised precipitate was analysed by SDS-PAGE and western blotting with monoclonal antibodies to the viral structural proteins.

Results: Following fractionation of the liver macerate on iodixanol density gradients all the HCV RNA was recovered in fractions of density of 1.13 g/ml and below suggesting that the RNA is associated with host lipoprotein. In line with this, manganese/heparin treatment precipitated essentially all of the HCV RNA. SDS-polyacrylamide gel electrophoresis of the proteins present in manganese/heparin precipitates and western blotting revealed a single band of core protein of molecular weight 21 kDa and an E1 band of 31 kDa which migrated in approximately the same position in the gel as the corresponding proteins transiently expressed from a recombinant vaccinia virus system. A single E2 band, however, migrated with a molecular weight of 62 kDa some 8 kDa smaller than the equivalent band expressed from recombinant vaccinia virus.

Conclusions: The molecular weights of the HCV structural proteins recovered from beta-lipoprotein associated virions suggest that processing of the virus polyprotein in the liver may differ from that recombinant vaccinia virus expression system.

2.30 **Characterization of cytoplasmic α -synuclein aggregates**

Lee, H-J. and Lee, S-J.

J. Biol. Chem., **277**, 48976-48983 (2002)

The α -synuclein fibrillation process has been associated with the pathogenesis of several neuro-degenerative diseases. Here, we have characterized the cytoplasmic α -synuclein aggregates using a fractionation procedure with which different aggregate species can be separated. Overexpression of α -synuclein in cells produce two distinct types of aggregates: large juxtannuclear inclusion bodies and small punctate aggregates scattered throughout the cytoplasm. Biochemical fractionation results in an inclusion-enriched fraction and two small aggregate fractions. Electron microscopy and thioflavin S reactivity of the fractions show that the juxtannuclear inclusion bodies are filled with amyloidlike α -synuclein fibrils, whereas both the small aggregate fractions contain non-fibrillar spherical aggregates with distinct size distributions. These aggregates appear sequentially, with the smallest population appearing the earliest and the fibrillar inclusions the latest. Based on the structural and kinetic properties, we suggest that the small spherical aggregates are the cellular equivalents of the protofibrils. The proteins that co-exist in the Lewy bodies, such as proteasome subunit, ubiquitin, and hsp70 chaperone, are present in the fibrillar inclusions but absent in the protofibrils, suggesting that these proteins may not be directly involved in the early aggregation stage. As predicted in the aggresome model, disruption of microtubules with nocodazole reduced the number of inclusions and increased the size of the protofibrils. Despite the increased size, the protofibrils remained non-fibrillar, suggesting that the deposition of the protofibrils in the juxtannuclear region is important in fibril formation. This study provides evidence that the cellular fibrillation also involves nonfibrillar intermediate species, and the microtubule-dependent inclusion-forming process is required for the protofibril-to-fibril conversion in cells.

2.31 **Properties of the chaperonin complexes from the halophilic archaeon *Haloferax volcanii***

Large, A.T., Kovacs, E. and Lund, P.A.

FEBS Lett., **532**, 309-312 (2002)

The halophilic archaeon *Haloferax volcanii* has three genes encoding type II chaperonins, named cct1, cct2 and cct3. We show here that the three CCT proteins are all expressed but not to the same level. All three proteins are further induced on heat shock. The CCT proteins were purified by ammonium sulphate precipitation, sucrose gradient centrifugation and hydrophobic interaction chromatography. This procedure yields a high molecular mass complex (or complexes). The complex has ATPase activity, which is magnesium dependent, low salt-sensitive and stable to at least 75°C. Activity requires high levels of potassium ions and was reduced in the presence of an increasing concentration of sodium ions.

2.32 **Methods for measuring lipid metabolism *in vivo***

Patterson, B. W.

Curr., Opin., Clin., Nutr., Metab. Care, **5**, 475-479 (2002)

Purpose of review

This review discusses diverse methods that have been used in several recent papers for the qualitative and quantitative analysis of lipids, studies of lipid oxidation, lipoprotein fractionation, and studies of lipid metabolism and metabolic kinetics using tracers. Papers for this review were selected on the basis of their timeliness, novelty, and/or their potential impact on diverse fields of lipid metabolism.

Recent findings

Many methods used for studies of lipid metabolism employ advanced chromatographic and mass spectrometric techniques to characterize lipids. In particular, the use of stable isotopically labeled tracers has become increasingly important to study metabolic kinetics.

Summary

Such developments in methodology will continue to advance studies of lipid metabolism in many areas of clinical interest, including heart disease, obesity, and diabetes.

2.33 **Functional reconstitution of purified metabotropic glutamate receptor expressed in the fly eye**

Eroglu, C., Croner, P., Panneels, V., Beaufils, P. and Sinning, I.

EMBO Reports, **3(5)**, 491-496 (2002)

G-protein-coupled receptors (GPCRs) form one of the largest superfamilies of membrane proteins. Obtaining high yields of GPCRs remains one of the major factors limiting a detailed understanding of their structure and function. Photoreceptor cells (PRCs) contain extensive stacks of specialized membranes

where high levels of rhodopsins are naturally present, which makes them ideal for the overexpression of GPCRs. We have generated transgenic flies expressing a number of GPCRs in the PRCs. *Drosophila melanogaster* metabotropic glutamate receptor (DmGluRA) expressed by this novel strategy was purified to homogeneity, giving at least 3-fold higher yields than conventional baculovirus expression systems due to the higher membrane content of the PRCs. Pure DmGluRA was then reconstituted into liposomes of varying composition. Interestingly, glutamate binding was strictly dependent on the presence of ergosterol.

2.34 Vesicular restriction of synaptobrevin suggests a role for calcium in membrane fusion

Hu, K. et al

Nature, **415**, 646-650 (2002)

Release of neurotransmitter occurs when synaptic vesicles fuse with the plasma membrane. This neuronal exocytosis is triggered by calcium and requires three SNARE (soluble-*N*-ethylmaleimide-sensitive factor attachment protein receptors) proteins: synaptobrevin (also known as VAMP) on the synaptic vesicle, and syntaxin and SNAP-25 on the plasma membrane. Neuronal SNARE proteins form a parallel four-helix bundle that is thought to drive the fusion of opposing membranes. As formation of this SNARE complex in solution does not require calcium, it is not clear what function calcium has in triggering SNARE-mediated membrane fusion. We now demonstrate that whereas syntaxin and SNAP-25 in target membranes are freely available for SNARE complex formation, availability of synaptobrevin on synaptic vesicles is very limited. Calcium at micromolar concentrations triggers SNARE complex formation and fusion between synaptic vesicles and reconstituted target membranes. Although calcium does promote interaction of SNARE proteins between opposing membranes, it does not act by releasing synaptobrevin from synaptic vesicle restriction. Rather, our data suggest a mechanism in which calcium-triggered membrane apposition enables syntaxin and SNAP-25 to engage synaptobrevin, leading to membrane fusion.

2.35 Massive and Selective Delivery of Lipid-Coated Cationic Lipoplexes of Oligonucleotides Targeted *in Vivo* to Hepatic Endothelial Cells

Bartsch, M., Weeke-Klump, A.H., Meijer, D.K.F., Scherphof, G.L. and Kamps, J.A.A.M.

Pharmaceutical Res., **19**(5), 676-680 (2002)

Purpose. Previously we reported on massive uptake of liposomes surface-modified with negatively charged aconitylated albumin (Aco-HSA) by liver sinusoidal endothelial cells (EC) *in vivo*. In the present work we applied this principle for the *in vivo* delivery of antisense oligonucleotides (ODN) to these cells.

Methods. Anti ICAM-1 ODN was complexed with the cationic lipid DOTAP and the complex was coated by an excess of neutral lipids including a lipid-anchored poly(ethylene glycol). Aco-HSA was coupled to the coated cationic lipoplexes (CCLs). Plasma disappearance, organ and intrahepatic distribution of Aco-HSA modified CCLs were determined in rats, using [³H]-cholesteryl oleyl ether and ³²P-labeled ODN as markers.

Results. The Aco-HSA coupled CCLs were <160 nm in size, contained 1.03 ±

0.35 nmol odn and 54 ± 18 μg Aco-HSA per μmol total lipid. These CCLs were rapidly eliminated from plasma, about 60% the injected dose of ³H- or ³²P-label being recovered in the liver after 30 min. Within the liver, the EC accounted for two thirds of total liver uptake. Control non-targeted CCLs were eliminated very slowly: after 30 min still >90% of the particles was in the blood.

Conclusions. Our results demonstrate efficient targeting of antisense ODN to EC *in vivo*, employing plasma-stable coated cationic lipoplexes, surface modified with negatively charged albumin. 40% of the injected ODN was delivered to the target cells within 30 min.

2.36 Antioxidant properties of aged garlic extract: an *in vitro* study incorporating human low density lipoprotein

Dillon, S.A., Burmi, R.S., Lowe, G.M., Billington, D. and Rahman, K.

Life Sciences, **72**, 1538-1594 (2003)

Oxidation of low-density lipoprotein (LDL) has been recognized as playing an important role in the development and progression of atherosclerotic heart disease. Human LDL was isolated and challenged with a range of oxidants either in the presence or absence of AGE or its diethyl ether extract. Oxidative modification of the LDL fraction using CuSO₄, 5-lipoxygenase and xanthine/xanthine oxidase was monitored by both the appearance of thiobarbituric-acid substances (TBA-RS) and an increase in electrophoretic mobility. This study indicates that AGE is an effective antioxidant as it scavenged superoxide ions and reduced lipid peroxide formation in cell free assays. Superoxide production was

completely inhibited in the presence of a 10% (v/v) aqueous preparation of AGE and reduced by 34% in the presence of a 10% (v/v) diethyl ether extract of AGE. The presence of 10% (v/v) diethyl ether extract of AGE significantly reduced Cu^{2+} and 15-lipoxygenase-mediated lipid peroxidation of isolated LDL by 81% and 37%, respectively. In addition, it was found that AGE also had the capacity to chelate copper ions. In contrast, the diethyl ether extract of AGE displayed no copper binding capacity, but demonstrated distinct antioxidant properties. These results support the view that AGE inhibits the in vitro oxidation of isolated LDL by scavenging superoxide and inhibiting the formation of lipid peroxides. AGE was also shown to reduce LDL oxidation by the chelation of Cu^{2+} . Thus, AGE may have a role to play in preventing the development and progression of atherosclerotic disease.

2.37 Separation of bovine plasma lipoproteins by a rapid ultracentrifugation method

Gardner, R.S., Ogden, N.H., Cripps, P.J. and Billington, D.
J. Comp. Path., **128**, 15-23 (2003)

The recently described method of centrifugation with iodixanol for the rapid separation of human plasma lipoproteins was adapted to separate bovine plasma lipoproteins. Density gradients were generated by mixing plasma with iodixanol 12% (w/v), followed by centrifugation at 350000g and 16 degrees C for 3h 10min in a vertical rotor. Gradients were unloaded dense-end first into 10 fractions. Human very low density lipoprotein (VLDL; density <1.011g/ml), low density lipoprotein (LDL; density = 1.016-1.039 g/ml) and high density lipoprotein (HDL; density = 1.039-1.090 g/ml) were resolved well at densities considerably lower than those traditionally reported in salt gradients. In gradients generated from 12% iodixanol, bovine LDL and HDL exhibited even lower densities (1.016-1.028 and 1.016-1.048g/ml, respectively) with all lipoproteins occurring at the lower density region of the gradient. In contrast, density gradients generated from layers of equal volumes of 6% and 12% iodixanol readily separated bovine HDL from VLDL, whilst LDL still overlapped with HDL. The latter accounts for >80% of all bovine lipoproteins and exists as two populations, namely light and heavy HDL. Gradients generated from two layers of iodixanol recovered bovine HDL in five fractions. The hypercholesterolaemia associated with lactation resulted in a modest shift in the profile of HDL cholesterol towards lipoprotein particles of lower density (light HDL). Significant between-farm differences were also detected in the density profiles of bovine plasma cholesterol. This new method is suitable for use in research and diagnosis in relation to lipoprotein metabolism disorders in cows.

2.38 Characterization of the genome and structural proteins of β -lipoprotein associated HCV extracted from infected human liver

Nielsen, S., Bassendine, M.F., Burt, A. And Toms, G.L.
GUT, Abstract from the British association for the study of liver meeting 2002, abstract 94 (2003)

Serum and liver macerates from a patient with common variable immunodeficiency and chronic HCV infection six weeks post-transplant were analysed by isopycnic centrifugation on isotonic iodixanol (**optiprep**) density gradients. All of the HCV RNA fractionated at the top of the gradient, in fractions of density of < 1.13 g/ml and was precipitable with manganese chloride and heparin (Mn/Hep) indicating that it is all associated with host beta-lipoprotein. Treatment with desoxycholate released putative hepatitis C virions with a density of 1.13 g/ml and treatment with 0.18% NP40 released putative virus cores with a density of 1.21 g/ml and a sedimentation coefficient of 150S. Northern blotting of Mn/Hep precipitates revealed a single band of HCV RNA of 9.4 kb corresponding in size to the full HCV genome. SDS-polyacrylamide gel electrophoresis and western blotting with monoclonal antibodies revealed a single 20 kDa band of core protein and a single 31 kDa E1 band reduced to 20 kDa after deglycosylation with endoglycosidase F. Both core and E1 bands co-migrated with corresponding bands derived from a recombinant vaccinia virus system expressing the HCV structural protein genes. Anti-E2 Mabs blotted a single 62 kDa band from Mn/Hep precipitates, approximately 7 kDa smaller than the anti-E2 staining band in the recombinant vaccinia virus system. Following deglycosylation, E2 glycoprotein ran with an apparent molecular weight of 36 kDa, co-migrating with E2, which forms a minor band in the deglycosylated vaccinia virus recombinant. No band equivalent to the major 40 kDa E2/P7 band in the deglycosylated vaccinia virus system was observed in the Mn/Hep precipitates, indicating that E2/P7 is not a structural protein. Under reducing conditions both E1 and E2 ran as monomers, suggesting that the two glycoproteins are not disulphide linked in the virion.

2.39 A novel role for CD36 in VLDL-enhanced platelet activation

Englyst, N.A., Taube, J.M., Aitman, T.J., Baglin, T.P. and Byrne C.D.
Diabetes, **52**, 1248-1255 (2003)

Type 2 diabetes is characterized by increased plasma triglyceride levels and a fourfold increase in ischemic heart disease, but the mechanism is unclear. CD36 is a receptor/transporter that binds fatty acids of lipoproteins. CD36 deficiency has been linked with insulin resistance. There is strong evidence of in vivo interaction between platelets and atherogenic lipoproteins suggesting that atherogenic triglyceride-rich lipoproteins, such as VLDL, that are increased in diabetic dyslipidemia are important in this process. This study demonstrates that VLDL binds to the platelet receptor CD36, enhances platelet thromboxane A2 production, and causes increased collagen-mediated platelet aggregation. VLDL enhanced collagen-induced platelet aggregation by 1) shortening the time taken for aggregation to begin (lag time) to 70% of control ($P = 0.001$); 2) increasing maximum aggregation to 170% of control ($P = 0.008$); and 3) increasing thromboxane production to 3,318% of control ($P = 0.004$), where control represents platelets stimulated with collagen (100%). A monoclonal antibody against CD36 attenuated VLDL-enhanced collagen-induced platelet aggregation by 1) inhibiting binding of VLDL to platelets by 75% ($P = 0.041$); 2) lengthening lag time to 190% ($P < 0.001$); and 3) decreasing thromboxane production to 8% of control ($P < 0.001$). In support of this finding, platelets from Cd36-deficient rats showed no increase in aggregation, thromboxane production, and VLDL binding in contrast to platelets from rats expressing CD36. These data suggest that platelet Cd36 has a key role in VLDL-induced collagen-mediated platelet aggregation, possibly contributing to atherothrombosis associated with increased VLDL levels.

2.40 Rapid separation of LDL subclasses by iodixanol gradient ultracentrifugation

Davies, I.G., Graham, J.M. and Griffin, B.A.
Clin. Chem., **49(11)**, 1865-1872 (2003)

Background: A predominance of small, dense LDL (sdLDL) confers in excess of a threefold increase in coronary heart disease (CHD) risk. The conventional method for the detection of sdLDL, salt density gradient ultracentrifugation (DGUC) has been superseded by more rapid techniques. This report presents novel methodology for the separation of sdLDL by a combination of iodixanol density gradient centrifugation and digital photography.

Methods: LDL subclasses were separated in 3 h from prestained plasma on a self-forming density gradient of iodixanol. LDL subclass profiles were generated by digital photography and gel-scan software. Plasma samples from 106 normo- and dyslipidemic individuals were used to optimize the gradient for the resolution of LDL heterogeneity. A subgroup of 47 LDL profiles were then compared with LDL subclasses separated by salt DGUC.

Results: The peak density of the predominant LDL band correlated significantly with the relative abundance (as a percentage) of sdLDL as resolved by salt DGUC ($P < 0.001$). As shown previously, LDL isolated at a lighter density in iodixanol compared with salt gradients. A predominance of sdLDL corresponded to a peak density on iodixanol of 1.028 kg/L. This density and the area under the LDL profile lying above this density were sensitive and specific markers for the prediction of a predominance of sdLDL ($P < 0.001$) and showed predictable associations with plasma triglycerides ($r = 0.59$; $P < 0.001$) and HDL ($r = -0.4$; $P < 0.001$).

Conclusions: This simple method for the detection of sdLDL can differentiate a predominance of sdLDL, is highly reproducible, and can be used preparatively to isolate sdLDL.

2.41 Protein-protein, protein-RNA and protein-lipid interactions of signal-recognition particle components in the hyperthermoacidophilic archeon *Arcidianus ambivalens*

Moll, R.G.
Biochem. J., **374**, 247-254 (2003)

The signal-recognition particle (SRP) of one of the most acidophilic and hyperthermophilic archaeal cells, *Arcidianus ambivalens*, and its putative receptor component, FtsY (prokaryotic SRP receptor), were investigated in detail. *A. ambivalens* Ffh (fifty-four-homologous protein) was shown to be a soluble protein with strong affinity to membranes. In its membrane-residing form, Ffh was extracted from plasma membranes with chaotropic agents like urea, but not with agents diminishing electrostatic interactions. Using unilamellar tetraether phospholipid vesicles, both Ffh and FtsY associate independently from each other in the absence of other factors, suggesting an equilibrium of soluble and membrane-bound protein forms under *in vivo* conditions. The Ffh protein precipitated from cytosolic cell supernatants with anti-Ffh antibodies, together with an 7 S-alike SRP-RNA, suggesting a stable core ribonucleoprotein composed of both components under native conditions. The SRP RNA of *A. ambivalens* depicted a size of about 309

nucleotides like the SRP RNA of the related organism *Sulfolobus acidocaldarius*. A stable heterodimeric complex composed of Ffh and FtsY was absent in cytosolic super-natants, indicating a transiently formed complex during archaeal SRP targeting. The FtsY protein precipitated in cytosolic super-natants with anti-FtsY antisera as a homomeric protein lacking accessory protein components. However, under *in vitro* conditions, recombinantly generated Ffh and FtsY associate in a nucleotide-independent manner, supporting a structural receptor model with two interacting apoproteins.

2.42 **The effect of CTAB concentration in cationic PLG microparticles on DNA adsorption and *in vivo* performance**

Singh, M. et al

Pharmaceut. Res., **20**(2), 247-251 (2003)

Purpose. Cationic PLG microparticles with adsorbed DNA have previously been shown to efficiently target antigen presenting cells *in vivo* for generating higher immune responses in comparison to naked DNA. In this study we tried to establish the role of surfactant (CTAB) concentration on the physical behavior of these formulations.

Methods. Cationic PLG microparticle formulations with adsorbed DNA were prepared using a solvent evaporation technique. Formulations with varying CTAB concentrations and a fixed DNA load were prepared. The loading efficiency and 24 h DNA release was evaluated for each formulation. Select formulations were tested *in vivo*.

Results. Higher CTAB concentration correlated with higher DNA binding efficiency on the microparticles and lower *in vitro* release rates. Surprisingly though, the *in vivo* performance of formulations with varying CTAB concentration was comparable to one another.

Conclusions. Cationic PLG microparticles with adsorbed DNA, as described here, offer a robust way of enhancing *in vivo* responses to plasmid DNA.

2.43 **Trans Unsaturated Fatty Acids Are Less Oxidizable than Cis Unsaturated Fatty Acids and Protect Endogenous Lipids from Oxidation in Lipoproteins and Lipid Bilayers**

Sargis, R.M. and Subbaiah, P.V.

Biochemistry, **42**, 11533-11543 (2003)

Epidemiological data suggest that dietary *trans* unsaturated fatty acids increase the risk of heart disease; however, the underlying mechanisms are unclear. In this study, we investigated one possible mechanism, namely, their effect on LDL oxidation. Supplementation of LDL with 10% 16:1 *trans*-cholesteryl ester (CE) inhibited the oxidation compared to that with 16:1 *cis*-CE. Total replacement of core lipids with 18:2 *trans,trans*-CE decreased the rate of LDL oxidation by 19% compared to replacement with 18:2 *cis,cis*-CE. When the surface phosphoglycerides were replaced with either 16:0-18:2 *cis,cis*-phosphatidylcholine (PC) or 16:0-18:2 *trans,trans*-PC, the latter was found to inhibit the rate and increase the lag time of oxidation to a greater extent than the former. To confirm these findings, we studied the oxidation of PC liposomes by assessing the formation of conjugated dienes or the degradation of a fluorescently labeled PC. By both methods, the 16:0-18:2 *trans,trans*-PC exhibited greater resistance to oxidation than the 16:0-18:2 *cis,cis*-PC. Eliminating the fluidity differences did not completely eliminate the differences in oxidation rates, suggesting that the *trans* double bond is inherently resistant to oxidation. The composition of the conjugated hydroperoxy products formed after oxidation differed markedly for the two 18:2 isomers. Supplementation of 16:0-18:2 *cis,cis*-PC liposomes with 20 mol % di16:1 *trans*-PC retarded oxidation rates to a greater extent than supplementation with di16:1 *cis*-PC. These studies show that dietary *trans* unsaturated fatty acids decrease the rate of lipid peroxidation, an effect that may mitigate the atherogenic effect of these fatty acids.

2.44 **Copper-mediated LDL oxidation by homocysteine and related compounds depends largely on copper ligation**

Nakano, E., Williamson, M.P., Williams, N.H. and Powers, H.J.

Biochim. Biophys. Acta, **1688**, 33-42 (2004)

Oxidation of low-density lipoprotein (LDL) is thought to be a major factor in the pathophysiology of atherosclerosis. Elevated plasma homocysteine is an accepted risk factor for atherosclerosis, and may act through LDL oxidation, although this is controversial. In this study, homocysteine at physiological concentrations is shown to act as a pro-oxidant for three stages of copper-mediated LDL oxidation (initiation, conjugated diene formation and aldehyde formation), whereas at high concentration, it acts as an antioxidant. The affinity for copper of homocysteine and related copper ligands homocysteine,

cystathionine and djenkolate was measured, showing that at high concentrations (100 μ M) under our assay conditions, they bind essentially all of the copper present. This is used to rationalise the behaviour of these ligands, which stimulate LDL oxidation at low concentration but generally inhibit it at high concentration. Albumin strongly reduced the effect of homocystine on lag time for LDL oxidation, suggesting that the effects of homocystine are due to copper binding. In contrast, copper binding does not fully explain the pro-oxidant behaviour of low concentrations of homocysteine towards LDL, which appears in part at least to be due to stimulation of free radical production. The likely role of homocysteine in LDL oxidation in vivo is discussed in the light of these results.

2.45 Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment

Ceriello, A. et al

Diabetes, **53**, 701-710 (2004)

Adhesion molecules, particularly intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin, have been associated with cardiovascular disease. Elevated levels of these molecules have been reported in diabetic patients. Postprandial hypertriglyceridemia and hyperglycemia are considered risk factors for cardiovascular disease, and evidence suggests that postprandial hypertriglyceridemia and hyperglycemia may induce an increase in circulating adhesion molecules. However, the distinct role of these two factors is a matter of debate. Thirty type 2 diabetic patients and 20 normal subjects ate three different meals: a high-fat meal, 75 g of glucose alone, and a high-fat meal plus glucose. Glycemia, triglyceridemia, plasma nitrotyrosine, ICAM-1, VCAM-1, and E-selectin were assayed during the tests. Subsequently, diabetic subjects took simvastatin 40 mg/day or placebo for 12 weeks. The three tests were performed again at baseline, between 3 and 6 days after starting the study, and at the end of each study. High-fat load and glucose alone produced an increase of nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels in normal and diabetic subjects. These effects were more pronounced when high fat and glucose were combined. Short-term simvastatin treatment had no effect on lipid parameters, but reduced the effect on adhesion molecules and nitrotyrosine, which was observed during every different test. Long-term simvastatin treatment was accompanied by a lower increase in postprandial triglycerides, which was followed by smaller variations in ICAM-1, VCAM-1, E-selectin, and nitrotyrosine during the tests. This study shows an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on ICAM-1, VCAM-1, and E-selectin plasma levels, suggesting oxidative stress as a common mediator of such effects. Simvastatin shows a beneficial effect on oxidative stress and the plasma levels of adhesion molecules, which may be ascribed to a direct effect in addition to the lipid-lowering action of the drug.

2.46 Effects of Rosiglitazone on endothelial function in men with coronary artery disease without diabetes mellitus

Sidhu, J.S., Cowan, D. and Kaski, J.C.

Am. J. Cardiol., **94**, 151-156 (2004)

Recent data have shown that peroxisome proliferator-activated receptor- γ agonists may exert protective effects on the vascular endothelium by amelioration of insulin resistance and through direct anti-inflammatory effects. In this study we assessed the effect of rosiglitazone on biochemical and biophysical indexes of endothelial function in male, nondiabetic patients with coronary artery disease. Consecutive male subjects ($n = 71$) with clinically stable, angiographically documented coronary artery disease and without diabetes mellitus were investigated. Patients were randomized in a double-blind manner to placebo or rosiglitazone for a total of 24 weeks. Flow-mediated dilation (FMD) of the brachial artery, C-reactive protein, von Willebrand factor, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 levels, and parameters of glucose and lipid metabolism were measured at baseline and after 12 and 24 weeks of treatment. Rosiglitazone treatment significantly reduced C-reactive protein (median 0.56 mg/L [interquartile range 0.33 to 1.02] to 0.33 mg/L [interquartile range 0.26 to 0.40], $p < 0.01$), von Willebrand factor (139 ± 47 to 132 ± 44 IU/dl, $P = 0.02$), insulin resistance index ($p = 0.05$), and mean low-density lipoprotein (LDL) density ($p < 0.001$) compared with placebo. However, no significant differences were seen between the rosiglitazone and placebo groups with regard to brachial artery FMD, intercellular adhesion molecule-1, or vascular cell adhesion molecule-1 levels. Rosiglitazone treatment significantly increased LDL (2.62 ± 0.72 to 2.95 ± 0.84 mmol/L, $P = 0.03$) and triglyceride (1.23 ± 0.63 to 1.56 ± 0.98 mmol/L, $P = 0.04$) levels. Thus, rosiglitazone reduced markers of inflammation and endothelial activation, but this did not translate into an improvement in FMD. Increased LDL and triglyceride levels may have played a role.

- 2.47 Plasma appearance of unesterified astaxanthin geometrical E/Z and optical R/S isomers in men given single doses of a mixture of optical 3 and 3'R/S isomers of astaxanthin fatty acyl diesters**
Coral-Hinostroza, G.N., Ytrestøyl, T., Ruyter, B. and Bjerkeng, B.
Comp. Biochem. Biophys. Part C, **139**, 99-110 (2004)

Appearance, pharmacokinetics and distribution of astaxanthin all-*E*-, 9*Z*- and 13*Z*-geometrical and (3*R*,3'*R*)-, (3*R*,3'*S*)- and (3*S*,3'*S*)-optical isomers in plasma fractions were studied in three middle-aged male volunteers (41–50 years) after ingestion of a single meal containing first a 10-mg dose equivalent of astaxanthin from astaxanthin diesters, followed by a dose of 100 mg astaxanthin equivalents after 4 weeks. Direct resolution of geometrical isomers and optical isomers of astaxanthin dicamphanates by HPLC after saponification showed that the astaxanthin consisted of 95.2% all-*E*-, 1.2% 9*Z*- and 3.6% 13*Z*-astaxanthin, of (3*R*,3'*R*)-, (3*R*,3'*S*; *meso*)- and (3*S*,3'*S*)-astaxanthin in a 31:49:20 ratio. The plasma astaxanthin concentration–time curves were measured during 76 h. Astaxanthin esters were not detected in plasma. Maximum levels of astaxanthin ($C_{\max}=0.28\pm 0.1$ mg/l) were reached 11.5 h after administration and the plasma astaxanthin elimination half-life was 52 ± 40 h. The C_{\max} at the low dose was 0.08 mg/l and showed that, the dose response was non-linear. The (3*R*,3'*R*)-astaxanthin optical isomer accumulated selectively in plasma compared to the (3*R*,3'*S*)- and (3*S*,3'*S*)-isomers, and comprised 54% of total astaxanthin in the blood and only 31% of total astaxanthin in the administered dose. The astaxanthin *Z*-isomers were absorbed selectively into plasma and comprised $\sim 32\%$ of total astaxanthin 6–7.5 h postprandially. The proportion of all-*E*-astaxanthin was significantly higher in the very low density lipoproteins and chylomicrons (VLDL/CM) plasma lipoprotein fraction than in the high density lipoproteins (HDL) and low density lipoproteins (LDL) fractions ($P<0.05$). The results indicate that a selective process increase the relative proportion of astaxanthin *Z*-isomers compared to the all-*E*-astaxanthin before uptake in blood and that the astaxanthin esters are hydrolyzed selectively during absorption.

- 2.48 Prevention of Alzheimer's disease-associated A β aggregation by rationally designed nonpeptidic β -sheet ligands**
Rzepecki, P. et al
J. Biol. Chem., **279**(46), 47497-47505 (2004)

A new concept is introduced for the rational design of β -sheet ligands, which prevent protein aggregation. Oligomeric acylated aminopyrazoles with a donor-acceptor-donor (DAD) hydrogen bond pattern complementary to that of a β -sheet efficiently block the solvent-exposed β -sheet portions in A β -(1–40) and thereby prevent formation of insoluble protein aggregates. Density gradient centrifugation revealed that in the initial phase, the size of A β aggregates was efficiently kept between the trimeric and 15-meric state, whereas after 5 days an additional high molecular weight fraction appeared. With fluorescence correlation spectroscopy (FCS) exactly those two, *i.e.* a dimeric aminopyrazole with an oxalyl spacer and a trimeric head-to-tail connected aminopyrazole, of nine similar aminopyrazole ligands were identified as efficient aggregation retardants whose minimum energy conformations showed a perfect complementarity to a β -sheet. The concentration dependence of the inhibitory effect of a trimeric aminopyrazole derivative allowed an estimation of the dissociation constant in the range of 10^{-5} M. Finally, electrospray ionization mass spectrometry (ESI-MS) was used to determine the aggregation kinetics of A β -(1–40) in the absence and in the presence of the ligands. From the comparable decrease in A β monomer concentration, we conclude that these β -sheet ligands do not prevent the initial oligomerization of monomeric A β but rather block further aggregation of spontaneously formed small oligomers. Together with the results from density gradient centrifugation and fluorescence correlation spectroscopy it is now possible to restrict the approximate size of soluble A β aggregates formed in the presence of both inhibitors from 3- to 15-mers.

- 2.49 A common mechanism for the regulation of vesicular SNAREs on phospholipid membranes**
Hu, K., Rickman, C., Carroll, J. and Davletov, B.
Biochem. J., **377**, 781-795 (2004)

The SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor) family of proteins is essential for membrane fusion in intracellular traffic in eukaryotic organisms. v-SNAREs (vesicular SNAREs) must engage target SNAREs in the opposing membrane to form the fusogenic SNARE complex. Temporal and spatial control of membrane fusion is important for many aspects of cell physiology and may involve the regulation of the SNAREs resident on intracellular membranes. Here we

show that the v-SNARE synaptobrevin 2, also known as VAMP (vesicle-associated membrane protein) 2, is restricted from forming the SNARE complex in chromaffin granules from adrenal medullae to the same degree as in brain-purified synaptic vesicles. Our analysis indicates that the previously reported synaptophysin–synaptobrevin interaction is not likely to be involved in regulation of the v-SNARE. Indeed, the restriction can be reproduced for two distinct v-SNARE homologues, synaptobrevin 2 and cellubrevin/VAMP3, by reconstituting them in pure liposomal membranes. Overall, our data uncover a common mechanism for the control of SNARE engagement where intact phospholipid membranes rather than proteins down-regulate vesicular SNAREs in different cellular organelles.

2.50 Hyperlipidemic subjects have reduced uptake of newly absorbed vitamin E into their plasma lipoproteins, erythrocytes, platelets, and lymphocytes, as studied by deuterium-labeled α -tocopherol biokinetics

Hall, W.L., Jeanes, Y.M. and Lodge, J.K.
J. Nutr., **135**, 58-63 (2005)

Vitamin E homeostasis in hyperlipidemia is poorly understood. The biokinetics of deuterated α -tocopherol (α -T) in blood components was investigated in normolipidemic (N; total cholesterol < 5.5 mmol/L and triglycerides < 1.5 mmol/L, $n = 9$), hypercholesterolemic (HC; total cholesterol > 6.5 mmol/L and triglycerides < 1.5 mmol/L, $n = 10$), and combined hypercholesterolemic and hypertriglyceridemic (HCT; total cholesterol > 6.5 mmol/L and triglycerides > 2.5 mmol/L, $n = 6$) subjects. Subjects ingested 150 mg hexadeuterated *RRR*- α -tocopheryl acetate, and blood was collected up to 48 h after ingestion. Labeled α -T was measured in plasma, lipoproteins, erythrocytes, platelets, and lymphocytes by liquid chromatography/mass spectroscopy. In plasma, HC had an earlier time of maximum concentration (6 h) compared with N and HCT (12 h) ($P < 0.05$). HCT had a lower uptake of labeled α -T ($P < 0.005$) and a longer half-life ($P < 0.05$). In chylomicrons, the maximum labeled α -T concentration was higher in HC compared with N and HCT ($P < 0.00005$); however, HCT had a lower uptake of labeled α -T in LDL. In all groups, the lowest density LDL subfraction contained more labeled α -T than denser subfractions ($P < 0.05$). In platelets, lymphocytes, and erythrocytes, the areas under the labeled α -T concentration vs. time curves were in the order N > HC > HCT. In lymphocytes, differences in labeled α -T were found at 6 and 48 h ($P < 0.05$). These data demonstrate that there are differences in the uptake of newly absorbed α -T into blood components in hyperlipidemia. Because these blood components are functionally affected by vitamin E, reduced uptake of α -T may be relevant to the pathogenesis of atherosclerosis.

2.51 Cell surface heparan sulfate proteoglycans contribute to intracellular lipid accumulation in adipocytes

Wilsie, L.C., Chanchani, S., Navaratna, D. and Orlando, R.A.
Lipids in Health and Disease, **4**(2), 1-15 (2005)

Background

Transport of fatty acids within the cytosol of adipocytes and their subsequent assimilation into lipid droplets has been thoroughly investigated; however, the mechanism by which fatty acids are transported across the plasma membrane from the extracellular environment remains unclear. Since triacylglycerol-rich lipoproteins represent an abundant source of fatty acids for adipocyte utilization, we have investigated the expression levels of cell surface lipoprotein receptors and their functional contributions toward intracellular lipid accumulation; these include very low density lipoprotein receptor (VLDL-R), low density lipoprotein receptor-related protein (LRP), and heparan sulfate proteoglycans (HSPG).

Results

We found that expression of these three lipoprotein receptors increased 5-fold, 2-fold, and 2.5-fold, respectively, during adipocyte differentiation. The major proteoglycans expressed by mature adipocytes are of high molecular weight (>500 kD) and contain both heparan and chondroitin sulfate moieties. Using ligand binding antagonists, we observed that HSPG, rather than VLDL-R or LRP, play a primary role in the uptake of DiI-labeled apoE-VLDL by mature adipocytes. In addition, inhibitors of HSPG maturation resulted in a significant reduction (>85%) in intracellular lipid accumulation.

Conclusions

These results suggest that cell surface HSPG is required for fatty acid transport across the plasma membrane of adipocytes.

2.52 Optimized targeting of polyethylene glycol-stabilized anti-intercellular adhesion molecule 1 oligonucleotide/lipid particles to liver sinusoidal endothelial cells

Bartsch, M. et al

We prepared polyethylene glycol (PEG)-stabilized antisense oligonucleotide (ODN)/lipid particles from a lipid mixture including the positively charged amphiphile 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and anti-intercellular adhesion molecule 1 (ICAM-1) antisense ODN by an extrusion method in the presence of 40% ethanol. These particles were targeted to scavenger receptors on liver endothelial cells by means of covalently coupled polyanionized albumin. Two types of such targeted particles were prepared, one with the albumin coupled to a maleimide group attached to the particle's lipid bilayer and the other with the protein coupled to a maleimide group attached at the distal end of added bilayer-anchored PEG chains. Upon intravenous injection, the ODN particles with bilayer-coupled albumin were cleared from the blood circulation at the same low rate as untargeted particles (<5% in 30 min). By contrast, the distal-end coupled particles were very rapidly cleared from the blood and preferentially taken up by the endothelial cells of the hepatic sinusoid (55% of injected dose after 30 min). Despite this substantial endothelial targeting, no consistent inhibition of ICAM-1 expression could be demonstrated in this cell type, either *in vivo* or *in vitro*. However, in J774 cells that also express scavenger receptors and ICAM-1, significant down-regulation of ICAM-1 mRNA was achieved with distal-end targeted lipid particles, as determined with real-time RT-PCR. It is concluded that massive delivery of ODN to cell types that express scavenger receptors can be achieved if lipid particles are provided with negatively charged albumin distally attached to bilayer anchored PEG chains.

2.53 Downstream effects on human low density lipoprotein of homocysteine exported from endothelial cells in an *in vitro* system

Nakano, E. et al

J. Lipid Res., **46**, 484-493 (2005)

A model system is presented using human umbilical vein endothelial cells (HUVECs) to investigate the role of homocysteine (Hcy) in atherosclerosis. HUVECs are shown to export Hcy at a rate determined by the flux through the methionine/Hcy pathway. Additional methionine increases intracellular methionine, decreases intracellular folate, and increases Hcy export, whereas additional folate inhibits export. An inverse relationship exists between intracellular folate and Hcy export. Hcy export may be regulated by intracellular *S*-adenosyl methionine rather than by Hcy. Human LDLs exposed to HUVECs exporting Hcy undergo time-related lipid oxidation, a process inhibited by the thiol trap dithionitrobenzoate. This is likely to be related to the generation of hydroxyl radicals, which we show are associated with Hcy export. Although Hcy is the major oxidant, cysteine also contributes, as shown by the effect of glutamate. Finally, the LDL oxidized in this system showed a time-dependent increase in uptake by human macrophages, implying an upregulation of the scavenger receptor.

These results suggest that continuous export of Hcy from endothelial cells contributes to the generation of extracellular hydroxyl radicals, with associated oxidative modification of LDL and incorporation into macrophages, a key step in atherosclerosis. Factors that regulate intracellular Hcy metabolism modulate these effects.

2.54 Evidence for the presence of three distinct binding sites for the thioflavin T class of Alzheimer's disease PET imaging agents on β -amyloid peptide fibrils

Lockhart, A. et al

J. Biol. Chem., **280**(9), 7677-7684 (2005)

Imaging the progression of Alzheimer's disease would greatly facilitate the discovery of therapeutics, and a wide range of ligands are currently under development for the detection of β -amyloid peptide ($A\beta$)-containing plaques by using positron emission tomography. Here we report an in-depth characterization of the binding of seven previously described ligands to *in vitro* generated $A\beta$ -(1-40) polymers. All of the compounds were derived from the benzothiazole compound thioflavin T and include 2-[4'-(methylamino)phenyl]benzothiazole and 2-(4'-(dimethylamino)-phenyl)-imidazo[1,2-*a*]-pyridine derivatives, 2-[4'-(dimethylamino)phenyl]-6-iodobenzothiazole and 2-[4'-(4''-methylpiperazin-1-yl)phenyl]-6-iodobenzothiazole, and a benzofuran compound (5-bromo-2-(4-dimethylaminophenyl)benzofuran). By using a range of fluorescent and radioligand binding assays, we find that these compounds display a more complex binding pattern than described previously and are consistent with three classes of binding sites on the $A\beta$ fibrils. All of the compounds bound with very high affinity (low nM K_d) to a low capacity site (BS3) (1 ligand-binding site per \sim 300 $A\beta$ -(1-40) monomers) consistent with the previously recognized binding site for these compounds on the fibrils. However, the compounds also bound with high affinity ($K_d \sim$ 100

nm) to either one of two additional binding sites on the A β -(1–40) polymer. The properties of these sites, BS1 and BS2, suggest they are adjacent or partially overlapping and have a higher capacity than BS3, occurring every \sim 35 or every \sim 4 monomers of A β -(1–40)-peptide, respectively. Compounds appear to display selectivity for BS2 based on the presence of a halogen substitution (2-[4'-(dimethylamino)phenyl]-6-iodobenzothiazole, 2-[4'-(4"-methylpiperazin-1-yl)phenyl]-6-iodobenzothiazole, and 5-bromo-2-(4-dimethylaminophenyl)benzofuran) on their aromatic ring system. The presence of additional ligand-binding sites presents potential new targets for ligand development and may allow a more complete modeling of the current positron emission tomography data.

2.55 **Distinct signaling particles containing ERK/MEK and B-Raf in PC12 cells**

MacCormick, M. et al

Biochem. J., **387**, 155-164 (2005)

Although several multiprotein complexes containing MAPKs (mitogen-activated protein kinases) have been identified using overexpression of kinases and scaffold proteins, the components of the complexes and their physical properties at endogenous expression levels have not been defined. We characterized a large protein complex containing a nerve-growth-factor-activated ERK (extracellular-signal-regulated kinase) and MEK (MAPK/ERK kinase) in rat pheochromocytoma (PC12) cells. This protein complex fractionated into a high-speed pellet and was resistant to non-ionic detergent treatments that solubilized membranes. Disruption of protein–protein interactions by treatment with high salt was required to facilitate immunoprecipitation of active ERK1 and co-precipitation of MEK1. Microtubule fragments were also present in the detergent-resistant high-speed pellet, and some kinases were bound to them, especially ERK1b (an alternatively spliced isoform of ERK1), which showed a strong preference for binding microtubules. The large protein complex containing ERK1 and MEK1 was resolved by velocity sedimentation from fragments of microtubules; however, it did not contain other scaffolding components known to bind ERK and MEK. B-Raf was also present in a distinct detergent-resistant, microtubule-independent protein complex slightly larger than that containing ERK and MEK. We conclude that there are two independent nerve growth factor-regulated ‘signalling particles’ with an estimated size of 60–75 S, one containing ERK1 and MEK1 and the other containing B-Raf. These signalling particles may have a role in the temporal and spatial regulation of kinase activity inside cells.

2.56 **A *Dictyostelium* homologue of WASP is required for polarized F-actin assembly during chemotaxis**

Myers, S.A., Han, J.W., Lee, Y., Firtel, R.A. and Chung, C.Y.

Mol. Biol. Cell, **16**, 2191-2206 (2005)

The actin cytoskeleton controls the overall structure of cells and is highly polarized in chemotaxing cells, with F-actin assembled predominantly in the anterior leading edge and to a lesser degree in the cell's posterior. Wiskott-Aldrich syndrome protein (WASP) has emerged as a central player in controlling actin polymerization. We have investigated WASP function and its regulation in chemotaxing *Dictyostelium* cells and demonstrated the specific and essential role of WASP in organizing polarized F-actin assembly in chemotaxing cells. Cells expressing very low levels of WASP show reduced F-actin levels and significant defects in polarized F-actin assembly, resulting in an inability to establish axial polarity during chemotaxis. GFP-WASP preferentially localizes at the leading edge and uropod of chemotaxing cells and the B domain of WASP is required for the localization of WASP. We demonstrated that the B domain binds to PI(4,5)P₂ and PI(3,4,5)P₃ with similar affinities. The interaction between the B domain and PI(3,4,5)P₃ plays an important role for the localization of WASP to the leading edge in chemotaxing cells. Our results suggest that the spatial and temporal control of WASP localization and activation is essential for the regulation of directional motility.

2.57 **Monomeric and dimeric states exhibited by the kinesin-related motor protein KIF1A**

Rashid, D.J., Bononi, J., Triplet, B.P., Hodges, R.S. and Pierce, D.W.

J. Peptide Res., **65**, 538-549 (2005)

KIF1A, a kinesin-related motor protein that transports pre-synaptic vesicles in neurons, was originally presumed to translocate along microtubules (MT) as a monomer. Protein structure predictions from its amino acid sequence failed to identify the long coiled-coil domains typical of kinesins, which led researchers to believe it does not oligomerize into the canonical kinesin dimer. However, mounting evidence using recombinant chimeric protein indicates that KIF1A, like conventional kinesin, requires dimerization for fast, unidirectional processive movement along MTs. Because these studies are somewhat indirect, we wished to test the oligomerization state of native KIF1A, and to compare that to full-length

recombinant protein. We have performed hydrodynamic analyses to determine the molecular weights of the respective complexes. Our results indicate that most native KIF1A is soluble and indeed monomeric, but recombinant KIF1A is a dimer. MT-binding studies also showed that native KIF1A did not bind to MTs in either the presence of AMP-PNP, apyrase, or adenosine triphosphate (ATP), but recombinant KIF1A bound to MTs most stably in the presence of ATP, indicating very different motor functional states. To further characterize KIF1A's dimerization potential, we prepared peptides corresponding to the neck domains of MmKIF1A and CeUnc104, and by circular dichroism spectroscopy compared these peptides for their ability to form coiled-coils. Interestingly, both MmKIF1A and CeUnc104 neck peptides formed homodimeric coiled-coils, with the MmKIF1A neck coiled-coil exhibiting the greater stability. Collectively, from our data and from previous studies, we predict that native KIF1A can exist as both an inactive monomer and an active homodimer formed in part through its neck coiled-coil domain.

2.58 Effect of Atorvastatin and Irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients

Ceriello, A. et al

Circulation, **111**, 2518-2524 (2005)

Background— Postprandial hypertriglyceridemia and hyperglycemia are considered risk factors for cardiovascular disease. Evidence suggests that postprandial hypertriglyceridemia and hyperglycemia induce endothelial dysfunction and inflammation through oxidative stress. Statins and angiotensin type 1 receptor blockers have been shown to reduce oxidative stress and inflammation, improving endothelial function.

Methods and Results— Twenty type 2 diabetic patients ate 3 different test meals: a high-fat meal, 75 g glucose alone, and a high-fat meal plus glucose. Glycemia, triglyceridemia, endothelial function, nitrotyrosine, C-reactive protein, intercellular adhesion molecule-1, and interleukin-6 were assayed during the tests. Subsequently, diabetics took atorvastatin 40 mg/d, irbesartan 300 mg/d, both, or placebo for 1 week. The 3 tests were performed again between 5 and 7 days after the start of each treatment. High-fat load and glucose alone produced a decrease in endothelial function and increases in nitrotyrosine, C-reactive protein, intercellular adhesion molecule-1, and interleukin-6. These effects were more pronounced when high-fat load and glucose were combined. Short-term atorvastatin and irbesartan treatments significantly counterbalanced these phenomena, and their combination was more effective than either therapy alone.

Conclusions— This study confirms an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial function and inflammation, suggesting oxidative stress as a common mediator of such an effect. Short-term treatment with atorvastatin and irbesartan may counterbalance this phenomenon; the combination of the 2 compounds is most effective.

2.59 Distribution of brevetoxin (PbTx-3) in mouse plasma: association with high-density lipoprotein

Woofter, R.T., Spiess, P.C. and Ramsdell, J.S.

Environ. Health Perspect., **113**(11), 1491-1496 (2005)

We investigated the brevetoxin congener PbTx-3 to determine its distribution among carrier proteins, including albumin and blood lipoproteins. Using a radiolabeled brevetoxin tracer (PbTx-3), we found that 39% of the radiolabel remained associated with components in mouse plasma after > 15 kDa cutoff dialysis. Of this portion, only 6.8% was bound to serum albumin. We also examined the binding of brevetoxin to various lipoprotein fractions. Plasma, either spiked with PbTx-3 or from mice treated for 30 min with PbTx-3, was fractionated into different-sized lipoproteins by iodixanol gradient ultracentrifugation. Each fraction was then characterized and quantified by agarose gel electrophoresis and brevetoxin radioimmunoassay, respectively. In both the in vitro and in vivo experiments, the majority of brevetoxin immunoreactivity was restricted to only those gradient fractions that contained high-density lipoproteins (HDLs). Independent confirmation of brevetoxin binding to HDLs was provided by high molecular weight (100 kDa cutoff) dialysis of [3H]PbTx-3 from lipoprotein fractions as well as a scintillation proximity assay using [3H]PbTx-3 and purified human HDLs. This information on the association of brevetoxins with HDLs provides a new foundation for understanding the process by which the toxin is delivered to and removed from tissues and may permit more effective therapeutic measures to treat intoxication from brevetoxins and the related ciguatoxins.

2.60 Phosphorylation-induced autoinhibition regulates the cytoskeletal protein lethal (2) giant larvae

Betschinger, J., Eisenhaber, F. and Knoblich, J.A.

Current Biol., **15**(3), 276-282 (2005)

During asymmetric cell division, cell fate determinants localize asymmetrically and segregate into one of the two daughter cells. In *Drosophila* neuroblasts, the asymmetric localization of cell fate determinants to the basal cell cortex requires aPKC. aPKC localizes to the apical cell cortex and phosphorylates the cytoskeletal protein Lethal (2) giant larvae (Lgl). Upon phosphorylation, Lgl dissociates from the cytoskeleton and becomes inactive. Here, we show that phosphorylation regulates Lgl by allowing an autoinhibitory interaction of the N terminus with the C terminus of the protein. We demonstrate that interaction with the cytoskeleton is mediated by a C-terminal domain while the N terminus is not required. Instead, the N terminus can bind to the C terminus and can compete for binding to the cytoskeleton. Interaction between the N- and C-terminal domains requires phosphorylation of Lgl by aPKC. Our results suggest that unphosphorylated, active Lgl exists in an open conformation that interacts with the cytoskeleton while phosphorylation changes the protein to an autoinhibited state.

2.61 E-Cadherin Tethered to Micropatterned Supported Lipid Bilayers as a Model for Cell Adhesion

Perez, T.D., Nelson, W.J., Boxer, S.G. and Kam, L.
Langmuir, **21**, 11963-11968 (2005)

Cell-cell adhesion is a dynamic process requiring recruitment, binding, and reorganization of signaling proteins in the plane of the plasma membrane. Here, we describe a new system for investigating how this lateral mobility influences cadherin-based cell signaling. This model is based on tethering of a GPI-modified E-cadherin protein (hEFG) to a supported lipid bilayer. In this report, membrane microfluidics and micropatterning techniques are used to adopt this tethered protein system for studies with the anchorage-dependent cells. As directly formed from proteoliposomes, hEFG exhibits a diffusion coefficient of $0.6 \pm 0.3 \mu\text{m}^2/\text{s}$ and mobile fraction of 30–60%. Lateral structuring of the supported lipid bilayer is used to isolate mobile proteins from this mixed mobile/immobile population, and should be widely applicable to other proteins. MCF-7 cells seeded onto hEFG-containing bilayers recognize and cluster this protein, but do not exhibit cell spreading required for survival. By micropatterning small anchors into the supported lipid bilayer, we have achieved cell spreading across the bilayer surface and concurrent interaction with mobile hEFG protein. Together, these techniques will allow more detailed analysis of the cellular dynamics involved in cadherin-dependent adhesion events.

2.62 A discoidal lipoprotein from the coelomic fluid of the polychaete *Nereis virens*

Schenk, S., Harris, J.R. and Hoeger, U.
Comp. Biochem. Physiol., Part B, **143**, 236-243 (2006)

A discoidal lipoprotein was isolated from the coelomic fluid of the polychaete, *Nereis virens*, by density gradient centrifugation. The lipoprotein was present in both sexes and moved as a uniform band in an agarose gel. The average diameter of the lipoprotein particles determined by electron microscopy was 42 nm with a thickness of 10 nm. SDS electrophoresis showed two apoprotein subunits with molecular masses of 247 and 85 kDa, respectively. In lectin blots, both apoproteins were reactive with Concanavalin A indicating the presence of *N*-glycans. The small subunit was also reactive with peanut lectin, indicating additional *O*-glycosylation. The total lipid content was 48% and consisted mainly of phospholipids and some diglycerides as judged by thin layer chromatography. The estimated native molecular mass of *N. virens* lipoprotein (~ 675 kDa) lies in the range of vertebrate high-density lipoprotein and insect lipophorins. The size of the apoproteins is similar to those found in insects, while the composition of the lipid fraction is more similar to that of crustacean lipoproteins.

2.63 Blood lipid concentrations and lipoprotein patterns in captive and wild American black bears (*Ursus americanus*)

Frank, N., Elliott, S.B., Allin, S.B. and Ramsay, E.C.
Am. J. Vet. Res., **67**(2), 335-341 (2006)

Objective-To compare blood lipid concentrations and lipoprotein patterns for captive and wild American black bears (*Ursus americanus*). Animals-7 captive and 9 wild adult (≥ 4 years old) black bears. Procedure-Blood was collected from 2 groups of captive black bears (groups A and B) and 1 group of wild black bears (group C). Blood triglyceride (TG) and cholesterol concentrations were compared among groups. Plasma lipoproteins were isolated by use of a self-generating gradient of iodixanol, and lipoprotein patterns were compared between groups A and B. Results-Captive bears (mean \pm SD, 187.8 \pm 44.4 kg) weighed significantly more than wild bears (mean, 104.8 \pm 41.4 kg), but mean body weight did not differ between groups A and B. Mean blood TG concentrations for groups B (216.8 \pm 16.0 mg/dL) and C

(190.7 +/- 34.0 mg/dL) were significantly higher than that of group A (103.9 +/- 25.3 mg/dL). Mean blood cholesterol concentration was also significantly higher for group B (227.8 +/- 8.2 mg/dL) than for groups A (171.7 +/- 35.5 mg/dL) or C (190.8 +/- 26.8 mg/dL). Mean very-low-density lipoprotein TG and low-density lipoprotein cholesterol concentrations were 2- and 3-fold higher, respectively, for group B, compared with concentrations for group A. Conclusions and Clinical Relevance-Blood lipid concentrations vary significantly among populations of black bears. Plasma lipoprotein patterns of captive bears differed significantly between colonies and may have reflected differences in diet or management practices.

2.64 Prolonged deterioration of endothelial dysfunction in response to postprandial lipaemia is attenuated by vitamin C in type 2 diabetes

Anderson, R.A. et al
Diabetic Med., 23, 258-264 (2006)

Background Endothelial dysfunction (ED) has been described in Type 2 diabetes (T2DM). We have described previously a diminution of flow-mediated arterial dilatation and, by implication, further ED in T2DM in response to postprandial lipaemia (PPL) at 4 h. This is possibly mediated by oxidative stress/alteration of the nitric oxide (NO) pathway. T2DM subjects tend to exhibit both exaggerated and prolonged PPL. We therefore studied the relationship of PPL to the duration of ED in T2DM subjects and oxidative stress with or without the antioxidant, vitamin C.

Methods Twenty subjects with T2DM with moderate glycaemic control (mean HbA_{1c} 8.4%) were studied. After an overnight fast, all subjects consumed a standard fat meal. Endothelial function (EF), lipid profiles, and venous free radicals were measured in the fasting, peak lipaemic phase (4 h) and postprandially to 8 h. The study was repeated in a double-blinded manner with placebo, vitamin C (1 g) therapy for 2 days prior to re-testing and with the fat meal. Oxidative stress was assessed by lipid-derived free radicals in plasma, *ex vivo* by electron paramagnetic resonance spectroscopy (EPR) and by markers of lipid peroxidation (TBARS). Endothelial function was assessed by flow-mediated vasodilatation (FMD) of the brachial artery.

Results There was a significant decrease in endothelial function in response to PPL from baseline (B) $1.3 \pm 1.3\%$ to 4 h $0.22 \pm 1.1\%$ ($P < 0.05$) and 8 h $0.7 \pm 0.9\%$ ($P < 0.05$) (mean \pm SEM). The endothelial dysfunction seen was attenuated at each time point with vitamin C. Baseline EF with vitamin C changed from (fasting) 3.8 ± 0.9 – 2.8 ± 0.8 (at 4 h) and 2.9 ± 1.3 (at 8 h) in response to PPL. Vitamin C attenuated postprandial (PP) oxidative stress significantly only at the 4-h time point [301.1 ± 118 (B) to 224.7 ± 72 ($P < 0.05$)] and not at 8 h 301.1 ± 118 (B) to 260 ± 183 ($P = \text{NS}$). There were no changes with placebo treatment in any variable. PPL was associated with a PP rise in TG levels (in mmol/l) from (B) 1.8 ± 1 to 2.7 ± 1 at 4 h and 1.95 ± 1.2 at 8 h ($P = 0.0002$ and 0.33 , respectively).

Conclusion PPL is associated with prolonged endothelial dysfunction for at least 8 h after a fatty meal. Vitamin C treatment improves endothelial dysfunction at all time points and attenuates PPL-induced oxidative stress. This highlights the importance of low-fat meals in T2DM and suggests a role for vitamin C therapy to improve endothelial function during meal ingestion.

2.65 Soy-isoflavone-enriched foods and markers of lipid and glucose metabolism in postmenopausal women: interactions with genotype and equol production

Hall, W.L. et al
Am. J. Clin. Nutr., 83, 592-600 (2006)

Background: The hypocholesterolemic effects of soy foods are well established, and it has been suggested that isoflavones are responsible for this effect. However, beneficial effects of isolated isoflavones on lipid biomarkers of cardiovascular disease risk have not yet been shown.

Objective: The objective was to investigate the effects of isolated soy isoflavones on metabolic biomarkers of cardiovascular disease risk, including plasma total, HDL, and LDL cholesterol; triacylglycerols; lipoprotein(a); the percentage of small dense LDL; glucose; nonesterified fatty acids; insulin; and the homeostasis model assessment of insulin resistance. Differences with respect to single nucleotide polymorphisms in selected genes [ie, estrogen receptor α (*Xba*I and *Pvu*II), estrogen receptor β (*Alu*I), and estrogen receptor β (cx) (*Tsp*509I), endothelial nitric oxide synthase (*Glu*298Asp), apolipoprotein E (*Apo E*2, *E*3, and *E*4), cholesteryl ester transfer protein (*Taq*IB), and leptin receptor (*Gln*223Arg)] and with respect to equol production were investigated.

Design: Healthy postmenopausal women ($n = 117$) participated in a randomized, double-blind, placebo-controlled, crossover dietary intervention trial. Isoflavone-enriched (genistein-to-daidzein ratio of 2:1; 50 mg/d) or placebo cereal bars were consumed for 8 wk, with a wash-out period of 8 wk before the crossover.

Results: Isoflavones did not have a significant beneficial effect on plasma concentrations of lipids, glucose, or insulin. A significant difference between the responses of HDL cholesterol to isoflavones and to placebo

was found with estrogen receptor β (cx) *Tsp509I* genotype AA, but not GG or GA.

Conclusions: Isoflavone supplementation, when provided in the form and dose used in this study, had no effect on lipid or other metabolic biomarkers of cardiovascular disease risk in postmenopausal women but may increase HDL cholesterol in an estrogen receptor β gene-polymorphic subgroup.

2.66 Inducible expression of Tau repeat domain in cell models of tauopathy

Khlistunova, I. et al

J. Biol. Chem., **281**(2), 1205-1214 (2006)

We generated several cell models of tauopathy in order to study the mechanisms of neurodegeneration in diseases involving abnormal changes of tau protein. N2a neuroblastoma cell lines were created that inducibly express different variants of the repeat domain of tau (τ_{RD}) when exposed to doxycycline (Tet-On system). The following three constructs were chosen: (i) the repeat domain of tau that coincides with the core of Alzheimer paired helical filaments; (ii) the repeat domain with the deletion mutation Δ K280 known from frontotemporal dementia and highly prone to spontaneous aggregation; and (iii) the repeat domain with Δ K280 and two proline point mutations that inhibit aggregation. The comparison of wild-type, pro-aggregation, and anti-aggregation mutants shows the following. (a) Aggregation of τ_{RD} is toxic to cells. (b) The degree of aggregation and toxicity depends on the propensity for β -structure. (c) Soluble mutants of τ_{RD} that cannot aggregate are not toxic. (d) Aggregation is preceded by fragmentation. (e) Fragmentation of τ_{RD} in cells is initially due to a thrombin-like protease activity. (f) Phosphorylation of τ_{RD} (at KXGS motifs) precedes aggregation but is not correlated with the degree of aggregation. (g) Aggregates of τ_{RD} disappear when the expression is silenced, showing that aggregation is reversible. (h) Aggregation can be prevented by drugs and even pre-formed aggregates can be dissolved again by drugs. Thus, the cell models open up new insights into the relationship between the structure, expression, phosphorylation, aggregation, and toxicity of τ_{RD} that can be used to test current hypotheses on tauopathy and to develop drugs that prevent the aggregation and degeneration of cells.

2.67 Effects of dairy products naturally enriched with cis-9, trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men

Tricon, S. et al

Am. J. Clin. Nutr., **83**, 744-753 (2006)

Background: Interest in the development of dairy products naturally enriched in conjugated linoleic acid (CLA) exists. However, feeding regimens that enhance the CLA content of milk also increase concentrations of *trans*-18:1 fatty acids. The implications for human health are not yet known.

Objective: This study investigated the effects of consuming dairy products naturally enriched in *cis*-9,*trans*-11 CLA (and *trans*-11 18:1) on the blood lipid profile, the atherogenicity of LDL, and markers of inflammation and insulin resistance in healthy middle-aged men.

Design: Healthy middle-aged men ($n = 32$) consumed ultra-heat-treated milk, butter, and cheese that provided 0.151 g/d (control) or 1.421 g/d (modified) *cis*-9,*trans*-11 CLA for 6 wk. This was followed by a 7-wk washout and a crossover to the other treatment.

Results: Consumption of dairy products enriched with *cis*-9,*trans*-11 CLA and *trans*-11 18:1 did not significantly affect body weight, inflammatory markers, insulin, glucose, triacylglycerols, or total, LDL, and HDL cholesterol but resulted in a small increase in the ratio of LDL to HDL cholesterol. The modified dairy products changed LDL fatty acid composition but had no significant effect on LDL particle size or the susceptibility of LDL to oxidation. Overall, increased consumption of full-fat dairy products and naturally derived *trans* fatty acids did not cause significant changes in cardiovascular disease risk variables, as may be expected on the basis of current health recommendations.

Conclusion: Dairy products naturally enriched with *cis*-9,*trans*-11 CLA and *trans*-11 18:1 do not appear to have a significant effect on the blood lipid profile.

2.68 Influence of an algal triacylglycerol containing docosahexaenoic acid (22: 6n-3) and docosapentaenoic acid (22: 5n-6) on cardiovascular risk factors in healthy men and women

Sanders, T.A.B., Gleason, K., Griffin, B. and Miller, G.J.

Br. J. Nutrition, **95**, 525-531 (2006)

The intake of long-chain n-3 PUFA, including DHA (22: 6n-3), is associated with a reduced risk of CVD. Schizochytrium sp. are an important primary source of DHA in the marine food chain but they also provide substantial quantities of the n-6 PUFA docosapentaenoic acid (22: 5n-6; DPA). The effect of this oil on cardiovascular risk factors was evaluated using a double-blind randomised placebo-controlled

parallel-design trial in thirty-nine men and forty women. Subjects received 4 g oil/d for 4 weeks; the active treatment provided 1[middle dot]5 g DHA and 0[middle dot]6 g DPA. Active treatment increased plasma concentrations of arachidonic acid, adrenic acid, DPA and DHA by 21, 11, 11 and 88 mg/l respectively and the proportions of DPA and DHA in erythrocyte phospholipids by 78 and 27 % respectively. Serum total, LDL- and HDL-cholesterol increased by 0[middle dot]33 mmol/l (7[middle dot]3 %), 0[middle dot]26 mmol/l (10[middle dot]4 %) and 0[middle dot]14 mmol/l (9[middle dot]0 %) compared with placebo (all $P \leq 0$ [middle dot]001). Factor VII (FVII) coagulant activity increased by 12 % following active treatment ($P = 0$ [middle dot]006). There were no significant differences between treatments in LDL size, blood pressure, plasma glucose, serum C-reactive protein, plasma FVII antigen, FVII activated, fibrinogen, von Willebrand factor, tocopherol or carotenoid concentrations, plasminogen activator inhibitor-1, creatine kinase or troponin-I activities, haematology or liver function tests or self-reported adverse effects. Overall, the oil was well tolerated and did not adversely affect cardiovascular risk.

2.69 Metformin prevents alcohol-induced liver injury in the mouse: critical role of plasminogen activator inhibitor-1

Bergheim, I. et al

Gastroenterology, 130, 2099-2112 (2006)

Background & Aims: The biguanide drug metformin has recently been found to improve steatosis and liver damage in animal models and in humans with nonalcoholic steatohepatitis. **Methods:** The aim of the present study was to determine whether metformin also prevents steatosis and liver damage in mouse models of acute and chronic alcohol exposure. **Results:** Acute ethanol exposure caused a >20-fold increase in hepatic lipids, peaking 12 hours after administration. Metformin treatment significantly blunted the ethanol effect by >60%. Although metformin is a known inducer of AMP kinase (AMPK) activity, the hepatoprotective property of metformin did not correlate with activation of AMPK or of AMPK-dependent pathways. Instead, the protective effects of metformin correlated with complete prevention of the upregulation of plasminogen activator inhibitor (PAI)-1 caused by ethanol. Indeed, a similar protective effect against acute alcohol-induced lipid accumulation was observed in PAI-1^{-/-} mice. Hepatic fat accumulation caused by chronic enteral ethanol feeding was also prevented by metformin or by knocking out PAI-1. Under these conditions, necroinflammatory changes caused by ethanol were also significantly attenuated. **Conclusions:** Taken together, these findings suggest a novel mechanism of action for metformin and identify a new role of PAI-1 in hepatic injury caused by ethanol.

2.70 Historical milestones in measurement of HDL-cholesterol: Impact on clinical and laboratory practice

Langlois, M.R. and Blaton, V.H.

Clin. Chem. Acta, 369, 168-178 (2006)

High-density lipoprotein cholesterol (HDL-C) comprises a family of particles with differing physicochemical characteristics. Continuing progress in improving HDL-C analysis has originated from two separate fields—one clinical, reflecting increased attention to HDL-C in estimating risk for coronary heart disease (CHD), and the other analytical, reflecting increased emphasis on finding more reliable and cost-effective HDL-C assays. Epidemiologic and prospective studies established the inverse association of HDL-C with CHD risk, a relationship that is consistent with protective mechanisms demonstrated in basic research and animal studies. Atheroprotective and less atheroprotective HDL subpopulations have been described. Guidelines on primary and secondary CHD prevention, which increased the workload in clinical laboratories, have led to a revolution in HDL-C assay technology. Many analytical techniques including ultracentrifugation, electrophoresis, chromatography, and polyanion precipitation methods have been developed to separate and quantify HDL-C and HDL subclasses. More recently developed homogeneous assays enable direct measurement of HDL-C on an automated analyzer, without the need for manual pretreatment to separate non-HDL. Although homogeneous assays show improved accuracy and precision in normal serum, discrepant results exist in samples with atypical lipoprotein characteristics. Hypertriglyceridemia and monoclonal paraproteins are important interfering factors. A novel approach is nuclear magnetic resonance spectroscopy that allows rapid and reliable analysis of lipoprotein subclasses, which may improve the identification of individuals at increased CHD risk. Apolipoprotein A-I, the major protein of HDL, has been proposed as an alternative cardioprotective marker avoiding the analytical limitations of HDL-C.

2.71 Syndecan-1 mediates internalization of apoE-VLDL through a low density lipoprotein receptor-related protein (LRP)-independent, non-clathrin-mediated pathway

Wilsie, L.C., Gonzales, M. and Orlando, R.A.

Lipids in Health and Disease, 5(23), 1-14 (2006)

Background

Triacylglycerol-rich very low density lipoprotein (VLDL) particles are the primary carriers of fatty acids in the circulation and as such serve as a rich energy source for peripheral tissues. Receptor-mediated uptake of these particles is dependent upon prior association with apolipoprotein E (apoE-VLDL) and is brought about by cell surface heparan sulfate proteoglycans (HSPG) in some cell types and by the low density lipoprotein receptor-related protein (LRP) in others. Although LRP's role in apoE-VLDL uptake has been well studied, the identity of the HSPG family member that mediates apoE-VLDL uptake has not been established. We investigated if syndecan-1 (Syn-1), a transmembrane cell surface HSPG, is able to mediate the internalization of apoE-VLDL and examined the relationship between Syn-1 and LRP toward apoE-VLDL uptake. For this study, we used a human fibroblast cell line (GM00701) that expresses large amounts of LRP, but possesses no LDL receptor activity to eliminate its contributions toward apoE-VLDL uptake.

Results

Although LRP in these cells is fully active as established by substantial α_2 macroglobulin binding and internalization, uptake of apoE-VLDL is absent. Expression of human Syn-1 cDNA restored apoE-VLDL binding and uptake by these cells. Competition for this uptake with an LRP ligand-binding antagonist had little or no effect, whereas co-incubation with heparin abolished apoE-VLDL internalization. Depleting Syn-1 expressing cells of K^+ , to block clathrin-mediated endocytosis, showed no inhibition of Syn-1 internalization of apoE-VLDL. By contrast, treatment of cells with nystatin to inhibit lipid raft function, prevented the uptake of apoE-VLDL by Syn-1.

Conclusion

These data demonstrate that Syn-1 is able to mediate apoE-VLDL uptake in human fibroblasts with little or no contribution from LRP and that the endocytic path taken by Syn-1 is clathrin-independent and relies upon lipid raft function. These data are consistent with previous studies demonstrating Syn-1 association with lipid raft domains.

2.72 Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study

Griffin, M.D. et al

Am. J. Clin. Nutr., 84, 1290-1298 (2006)

Background: Insulin resistance is associated with elevated plasma triacylglycerol, low HDL concentrations, elevated postprandial lipemia, and a predominance of small, dense LDLs (sdLDLs). It has been hypothesized that the dietary ratio of n-6 to n-3 (n-6:n-3) polyunsaturated fatty acids (PUFAs) may have favorable effects on these risk factors by increasing insulin sensitivity.

Objective: The objective was to measure changes in insulin sensitivity, lipoprotein size, and postprandial lipemia after a 6-mo alteration in n-6:n-3.

Design: In a randomized, parallel design in 258 subjects aged 45-70 y, we compared 4 diets providing 6% of energy as PUFAs with an n-6:n-3 between 5:1 and 3:1 with a control diet that had an n-6:n-3 of 10:1. The diets were enriched in α -linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or both. Insulin sensitivity was assessed with the homeostatic model assessment of insulin resistance and the revised quantitative insulin sensitivity test.

Results: Dietary intervention did not influence insulin sensitivity or postprandial lipase activities. Fasting and postprandial triacylglycerol concentrations were lower, and the proportion of sdLDLs decreased (by 12.7%; 95% CI: -22.9%, 2.4%), with an n-6:n-3 of \approx 3:1, which was achieved by the addition of long-chain n-3 PUFAs (EPA and DHA).

Conclusions: Decreasing the n-6:n-3 does not influence insulin sensitivity or lipase activities in older subjects. The reduction in plasma triacylglycerol after an increased intake of n-3 long-chain PUFAs results in favorable changes in LDL size.

2.73 Prolonged deterioration of endothelial dysfunction in response to postprandial lipaemia is attenuated by vitamin C in Type 2 diabetes

Anderson, R.A., Evans, L.M., Ellis, G.R., Khan, N., Morrissett, K., Jackson, S.K., Rees, A., Lewis, M.J. and Frenneaux, M.P.

Diabetic Med., 23(8), 258-264 (2006)

Background Endothelial dysfunction (ED) has been described in Type 2 diabetes (T2DM). We have described previously a diminution of flow-mediated arterial dilatation and, by implication, further ED in T2DM in response to postprandial lipaemia (PPL) at 4 h. This is possibly mediated by oxidative stress/alteration of the nitric oxide (NO) pathway. T2DM subjects tend to exhibit both exaggerated and prolonged PPL. We therefore studied the relationship of PPL to the duration of ED in T2DM subjects and oxidative stress with or without the antioxidant, vitamin C.

Methods Twenty subjects with T2DM with moderate glycaemic control (mean HbA_{1c} 8.4%) were studied. After an overnight fast, all subjects consumed a standard fat meal. Endothelial function (EF), lipid profiles, and venous free radicals were measured in the fasting, peak lipaemic phase (4 h) and postprandially to 8 h. The study was repeated in a double-blinded manner with placebo, vitamin C (1 g) therapy for 2 days prior to re-testing and with the fat meal. Oxidative stress was assessed by lipid-derived free radicals in plasma, *ex vivo* by electron paramagnetic resonance spectroscopy (EPR) and by markers of lipid peroxidation (TBARS). Endothelial function was assessed by flow-mediated vasodilatation (FMD) of the brachial artery.

Results There was a significant decrease in endothelial function in response to PPL from baseline (B) $1.3 \pm 1.3\%$ to 4 h $0.22 \pm 1.1\%$ ($P < 0.05$) and 8 h $0.7 \pm 0.9\%$ ($P < 0.05$) (mean \pm SEM). The endothelial dysfunction seen was attenuated at each time point with vitamin C. Baseline EF with vitamin C changed from (fasting) 3.8 ± 0.9 – 2.8 ± 0.8 (at 4 h) and 2.9 ± 1.3 (at 8 h) in response to PPL. Vitamin C attenuated postprandial (PP) oxidative stress significantly only at the 4-h time point [301.1 ± 118 (B) to 224.7 ± 72 $P < 0.05$] and not at 8 h 301.1 ± 118 (B) to 260 ± 183 ($P = \text{NS}$). There were no changes with placebo treatment in any variable. PPL was associated with a PP rise in TG levels (in mmol/l) from (B) 1.8 ± 1 to 2.7 ± 1 at 4 h and 1.95 ± 1.2 at 8 h ($P = 0.0002$ and 0.33 , respectively).

Conclusion PPL is associated with prolonged endothelial dysfunction for at least 8 h after a fatty meal. Vitamin C treatment improves endothelial dysfunction at all time points and attenuates PPL-induced oxidative stress. This highlights the importance of low-fat meals in T2DM and suggests a role for vitamin C therapy to improve endothelial function during meal ingestion.

2.74 **Novel Porphyrin Conjugates with a Potent Photodynamic Antitumor Effect: Differential Efficacy of Mono- and Bis- β -cyclodextrin Derivatives**

Kralova, J., Synytsya, A., Pouckova, P., Koc, M., Dvorak, M. and Kral, V.
Photochem. Photobiol., **82**(2), 432-438 (2006)

In the present study we investigated the photosensitizing properties of two novel mono- and bis-cyclodextrin tetrakis (pentafluorophenyl) porphyrin derivatives in several tumor cell lines and in BALB/c mice bearing subcutaneously transplanted syngeneic mouse mammary carcinoma 4T1. Both studied sensitizers were localized mainly in lysosomes and were found to induce cell death by triggering apoptosis in human leukemic cells HL-60. In 4T1 and other cell lines both apoptotic and necrotic modes of cell death occurred depending on drug and light doses. Mono-cyclodextrin porphyrin derivative P(β -CD)1 exhibited stronger *in vitro* phototoxic effect than bis-cyclodextrin derivative P(β -CD)2. However, *in vivo* P(β -CD)2 displayed faster tumor uptake with maximal accumulation 6 h after application, leading to complete and prolonged elimination of subcutaneous tumors within 3 days after irradiation (100 J cm^{-2}). In contrast, P(β -CD)1 uptake was slower (48 h) and the reduction of tumor mass was only transient, reaching the maximum at the 12 h interval when a favorable tumor-to-skin ratio appeared. Thus, P(β -CD)2 represents a new photosensitizing drug displaying fast and selective tumor uptake, strong antitumor activity and fast elimination from the body.

2.75 **Gasoline Exhaust Emissions Induce Vascular Remodeling Pathways Involved in Atherosclerosis**

Lund, A.K. et al
Toxicol. Sci., **95**(2), 485-494 (2007)

Epidemiological evidence indicates that environmental air pollutants are positively associated with the development of chronic vascular disease; however, the mechanisms involved have not been fully elucidated. In the present study we examined molecular pathways associated with chronic vascular disease in atherosclerosis-prone apolipoprotein E-deficient (ApoE^{-/-}) mice, including markers of vascular remodeling and oxidative stress, in response to exposure to the ubiquitous environmental pollutant, gasoline engine emissions. ApoE^{-/-} mice, on a high-fat diet, were exposed by inhalation to either filtered air; 8, 40, or 60 $\mu\text{g}/\text{m}^3$ particulate matter whole exhaust; or filtered exhaust with gases matching the 60- $\mu\text{g}/\text{m}^3$ concentration, for 7 weeks. Aortas and plasma were collected and assayed for changes in histochemical markers, real-time reverse transcriptase-polymerase chain reaction, and indicators of oxidative damage. Inhalational exposure to gasoline engine emissions resulted in increased aortic mRNA expression of matrix metalloproteinase-3 (MMP-3), MMP-7, and MMP-9, tissue inhibitor of

metalloproteinases-2, endothelin-1 and heme oxygenase-1 in ApoE^{-/-} mice; increased aortic MMP-9 protein levels were confirmed through immunohistochemistry. Elevated reactive oxygen species were also observed in arteries from exposed animals, despite absence of plasma markers. Similar findings were also observed in the aortas of ApoE^{-/-} mice exposed to particle-filtered atmosphere, implicating the gaseous components of the whole exhaust in mediating the expression of markers associated with the vasculopathy. These findings demonstrate that exposure to gasoline engine emissions results in the transcriptional upregulation of factors associated with vascular remodeling, as well as increased markers of vascular oxidative stress, which may contribute to the progression of atherosclerosis and reduced stability of vulnerable plaques.

2.76 The AAA+ protein ATAD3 has displacement loop binding properties and is involved in mitochondrial nucleoid organization

He, J. et al

J. Cell Biol., **176**(2), 141-146 (2007)

Many copies of mammalian mitochondrial DNA contain a short triple-stranded region, or displacement loop (D-loop), in the major noncoding region. In the 35 years since their discovery, no function has been assigned to mitochondrial D-loops. We purified mitochondrial nucleoprotein complexes from rat liver and identified a previously uncharacterized protein, ATAD3p. Localization studies suggested that human ATAD3 is a component of many, but not all, mitochondrial nucleoids. Gene silencing of ATAD3 by RNA interference altered the structure of mitochondrial nucleoids and led to the dissociation of mitochondrial DNA fragments held together by protein, specifically, ones containing the D-loop region. In vitro, a recombinant fragment of ATAD3p bound to supercoiled DNA molecules that contained a synthetic D-loop, with a marked preference over partially relaxed molecules with a D-loop or supercoiled DNA circles. These results suggest that mitochondrial D-loops serve to recruit ATAD3p for the purpose of forming or segregating mitochondrial nucleoids.

2.77 Functional refolding of a recombinant C-type lectin-like domain containing intramolecular disulfide bonds

Vohra, R., Murphy, J.E., Walker, J.H., Homer-Vanniasinkam, S. and Ponnambalam, S.

Protein Expression & Purification, **52**(2), 415-421 (2007)

The lectin-like oxidized low-density lipoprotein scavenger receptor (LOX-1) is a pro-inflammatory marker and Type II membrane protein expressed on vascular cells and tissues. The LOX-1 extracellular domain mediates recognition of oxidized low-density lipoprotein (oxLDL) particles that are implicated in the development of atherosclerotic plaques. To study the molecular basis for LOX-1-mediated ligand recognition, we have expressed, purified and refolded a recombinant LOX-1 protein and assayed for its biological activity using a novel fluorescence-based assay to monitor binding to lipid particles. Overexpression of a hexahistidine-tagged cysteine-rich LOX-1 extracellular domain in bacteria leads to the formation of aggregates that accumulated in bacterial inclusion bodies. The hexahistidine-tagged LOX-1 molecule was purified by affinity chromatography from solubilized inclusion bodies. A sequential dialysis procedure was used to refold the purified but inactive and denatured LOX-1 protein into a functionally active form that mediated recognition of oxLDL particles. This approach allowed slow LOX-1 refolding and assembly of correct intrachain disulfide bonds. Circular dichroism analysis of the refolded LOX-1 molecule demonstrated a folded state with substantial α -helical content. Using immobilized recombinant, refolded LOX-1 we demonstrated a 70-fold preferential recognition for oxLDL over native LDL particles. Thus, a protein domain containing intrachain disulfide bonds can be reconstituted into a functionally active state using a relatively simple dialysis-based technique.

2.78 Simultaneous Control of Hyperglycemia and Oxidative Stress Normalizes Endothelial Function in Type 1 Diabetes

Ceriello, A., Kumar, S., Piconi, L., Esposito, K. and Guigliano, D.

Diabetes Care, **30**(3), 649-654 (2007)

OBJECTIVE—Previous studies have shown that in type 1 diabetes endothelial dysfunction persists even when glycemia is normalized. Moreover, oxidative stress has recently been demonstrated to be the mediator of hyperglycemia-induced endothelial dysfunction.

RESEARCH DESIGN AND METHODS—Thirty-six type 1 diabetic patients and 12 control subjects were enrolled. The diabetic patients were divided into three groups. The first group was treated for 24 h with insulin, achieving a near-normalization of glycemia. After 12 h of this treatment, vitamin C was added

for the remaining 12 h. The second group was treated for 24 h with vitamin C. After 12 h of this treatment, insulin was started, with achievement of near-normalization of glycemia for the remaining 12 h. The third group was treated for 24 h with both vitamin C and insulin, achieving near-normalization of glycemia.

RESULTS—Neither normalization of glycemia nor vitamin C treatment alone was able to normalize endothelial dysfunction or oxidative stress. However, a combination of insulin and vitamin C normalized endothelial dysfunction and decreased oxidative stress to normal levels.

CONCLUSIONS—This study suggests that long-lasting hyperglycemia in type 1 diabetic patients induces permanent alterations in endothelial cells, which may contribute to endothelial dysfunction by increased oxidative stress even when hyperglycemia is normalized.

2.79 **Secretion of the glucose-regulated selenoprotein SEPS1 from hepatoma cells**

Gao, Y. et al

Biochem. Biophys. Res. Comm., **356**, 636-641 (2007)

SEPS1 (also called selenoprotein S, SelS, Tanis or VIMP) is a selenoprotein, localized predominantly in the ER membrane and also on the cell surface. In this report, we demonstrate that SEPS1 protein is also secreted from hepatoma cells but not from five other types of cells examined. The secretion can be abolished by the ER-Golgi transport inhibitor Brefeldin A and by the protein synthesis inhibitor cycloheximide. Using a sandwich ELISA, SEPS1 was detected in the sera of 65 out of 209 human subjects (31.1%, average = 15.7 ± 1.1 ng/mL). Fractionation of human serum indicated that SEPS1 was associated with LDL and possibly with VLDL. The function of plasma SEPS1 is unclear but may be related to lipoprotein metabolism.

2.80 **Differences in cell morphology, lipid and apo B secretory capacity in caco-2 cells following long term treatment with saturated and monounsaturated fatty acids**

Bateman, P.A., Jackson, K.G., Maitin, V., Yaqoob, P. and Williams, C.M.

Biochim. Biophys. Acta, **1771**, 475-485 (2007)

The suitability of the caco-2 cell line as a model for studying the long term impact of dietary fatty acids on intestinal lipid handling and chylomicron production was examined. Chronic supplementation of caco-2 cells with palmitic acid (PA) resulted in a lower triacylglycerol secretion than oleic acid (OA). This was coupled with a detrimental effect of PA, but not OA, on transepithelial electrical resistance (TER) measurements, suggesting a loss of structural integrity across the cell monolayer. Addition of OA reversed the adverse effects of PA and stearic acid on TER and increased the ability of cells to synthesise and accumulate lipid, but did not normalise the secretion of lipids by caco-2 cells. Increasing amounts of OA and decreasing amounts of PA in the incubation media markedly improved the ability of cells to synthesise apolipoprotein B and secrete lipids. Real time RT-PCR revealed a down regulation of genes involved in lipoprotein synthesis following PA than OA. Electron microscopy showed adverse effects of PA on cellular morphology consistent with immature enterocytes such as stunted microvilli and poor tight junction formation. In conclusion, previously reported differences in lipoprotein secretion by caco-2 cells supplemented with saturated fatty acids (SFA) and OA may partly reflect early cytotoxic effects of SFA on cellular integrity and function.

2.81 **Large-scale preparation of human low- and high-density lipoproteins by density gradient centrifugation using iodixanol**

Billington, D., Maxwell, E., Graham, J.M. and Newland, P.

Anal. Biochem., **367**(1), 137-139 (2007)

No abstract available

2.82 **Telmisartan Shows an Equivalent Effect of Vitamin C in Further Improving Endothelial Dysfunction After Glycemia Normalization in Type 1 Diabetes**

Ceriello, A., Piconi, L., Esposito, K. and Gugliano, D.

Diabetes Care, **30**(7), 1694-1698 (2007)

OBJECTIVE— Long-lasting hyperglycemia in type 1 diabetic patients induces permanent alterations of endothelial function by increased oxidative stress, even when glycemia is normalized.

RESEARCH DESIGN AND METHODS— In this study, 36 type 1 diabetic patients and 12 control subjects were enrolled. The diabetic patients were divided into three groups. The first group was treated for 24 h with insulin, achieving a near normalization of glycemia. After 12 h of this treatment, vitamin C was

added for the remaining 12 h. The second group was treated for 24 h with vitamin C. After 12 h of this treatment, insulin was started, achieving a near normalization of glycemia for the remaining 12 h. The third group was treated for 24 h with both vitamin C and insulin, achieving near normalization of glycemia. The same protocols were performed after 1 month of telmisartan or placebo.

RESULTS— Neither normalization of glycemia nor vitamin C treatment alone was able to normalize endothelial dysfunction or oxidative stress. Combining insulin and vitamin C normalized endothelial dysfunction and decreased oxidative stress to normal levels. Telmisartan significantly improved basal endothelial function and decreased nitrotyrosine plasma levels. In patients treated with telmisartan, a near normalization of both flow-mediated vasodilation and oxidative stress was achieved when glycemia was normalized, whereas adding vitamin C infusion did not show further effect on endothelial function or nitrotyrosine plasma levels.

CONCLUSIONS— These data indicate that combining the normalization of glycemia with an antioxidant can normalize endothelial function in type 1 diabetic patients and that telmisartan works as an antioxidant like vitamin C.

2.83 Separation of the principal HDL subclasses by iodixanol gradient ultracentrifugation

Harman, N.L., Davies, I.G. and Griffin, B.A.

Atherosclerosis, **194**(1), 283-284 (2007)

Introduction: HDL is the smallest and most dense lipoprotein, its main function is in reverse cholesterol transport and as such it can be described as an anti-atherogenic lipoprotein. HDL exists as a number of subclasses differing in size and density; it has been proposed that these subclasses show a variable relationship with CVD risk. The use of HDL subclasses as a CVD risk marker is limited due to the expensive and time consuming nature of current techniques. The aim of the current study was to develop a rapid and cost effective method for the measurement of the principal plasma HDL subclasses.

Methods: HDL subclasses were separated by ◀iodixanol▶ gradient ultracentrifugation (I_xDGUC) from pre-stained plasma, in a run time of 2 h 30 min. A digital image of the separated HDL bands was downloaded and analysed by TotalLab 1D gel scan software to generate HDL subclass profiles. These profiles were validated by co-isolation of the HDL fraction on gradient gel electrophoresis (GGE). HDL was further fractionated and characterised by the distribution of particle size and apo A-1.

Results: Delineation between HDL₂ and HDL₃ was at a density cut-off of ≈ 1.059 g/L. There was a significant correlation between HDL subclass for both GGE and I_xDGUC methods as measured by area under the curve [HDL₃ $r = 0.77$; $P < 0.01$]. HDL₃% correlated inversely with total HDL-cholesterol [$r = -0.64$; $P < 0.01$].

Conclusion: I_xDGUC provides a rapid, high throughput and cost effective method for the separation of HDL₂ and HDL₃ subclasses.

2.84 Characterization of hepatitis C virus associated with very low density lipoprotein (VLDL) in infected human serum and liver

Nielsen, S., Bassendine, M., Neely, D., Ibrahim, S. and Toms, G.

Atherosclerosis, **194**(1), 284 (2007)

Hepatitis C virus (HCV) particles in serum are complexed with host very low density lipoprotein (VLDL) in lipo-viro particles. These structures are fragile and heterogeneous in density and size but, hitherto, their titres have been too low for detailed structural studies. Our group has previously described a unique liver transplant patient with an unusually high titre of HCV, sufficient for comprehensive biochemical and biophysical characterization.

In order to characterize native HCV lipo-viro particles in this material, serum and liver macerate was first fractionated on iodixanol▶ density gradients. HCV containing fractions with densities below 1.10 g/ml were further characterized by gel filtration, which separates VLDL, LDL and HDL according to size. HCV RNA in serum was found in particles, which co-eluted with large VLDL particles. These are the putative lipo-viro particles and support our earlier findings that HCV circulates in blood in association with VLDL. HCV in liver was found to co-elute with endoplasmic reticulum membranes. However, particles of a similar size to HCV lipo-viro particles in serum contained a high ratio of positive to negative strand HCV RNA suggesting that these fractions are enriched in HCV virus particles associated with VLDL.

2.85 Endurance swimming activates trout lipoprotein lipase: plasma lipids as a fuel for muscle

Magnoni, L. and Weber, J-M.

J. Exp. Biol., **210**, 4016-4023 (2007)

Fish endurance swimming is primarily powered by lipids supplied to red muscle by the circulation, but the mechanism of delivery remains unknown. By analogy to mammals, previous studies have focused on non-esterified fatty acids (NEFA bound to albumin), but lipoproteins have not been considered as an energy shuttle to working muscles. The effects of exercise on fish lipoprotein lipase (LPL) have never been investigated. We hypothesized that LPL and circulating lipoproteins would be modified by prolonged swimming. Because LPL is naturally bound to the endothelium, we have used heparin to release the enzyme in the circulation and to characterize reserve capacity for lipoprotein catabolism. The effects of exercise (4 days at 1.5 body lengths s^{-1} in a swim tunnel) were measured for red muscle LPL, post-heparin plasma LPL, and lipoprotein concentration/composition. Red muscle LPL activity increased from 18 ± 5 (rest) to 49 ± 9 nmol fatty acids $min^{-1} g^{-1}$ (swimming). In resting fish, heparin administration caused a 27-fold increase in plasma LPL activity that reached a maximum of 1.32 ± 0.67 μ mol fatty acids $min^{-1} ml^{-1}$ plasma. This heparin-induced response of plasma LPL was not different between resting controls and exercised fish. Heparin or prolonged swimming had no effect on the concentration/composition of lipoproteins that contain 92% of the energy in total plasma lipids. We conclude that (1) red muscle LPL is strongly activated by endurance swimming, (2) rainbow trout have a high reserve capacity for hydrolyzing lipoproteins, and (3) future studies should aim to measure lipoprotein flux because their concentration does not reflect changes in flux. These novel characteristics of fish LPL imply that lipoproteins are used as a metabolic shuttle between fat reserves and working muscles, a strategy exploiting an abundant source of energy in rainbow trout.

2.86 Targeting of stabilized plasmid lipid particles to hepatocytes *in vivo* by means of coupled lactoferrin

Weeke-Klimp, A.H. et al
J. Drug Targeting, **15(9)**, 585-594 (2007)

For non-viral gene delivery we prepared stabilized plasmid lipid particles (SPLPs), to which lactoferrin (LF) was coupled as a hepatocyte specific targeting ligand. LF-SPLPs and untargeted SPLPs labeled with [3 H]cholesteryloleylether were injected into rats. About 87% of the LF-SPLPs were eliminated from the blood within 5 min, while 80% of untargeted SPLPs were still circulating after 2 h. Fifty-two percent of the LF-SPLPs were taken up by hepatocytes, while non-parenchymal liver cells accounted for 16% of the uptake. Despite the efficient targeting of LF-SPLPs to hepatocytes and their capacity to transfect HepG2 and COS-7 cells *in vitro*, expression of a reporter gene was not detected *in vivo*. Overall, covalent coupling of LF to SPLPs leads to massive delivery in hepatocytes after systemic administration. However, these LF-SPLPs are not able to transfect these cells *in vivo*.

2.87 Lipoprotein-Heparan Sulfate Interactions in the Hh Pathway

Eugster, C., Panakova, D., Mahmoud, A. and Eaton, S.
Developmental Cell, **13(1)**, 57-71 (2007)

The *Drosophila* lipoprotein particle, Lipophorin, bears lipid-linked morphogens on its surface and is required for long-range signaling activity of Wingless and Hedgehog. Heparan sulfate proteoglycans are also critical for trafficking and signaling of these morphogens. Here we show that Lipophorin interacts with the heparan sulfate moieties of the glypicans Dally and Dally-like. Membrane-associated glypicans can recruit Lipophorin to disc tissue, and remain associated with these particles after they are released from the membrane by cleavage of their gpi anchors. The released form of Dally colocalizes with Patched, Hedgehog, and Lipophorin in endosomes and increases Hedgehog signaling efficiency without affecting its distribution. These data suggest that heparan sulfate proteoglycans may influence lipid-linked morphogen signaling, at least in part, by binding to Lipophorin. They further suggest that the complement of proteins present on lipoprotein particles can regulate the activity of morphogens.

2.88 Binding of liver derived, low density hepatitis C virus to human hepatoma cells

Martin, C., Nielsen, S.U., Ibrahim, S., Bassendine, M.F. and Toms, G.L.
J. Med. Virol., **80**, 816-823 (2008)

HCV recovered from low density fractions of infected blood is associated with lipid and host apo-lipoproteins in lipo-viro-particles (LVP). It has been proposed that these particles are capable of binding and entering hepatocytes by viral glycoprotein independent mechanisms utilizing uptake pathways of normal host lipoproteins after binding to cell surface glycosaminoglycans (GAG), the low density lipoprotein receptor (LDL-r) or scavenger receptor B1 (SR-B1). In this study binding to human hepatoma cells of HCV low density RNA containing particles, semi-purified from macerates of infected human liver, is compared with that of normal host low density lipoprotein (LDL). Binding of both LDL and HCV low

density RNA containing particles paralleled LDL-r but not SR-B1 expression on the recipient cells. Binding of both particle types was sensitive to suramin at 0°C but less so at 37°C suggesting that they both bind initially to GAG but, at 37°C, are internalized or transferred to a suramin resistant receptor. Suramin resistant uptake of both particles was blocked in the presence of excess LDL or oxidized LDL. However, whilst LDL uptake was blocked by anti-apoB-100, HCV low density RNA uptake was enhanced by anti-apoB100 and further enhanced by a cocktail of anti-apo-B100 and anti-apoE. Pre-incubation of HCV low density RNA containing particles with antibodies to the E2 glycoprotein had little or no effect on uptake. These data indicate that whilst liver derived HCV RNA containing particles are taken up by HepG2 cells by a virus glycoprotein independent mechanism, the mechanism differs from that of LDL uptake.

2.89 TRANSPORT OF GHRELIN AND OBESTATIN IN PLASMA

Holmes, E., Davies, I., Lowe, G. and Ranganath, L.
Atherosclerosis Supplements, **9(1)**, 26-27 (2008)

Introduction: Since its discovery in 1999, ghrelin has emerged as a key player in central appetite regulation, and also has cardiovascular actions. Roles for ghrelin in the development of atherosclerosis have also been described. Ghrelin is secreted in an active, acylated form, which is rapidly des-acylated in plasma. Obestatin, a peptide produced from the cleavage of pre-proghrelin, is also emerging as a metabolic regulator. While it has been reported that ghrelin can bind to HDL, knowledge about ghrelin and obestatin transport and metabolism is limited.

Aims: To investigate the transport of ghrelin and obestatin by lipoproteins.

Methods: Ghrelin and obestatin were measured by EIA in plasma fractions generated using an iodixanol gradient.

Results: Acylated ghrelin (AG) bound to all lipoproteins and was present as a plasma protein (VLDL 12%, LDL 33%, HDL 23%, protein 32% of total AG). In contrast, unacylated ghrelin (UAG) was bound more specifically to HDL (LDL 5%, HDL 43%, protein 51%). Obestatin did not bind to lipoproteins.

Discussion: AG binding to lipoproteins, may be due to a non-specific, hydrophobic interaction between the acyl group and the phospholipid membrane. The interaction between UAG and HDL may indicate a more specific interaction; possibly due to des-acylation of ghrelin by a HDL bound enzyme. The implications of these various forms of Ghrelin in the context of obesity and cardiovascular disease is unknown.

2.90 Lipoprotein separation in a novel iodixanol density gradient, for composition, density, and phenotype analysis

Yee, M.S. et al
J. Lipid Res., **49**, 1364-1371 (2008)

Separation of lipoproteins by traditional sequential salt density floatation is a prolonged process (~72 h) with variable recovery, whereas **iodixanol**-based, self-generating density gradients provide a rapid (~4 h) alternative. A novel, three-layered **iodixanol** gradient was evaluated for its ability to separate lipoprotein fractions in 63 subjects with varying degrees of dyslipidemia. Lipoprotein cholesterol, triglycerides, and apolipoproteins were measured in 21 successive **iodixanol** density fractions. **Iodixanol** fractionation was compared with sequential floatation ultracentrifugation. **Iodixanol** gradient formation showed a coefficient of variation of 0.29% and total lipid recovery from the gradient of 95.4% for cholesterol and 84.7% for triglyceride. Recoveries for VLDL-, LDL-, and HDL-cholesterol, triglycerides, and apolipoproteins were approximately 10% higher with **iodixanol** compared with sequential floatation. The **iodixanol** gradient effectively discriminated classic lipoproteins and their subfractions, and there was evidence for improved resolution of lipoproteins with the **iodixanol** gradient. LDL particles subfractionated by the gradient showed good correlation between density and particle size with small, dense LDL (<25.5 nm) separated in fractions with density >1.028 g/dl. The new **iodixanol** density gradient enabled rapid separation with improved resolution and recovery of all lipoproteins and their subfractions, providing important information with regard to LDL phenotype from a single centrifugation step with minimal in-vitro modification of lipoproteins.

2.91 Phenolsulfonphthalein, but Not Phenolphthalein, Inhibits Amyloid Fibril Formation: Implications for the Modulation of Amyloid Self-Assembly

Levy, M., Porat, Y., Bacharach, E., Shalev, D.E. and Gazit, E.
Biochemistry, **47**, 5896-5904 (2008)

The study of the mechanism of amyloid fibril formation and its inhibition is of key medical importance due to the lack of amyloid assembly inhibitors that are approved for clinical use. We have previously

demonstrated the potent inhibitory potential of phenolsulfonphthalein, a nontoxic compound that was approved for diagnostic use in human subjects, on aggregation of islet amyloid polypeptide (IAPP) that is associated with type 2 diabetes. Here, we extend our studies on the mechanism of action of phenolsulfonphthalein by comparing its antiamyloidogenic effect to a very similar compound that is also approved for human use, phenolphthalein. While these compounds have very similar primary chemical structures, they significantly differ in their three-dimensional conformation. Our results clearly demonstrated that these two compounds had completely different inhibitory potencies: While phenolsulfonphthalein was a very potent inhibitor of amyloid fibril formation by IAPP, phenolphthalein did not show significant antiamyloidogenic activity. This behavior was observed with a short amyloid fragment of IAPP and also with the full-length polypeptide. The NMR spectrum of IAPP_{20–29} in the presence of phenolsulfonphthalein showed chemical shift deviations that were different from the unbound or phenolphthalein-bound peptide. Differential activity was also observed in the inhibition of insulin amyloid formation by these two compounds, and density-gradient experiments clearly demonstrated the different inhibitory effect of the two compounds on the formation of prefibrillar assemblies. Taken together, our studies suggest that the three-dimensional arrangement of the polyphenol phenolsulfonphthalein has a central role in its amyloid formation inhibition activity.

2.92 **Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss**

Harman, N.L., Leeds, A.R. and Griffin, B.A.
Eur. J. Nutr., **47**, 287-293 (2008)

Background Diets enriched with dietary cholesterol, frequently from eggs, have been shown to produce a small but variable increase in plasma low density lipoprotein (LDL) cholesterol. There is evidence to suggest that energy-restricted diets, that may contain a relatively high proportion of fat and cholesterol, can attenuate the cholesterol-raising effect of dietary cholesterol on plasma LDL.

Aim of the study To determine the combined effects of increased dietary cholesterol and weight loss produced by energy restriction on plasma LDL cholesterol and lipoproteins.

Methods A randomized, controlled, parallel study was performed in two groups of free-living volunteers on an energy-restricted diet for 12 weeks, one group was instructed to consume two eggs a day ($n = 24$), the other, to exclude eggs ($n = 21$). Dietary advice on energy restriction was based on the British Heart Foundation guidelines on how to lose weight for men and women.

Results Energy intake fell by 25 and 29% in the egg-fed and non-egg-fed groups, resulting in a moderate weight loss of 3.4 kg ($P < 0.05$) and 4.4 kg ($P < 0.05$), respectively. The daily intake of dietary cholesterol increased significantly in the egg-fed group from 278 to 582 mg after 6 weeks. The concentration of plasma LDL cholesterol decreased in the non-egg-fed groups after 6 weeks ($P < 0.01$) and in the egg-fed and non-egg-fed at 12 weeks relative to baseline. There were no other significant changes in plasma lipoproteins or LDL particle size.

Conclusions An increased intake of dietary cholesterol from two eggs a day, does not increase total plasma or LDL cholesterol when accompanied by moderate weight loss. These findings suggest that cholesterol-rich foods should not be excluded from dietary advice to lose weight on account of an unfavorable influence on plasma LDL cholesterol.

2.93 **The Potential for β -Structure in the Repeat Domain of Tau Protein Determines Aggregation, Synaptic Decay, Neuronal Loss, and Coassembly with Endogenous Tau in Inducible Mouse Models of Tauopathy**

Mocanu, M-M. et al
J. Neurosci., **28**(3), 737-748 (2008)

We describe two new transgenic mouse lines for studying pathological changes of Tau protein related to Alzheimer's disease. They are based on the regulatable expression of the four-repeat domain of human Tau carrying the FTDP17 (frontotemporal dementia and parkinsonism linked to chromosome 17) mutation Δ K280 (Tau_{RD}/ Δ K280), or the Δ K280 plus two proline mutations in the hexapeptide motifs (Tau_{RD}/ Δ K280/I277P/I308P). The Δ K280 mutation accelerates aggregation ("proaggregation mutant"), whereas the proline mutations inhibit Tau aggregation *in vitro* and in cell models ("antiaggregation mutant"). The inducible transgene expression was driven by the forebrain-specific CaMKII α (calcium/calmodulin-dependent protein kinase II α) promoter. The proaggregation mutant leads to Tau aggregates and tangles as early as 2–3 months after gene expression, even at low expression (70% of endogenous mouse Tau). The antiaggregation mutant does not aggregate even after 22 months of gene expression. Both mutants show missorting of Tau in the somatodendritic compartment and hyperphosphorylation in the repeat domain

[KXGS motifs, targets of the kinase MARK (microtubule affinity regulating kinase)]. This indicates that these changes are related to Tau expression rather than aggregation. The proaggregation mutant causes astrogliosis, loss of synapses and neurons from 5 months of gene expression onward, arguing that Tau toxicity is related to aggregation. Remarkably, the human proaggregation mutant Tau_{RD} coaggregates with mouse Tau, coupled with missorting and hyperphosphorylation at multiple sites. When expression of proaggregation Tau_{RD} is switched off, soluble and aggregated exogenous Tau_{RD} disappears within 1.5 months. However, tangles of mouse Tau, hyperphosphorylation, and missorting remain, suggesting an extended lifetime of aggregated wild-type Tau once a pathological conformation and aggregation is induced by a proaggregation Tau species.

2.94 mRNA Translation Regulation by the Gly-Ala Repeat of Epstein-Barr Virus Nuclear Antigen 1

Apcher, S., Komarova, A., Daskalogianni, C., Yin, Y., Malbert-Colas, L. And Fähræes, R.
J. Virol., **83**(3), 1289-1298 (2009)

The glycine-alanine repeat (GAR) sequence of the Epstein-Barr virus-encoded EBNA-1 prevents presentation of antigenic peptides to major histocompatibility complex class I molecules. This has been attributed to its capacity to suppress mRNA translation *in cis*. However, the underlying mechanism of this function remains largely unknown. Here, we have further investigated the effect of the GAR as a regulator of mRNA translation. Introduction of silent mutations in each codon of a 30-amino-acid GAR sequence does not significantly affect the translation-inhibitory capacity, whereas minimal alterations in the amino acid composition have strong effects, which underscores the observation that the amino acid sequence and not the mRNA sequence mediates GAR-dependent translation suppression. The capacity of the GAR to repress translation is dose and position dependent and leads to a relative accumulation of preinitiation complexes on the mRNA. Taken together with the surprising observation that fusion of the 5' untranslated region (UTR) of the c-myc mRNA to the 5' UTR of GAR-carrying mRNAs specifically inactivates the effect of the GAR, these results indicate that the GAR targets components of the translation initiation process. We propose a model in which the nascent GAR peptide delays the assembly of the initiation complex on its own mRNA.

2.95 Flow-mediated vasodilatation: variation and interrelationships with plasma lipids and lipoproteins

Rasmussen, J.G., Eschen, R.B., Aardestrup, I.V., Dethlefsen, C., Griffin, B.A. and Schmidt, E.B.
Scand. J. Clin. Lab. Invest., **69**(1), 156-160 (2009)

Objective . Endothelial dysfunction is a critical, prerequisite step in atherosclerosis, and may be evaluated by flow-mediated vasodilatation (FMD). The objective of this study was to examine interrelationships between FMD and plasma lipids and lipoproteins, and to determine the between-operator and within-subject variability associated with this technique. Material and methods. FMD, plasma lipids and lipoproteins, including small dense LDL (sdLDL), were measured twice in 40 healthy volunteers, 4 weeks apart. Interrelationships between mean FMD responses and plasma lipids and lipoproteins were examined by correlation analysis. FMD measurements were taken by two independent operators, allowing determination of between-operator variability. Within-subject variability was determined by obtaining two measurements, 4 weeks apart, in every subject, and carried out by the same operator. Results. FMD was inversely related to plasma triglycerides ($r = -0.47$, $p = 0.002$), total cholesterol/HDL cholesterol ($r = -0.35$, $p = 0.03$) and apolipoprotein B ($r = -0.36$, $p = 0.02$), but not to other plasma lipids and lipoproteins. When measuring variation in FMD, the following results were found: Between operators (SD = 4.0 FMD%) and within subjects (SD = 2.9 FMD%). Conclusions. The associations between FMD, plasma triglycerides and apoB provide evidence supporting a role for triglyceride-rich lipoproteins in endothelial dysfunction.

2.96 Phosphotyrosine-dependent in vitro reconstitution of recombinant LAT-nucleated multiprotein signalling complexes on liposomes

Sangani, D., Venien-Bryan, C. and Harder, T.
Mol. Med. Biol., **26**(2), 159-170 (2009)

Numerous cell surface receptors propagate activation signals to the interior of the cell via tyrosine phosphorylation of transmembrane proteins. This leads to the phosphotyrosine (PiY)-mediated recruitment of cytoplasmic signalling protein complexes which catalyze crucial biochemical signalling reactions. Here we describe the first *in vitro* reconstitution of such PiY-nucleated protein complexes on an artificial lipid membrane. A tyrosine phosphorylated recombinant variant of the transmembrane adaptor protein Linker for Activation of T cells (PiYLAT) was anchored in liposomes. These PiYLAT proteoliposomes

specifically recruited cooperative high avidity signalling protein complexes from Jurkat cytosol. Nucleation of signalling protein assemblies readily occurred on PiYLAT liposomes composed of phosphatidylserine, but not on PiYLAT liposomes composed of phosphatidylcholine. Purified recombinant grb2 alone did not stably associate with tyrosine phosphorylated LAT proteoliposomes. However, when grb2 was presented to the PiYLAT proteoliposomes in the context of Jurkat cytosol it was incorporated into multiprotein signalling complexes. Together the data suggest that these reconstituted high-avidity signalling protein complexes represent a cooperative protein network. This novel *in vitro* approach offers a novel technology permitting biochemical, structural, and pharmacological analyses of plasma membrane receptor signalling complexes.

2.97 **The scavenger receptor CD36 plays a role in cytokine-induced macrophage fusion**

Helming, L., Winter, J. and Gordon, S.
J. Cell Sci., **122**, 453-459 (2009)

Multinucleated giant cells, characteristic of granulomatous infections, originate from the fusion of macrophages. Using an antibody screening strategy we found that the scavenger receptor CD36 participates in macrophage fusion induced by the cytokines IL-4 and GM-CSF. Our results demonstrate that exposure of phosphatidylserine on the cell surface and lipid recognition by CD36 are required for cytokine-induced fusion of macrophages. We also show that CD36 acts in a heterotypic manner during giant-cell formation and that the formation of osteoclasts is independent of CD36. The discovery of molecules involved in the formation of multinucleated giant cells will enable us to determine their functional significance. Furthermore, our results suggest that lipid capture by cell surface receptors may be a general feature of cell fusion.

2.98 **Loss of Modifier of Cell Adhesion Reveals a Pathway Leading to Axonal Degeneration**

Chen, Q., Peto, C.A., Shelton, G.D., Mizisin, A., Sawchenko, P.E. and Schubert, D.
J. Neurosci., **29**(1), 118-130 (2009)

Axonal dysfunction is the major phenotypic change in many neurodegenerative diseases, but the processes underlying this impairment are not clear. Modifier of cell adhesion (MOCA) is a presenilin binding protein that functions as a guanine nucleotide exchange factor for Rac1. The loss of MOCA in mice leads to axonal degeneration and causes sensorimotor impairments by decreasing cofilin phosphorylation and altering its upstream signaling partners LIM kinase and p21-activated kinase, an enzyme directly downstream of Rac1. The dystrophic axons found in MOCA-deficient mice are associated with abnormal aggregates of neurofilament protein, the disorganization of the axonal cytoskeleton, and the accumulation of autophagic vacuoles and polyubiquitinated proteins. Furthermore, MOCA deficiency causes an alteration in the actin cytoskeleton and the formation of cofilin-containing rod-like structures. The dystrophic axons show functional abnormalities, including impaired axonal transport. These findings demonstrate that MOCA is required for maintaining the functional integrity of axons and define a model for the steps leading to axonal degeneration.

2.99 **DDX3 DEAD-Box RNA Helicase Inhibits Hepatitis B Virus Reverse Transcription by Incorporation into Nucleocapsids**

Wang, H., Kim, S. and Ryu, W-S.
J. Virol., **83**(11), 5815-5824 (2009)

Viruses utilize host factors in many steps of their life cycles. Yet, little is known about host factors that contribute to the life cycle of hepatitis B virus (HBV), which replicates its genome by reverse transcription. To identify host factors that contribute to viral reverse transcription, we sought to identify cellular proteins that interact with HBV polymerase (Pol) by using affinity purification coupled with mass spectrometry. One of the HBV Pol-interacting host factors identified was DDX3 DEAD-box RNA helicase, which unwinds RNA in an ATPase-dependent manner. Recently, it was shown that DDX3 is essential for both human immunodeficiency virus and hepatitis C virus infection. In contrast, we found that the ectopic expression of DDX3 led to significantly reduced viral DNA synthesis. The DDX3-mediated inhibition of viral DNA synthesis did not affect RNA encapsidation, a step prior to reverse transcription, and indicated that DDX3 inhibits HBV reverse transcription. Mutational analysis revealed that mutant DDX3 with an inactive ATPase motif, but not that with an inactive RNA helicase motif, failed to inhibit viral DNA synthesis. Our interpretation is that DDX3 inhibits viral DNA synthesis at a step following ATP hydrolysis but prior to RNA unwinding. Finally, **OptiPrep** density gradient analysis revealed that DDX3 was incorporated into nucleocapsids, suggesting that DDX3 inhibits viral reverse transcription following

nucleocapsid assembly. Thus, DDX3 represents a novel host restriction factor that limits HBV infection.

2.100 **Photoinitiated Destruction of Composite Porphyrin–Protein Polymersomes**

Robbins, G.P., Jimbo, M., Swift, J., Therien, M.J., Hammer, D.A. and Dmochowski, I.J.
J. Am. Chem. Soc., **131**, 3872-3874 (2009)

Bilayer vesicles assembled from amphiphilic diblock copolymers (polymersomes) adopt asymmetric structures when loaded with moderate concentrations (≥ 1.5 mg/mL) of horse spleen ferritin (HSF) or its iron-free variant (HSAF). Incorporation of both ferritin and a zinc porphyrin dimer (PZn₂) generates photoresponsive vesicles: irradiation with focused light of near-UV to near-IR wavelengths induces polymersome deformation and destruction on the minute time scale. To investigate this phenomenon, polymersomes were loaded with dye-labeled ferritin and PZn₂. Confocal microscopy identified BODIPY-FL-labeled ferritin at the membrane, whereas Cy3-labeled ferritin was found both at the membrane and throughout the aqueous core. Fluorescence recovery after photobleaching (FRAP) experiments confirmed that Cy3- and BODIPY-FL-labeled ferritin and PZn₂ exhibited slow diffusion at the membrane, consistent with membrane association. Furthermore, micropipette aspiration experiments revealed increased elastic moduli and altered bending rigidity in vesicles incorporating HSAF. Finally, a small molecule (biocytin) was encapsulated within the ferritin–PZn₂ vesicles and released upon exposure to light. These data indicate synergy between ferritin, whose membrane association lowers the barrier to deformation, and PZn₂, which embeds in the membrane, harvests light energy and produces local heating that may lead to membrane budding. This appears to be a general protein–polymer membrane phenomenon, as replacement of ferritin with bovine serum albumin or equine skeletal myoglobin resulted in vesicles with similar asymmetric morphology and photosensitivity.

2.101 **Four Conserved Cysteine Residues of the Hepatitis B Virus Polymerase Are Critical for RNA Pregenome Encapsidation**

Kim, S., Lee, J. and Ryu, W-S.
J. Virol., **83**(16), 8032-8040 (2009)

Hepadnaviruses replicate via reverse transcription of an RNA template, the pregenomic RNA (pgRNA). Although hepadnaviral polymerase (Pol) and retroviral reverse transcriptase are distantly related, some of their features are distinct. In particular, Pol contains two additional N-terminal subdomains, the terminal protein and spacer subdomains. Since much of the spacer subdomain can be deleted without detrimental effects to hepatitis B virus (HBV) replication, this subdomain was previously thought to serve only as a spacer that links the terminal protein and reverse transcriptase subdomains. Unexpectedly, we found that the C terminus of the spacer subdomain is indispensable for the encapsidation of pgRNA. Alanine-scanning mutagenesis revealed that four conserved cysteine residues, three at the C terminus of the spacer subdomain and one at the N terminus of the reverse transcriptase subdomain, are critical for encapsidation. The inability of the mutant Pol proteins to incorporate into nucleocapsid particles, together with other evidence, argued that the four conserved cysteine residues are critical for RNA binding. One implication is that these four cysteine residues might form a putative zinc finger motif. Based on these findings, we speculate that the RNA binding activity of HBV Pol may be mediated by this newly identified putative zinc finger motif.

2.102 **Effect of hyperthermia on plasma lipids and gene expression in Atlantic Cod. (*Gadus Morhua* I.)**

Aursnes, I.A.S., Gjoen, T. and Rishovd, A-L.
Toxico. Lett., **189S**, S57 (2009)

Purpose: Wild fish occupy their preferred temperature range by vertical movements in the water column. During fish farming, this migration may be restricted leading to thermal stress. We have investigated stress responses in Atlantic Cod (*Gadus Morhua*) exposed to elevated water temperature. The main goal was to identify stable housekeeping genes that can be used for internal normalization for quantitative real-time PCR analyses across treatment groups in experiments. The software used was Normfinder and REST. We also obtained blood samples and analyzed for differences in plasma lipid concentrations.

Methods: Wild Atlantic cod (*Gadus Morhua*) weighing 200–500 g was divided into two groups (5 fish in each tank) were one served as control (kept at 12 °C) and one group in which the water temperature was increased from 12 °C to 17 °C. Tissue samples and blood samples were withdrawn from all individuals. Blood was analyzed by separation on a continuous gradient using iodixanol and divided into fractions which was analyzed for triglycerides, cholesterol and level of oxidation. The tissue samples were prepared for RNA extraction and RT-qPCR analyses.

Results: Plasma lipid concentrations (total cholesterol and triglycerides), and oxidation levels in the hyperthermally stressed fish were significantly increased compared to the control group. Across both groups and all tissues Normfinder ranked the gene analyzed in this order, from most stable to least stable EF1 α > 18s > ubiquitin > β -actin > β -2-microglobulin > tubulin α > ARP > G6PDH. In liver tissue Normfinder identified ubiquitin as the most stable gene. REST analysis of relative expression revealed that in liver HSP90, IL-1 β and TNF α was significantly upregulated after hyperthermia.

2.103 **Comparability of methods for LDL subfraction determination: A systematic review**

Chung, M., Lichtenstein, A.H., Ip, S., Lau, J. and Balk, E.M.

Atherosclerosis, **205**, 342-348 (2009)

Identifying and aggressively treating individuals at elevated risk of developing cardiovascular disease (CVD) is critical to optimizing health outcomes. The CVD risk factors defined by the National Cholesterol Education Program do not fully predict individuals at high risk of developing CVD. Validation of potential methodologies against a reference method is essential to the adoption of a potential new risk factor to improve risk prediction. Low-density lipoprotein (LDL) subfraction has been advanced as a potential additional CVD risk factor. Currently, there is no reference method for determining LDL subfractions or standardizing the different methods used to measure LDL subfractions. We conducted a systematic review to identify reports comparing two or more methods of measuring LDL subfractions. Nine articles were identified that separated and quantified LDL subfractions by at least two methods. Comparative data were available for nuclear magnetic resonance vs. gel electrophoresis (GE), LipoPrint[®] vs. other GE methods, ultracentrifugation vs. GE, and high performance gel filtration chromatography vs. GE. We found a wide range of agreement (from 7 to 94% concordance for classifying LDL patterns) among methods for LDL subfraction determinations. Different criteria and definitions were used among the articles to classify individuals with respect to CVD risk. No study used CVD or other clinical outcomes as an outcome measure. In summary, the currently available literature does not provide adequate data about comparability in terms of test performance to choose one or another method to serve as a standard nor are data on comparability in terms of predicting CVD outcomes.

2.104 **Changes in lipoprotein profile and urinary albumin excretion in familial LCAT deficiency with lipid lowering therapy**

Yee, M.S., Pavitt, D.V., Richmond, W., Cook, H.T., McLean, A.G., Valabhji, J. and Elkeles, R.S.

Atherosclerosis, **205**, 528-532 (2009)

Familial lecithin:cholesterol acyltransferase deficiency (FLD) is a monogenic autosomal recessive condition, affecting cholesterol esterification and leads to progressive renal impairment and end-stage renal failure, probably due to the abnormal lipoprotein (X) (Lp(X)).

We report a case of FLD, whom we treated with a combination of nicotinic acid 1.5 g nocte and fenofibrate M/R 160 mg od and report changes in lipid profile and Lp(X), after six weeks and serum creatinine and urine albumin/creatinine ratio after 12 months. We assessed the cardiovascular risk using electron beam computed tomography.

At baseline total cholesterol was 6.61 mmol/L; HDL cholesterol 0.57 mmol/L; Lp(X) cholesterol 3.24 mmol/L; triglyceride 4.13 mmol/L; apolipoprotein A1 46 mg/dL; and apolipoprotein B 53 mg/dL. After six weeks of treatment his total cholesterol was 4.16; HDL cholesterol 0.52; Lp(X) cholesterol 1.73 mmol/L; triglyceride 1.80 mmol/L; apolipoprotein A1 36 mg/dL; and apolipoprotein B 50 mg/dL. Baseline serum creatinine was 106 μ mol/L and urine albumin/creatinine ratio was 127.3 mg/mmol and after 12 months was 101 μ mol/L and 31.5 mg/mmol respectively. His coronary artery calcification score was zero.

We have shown, we believe for the first time, that combination lipid modifying therapy in FLD leads to a reduction in Lp(X) concentration and an associated reduction in urine albumin excretion at 12 months.

2.105 **Long-Term Glycemic Control Influences the Long-Lasting Effect of Hyperglycemia on Endothelial Function in Type 1 Diabetes**

Ceriello, A., Esposito, K., Ihnat, M., Thorpe, J. and Giugliano, D.

J. Clin. Endocrinol. Metab., **94**(8), 2751-2756 (2009)

Objective: The objective of the study was to investigate the effect of different periods of hyperglycemia on the reversal of endothelial dysfunction by glucose normalization and antioxidant therapy.

Research Design and Methods: Ten healthy subjects and three subgroups of 10 type 1 diabetic subjects were enrolled as follows: 1) patients within 1 month of diagnosis; 2) patients between 4.5 and 5.2 yr from

diagnosis and with glycosylated hemoglobin levels 7% or greater since diagnosis; 3) patients between 4.8 and 5.4 yr from diagnosis and with glycosylated hemoglobin levels greater than 7% since diagnosis. Each patient participated in three experiments: 1) 24-h insulin treatment, achieving a near normalization of glycemia, together with the addition of the antioxidant vitamin C during the last 12 h; 2) 24-h vitamin C treatment with insulin treatment for the last 12 h; and 3) treatment with both vitamin C and insulin for 24 h. **Results:** Endothelial function, as measured by flow-mediated vasodilation of the brachial artery and levels of nitrotyrosine, an oxidative stress marker, were normalized by each treatment in subgroups 1 and 2. In the third subgroup, neither glucose normalization nor vitamin C treatment alone was able to normalize endothelial dysfunction or oxidative stress. Combining insulin and vitamin C, however, normalized endothelial dysfunction and nitrotyrosine. **Conclusions:** This study suggests that long-lasting hyperglycemia in type 1 diabetic patients induces long-term alterations in endothelial cells, which may contribute to endothelial dysfunction and is interrupted only by both glucose and oxidative stress normalization.

2.106 The accessory subunit of mitochondrial DNA polymerase γ determines the DNA content of mitochondrial nucleoids in human cultured cells

Re, M.D., Sembongi, H., He, J., Reyes, J.H., Yasukawa, T., Martinsson, P., Bailey, L.J., Goffart, S., Boyd-Kirkup, J.D., Wong, T.S., Fersht, A.R., Spelbrink, J.N. and Holt, I.J.
Nucleic Acids Res., **37**(17), 5701-5713 (2009)

The accessory subunit of mitochondrial DNA polymerase γ , POLG β , functions as a processivity factor *in vitro*. Here we show POLG β has additional roles in mitochondrial DNA metabolism. Mitochondrial DNA is arranged in nucleoprotein complexes, or nucleoids, which often contain multiple copies of the mitochondrial genome. Gene-silencing of POLG β increased nucleoid numbers, whereas over-expression of POLG β reduced the number and increased the size of mitochondrial nucleoids. Both increased and decreased expression of POLG β altered nucleoid structure and precipitated a marked decrease in 7S DNA molecules, which form short displacement-loops on mitochondrial DNA. Recombinant POLG β preferentially bound to plasmids with a short displacement-loop, in contrast to POLG α . These findings support the view that the mitochondrial D-loop acts as a protein recruitment centre, and suggest POLG β is a key factor in the organization of mitochondrial DNA in multigenomic nucleoprotein complexes.

2.107 Ultrastructures and strain comparison of under-glycosylated scrapie prion fibrils

Sim, V.L. and Caughey, B.
Neurobiology of Aging, **30**, 2031-2042 (2009)

Prions, composed primarily of misfolded, often fibrillar, polymers of prion protein, have poorly understood structures. Heavy surface glycosylation may obscure visualization of their fibrillar cores, so we purified severely under-glycosylated prion protein fibrils from scrapie-infected transgenic mice expressing anchorless prion protein. Using electron and atomic force microscopy, we obtained dimensions and morphological information about prion protein core protofilaments which variably intertwined to form scrapie fibrils. Occasional isolated protofilaments were observed, suggesting that the lateral association of protofilaments is neither essential nor invariant in prion protein polymerization. Strain comparisons suggested basic structural differences; ME7 and 22L fibrils contained thinner protofilaments, 22L fibrils preferred left-handed twists, and 22L fibril periodicities averaged 106 nm per half-turn, compared with 64 and 66 nm for RML and ME7 fibrils, respectively. The strains displayed overlapping fibril morphologies, providing evidence that prion fibril morphology is influenced, but not dictated, by strain-dependent differences in protofilament structure. These measurements of the amyloid core of scrapie fibrils should aid development of models of prion structure and strain determination.

2.108 Phytosterol-Enriched Yogurt Increases LDL Affinity and Reduces CD36 Expression in Polygenic Hypercholesterolemia

Ruiu, G., Pinach, S., Veglia, F., Gambino, R., Marena, S., Uberti, B., Alemanno, N., Burt, D., Pagano, G. and Cassader, M.
Lipids, **44**, 153-160 (2009)

Dietary enrichment with phytosterols (plant sterols similar to cholesterol) is able to reduce plasma cholesterol levels due to reduced intestinal absorption. The aim of this study was to investigate the effect of phytosterol-enriched yogurt consumption on the major serum lipid parameters, low density lipoprotein (LDL) receptor activity, LDL-receptor affinity, and CD36 expression in hypercholesterolemic subjects. Fifteen patients affected by polygenic hypercholesterolemia were evaluated in a single-blind randomized

crossover study after a 4 weeks treatment with a phytosterol-enriched yogurt containing 1.6 g esterified phytosterols (equivalent to 1.0 g free phytosterol). Lipid parameters were compared with a phytosterol-free placebo-controlled diet. The effect of the two treatments on each variable, measured as percentage change, was compared by paired samples t test and covariance analysis. The treatment induced a modest but significant decrease in LDL-cholesterol levels (4.3%, $P = 0.03$) and a significant increase in high density lipoprotein (HDL) 3-cholesterol (17.1%, $P = 0.01$). Phytosterol consumption had no effect on LDL-receptor activity whereas patient LDL-receptor affinity significantly increased (9.7%, $P = 0.01$) and CD36 expression showed a marked significant decrease (18.2%, $P = 0.01$) in the phytosterol-enriched yoghurt patients. Our data show that the oral administration of a phytosterol-enriched yogurt has modest but significant effects on commonly measured lipid parameters. The improvement of LDL-receptor affinity and the reduction in CD36 expression may reflect an important antiatherogenic effect.

2.109 **Circulating ghrelin exists in both lipoprotein bound and free forms**

Holmes, E., Davies, I., Lowe, G. and Ranganath, L.R.
Ann. Clin. Biochem., **46**, 514-516 (2009)

Introduction: Ghrelin is a gastric peptide that has been implicated in the development of obesity and cardiovascular disease. It has been reported that ghrelin binds to lipoproteins, although the different binding patterns of acylated ghrelin (AG) and unacylated ghrelin (UAG) are still to be determined.

Methods: Lipoprotein fractions were generated using a self-generating **iodixanol** gradient. AG and UAG were measured using specific enzyme immunoassays.

Results: AG bound to all lipoproteins in approximately equal concentrations (VLDL 26%, LDL 22%, HDL 23%) and was present as a plasma protein (27%). UAG bound more specifically to HDL (49%) and was present as a plasma protein (48%).

Conclusions: The different binding patterns of AG and UAG may have significant implications for their biological effects, including roles in energy metabolism, the development of obesity and potentially in the modulation of cardiovascular disease.

2.110 **Impact of Saturated, Polyunsaturated and Monounsaturated Fatty Acid-Rich Micelles on Lipoprotein Synthesis and Secretion in Caco-2**

Jackson, K.G., Bateman, P.A., Yaqoob, P. and Williams, C.M.
Lipids, **44**, 1081-1089 (2009)

Meal fatty acids have been shown to modulate the size and composition of triacylglycerol (TAG)-rich lipoproteins influencing the magnitude and duration of the postprandial plasma TAG response. As a result there is considerable interest in the origin of these meal fatty-acid induced differences in particle composition. Caco-2 cells were incubated over 4 days with fatty acid mixtures resembling the composition of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA)-rich meals fed in a previous postprandial study to determine their impact on lipoprotein synthesis and secretion. The MUFA- and PUFA-rich mixtures supported greater intracellular TAG, but not cholesterol accumulation compared with the SFA-rich mixture ($P < 0.001$). The MUFA-rich mixture promoted significantly greater TAG and cholesterol secretion than the other mixtures and significantly more apolipoprotein B-100 secretion than the PUFA-rich mixture ($P < 0.05$). Electron microscopy revealed the SFA-rich mixture had led to unfavourable effects on cellular morphology, compared with the unsaturated fatty acid-rich mixtures. Our findings suggest the MUFA-rich mixture, may support the formation of a greater number of TAG-rich lipoproteins, which is consistent with indirect observations from our human study. Our electron micrographs are suggestive that some endocytotic uptake of MUFA-rich taurocholate micelles may promote greater lipoprotein synthesis and secretion in Caco-2 cells.

2.111 **Incorporation of Eukaryotic Translation Initiation Factor eIF4E into Viral Nucleocapsids via Interaction with Hepatitis B Virus Polymerase**

Kim, S., Wang, H. and Ryu, W-S.
J. Virol., **84**(1), 52-58 (2010)

The DNA genome of hepatitis B virus (HBV) replicates via reverse transcription within capsids following the encapsidation of an RNA template, the pregenomic RNA (pgRNA). We previously demonstrated that the 5' cap proximity of the stem-loop structure (ϵ or epsilon), an encapsidation signal, is critically important for the encapsidation of the pgRNA (J. K. Jeong, G. S. Yoon, and W. S. Ryu, *J. Virol.* 74:5502-5508, 2000). Therefore, we speculated that the viral polymerase (Pol), while bound to the 5' ϵ -stem-loop structure, could recognize the cap via its interaction with eIF4E, a eukaryotic translation initiation factor.

Our data showed the direct interaction between HBV Pol and eIF4E, as measured by coimmunoprecipitation. Further, we demonstrated that eIF4E interacts with the Pol- ϵ ribonucleoprotein complex (RNP) rather than Pol alone, resulting in eIF4E-Pol- ϵ RNP complex formation. In addition, we asked whether eIF4E remains engaged to the Pol- ϵ RNP complex during nucleocapsid assembly. Density gradient analysis revealed that eIF4E indeed was incorporated into nucleocapsids. It is of great importance to uncover whether the incorporated eIF4E contributes to viral reverse transcription or other steps in the HBV life cycle.

2.112 **Unpacking a gel-forming mucin: a view of MUC5B organization after granular release**

Kesimer, M., Makhov, A.M., Griffith, J.D., Verdugo, P. and Sheehan, J.K.

Am. J. Physiol. Lung Cell Mol. Physiol., **298**, L15-L22 (2010)

Gel-forming mucins are the largest complex glycoprotein macromolecules in the body. They form the matrix of gels protecting all the surface epithelia and are secreted as disulfide-bonded polymeric structures. The mechanisms by which they are formed and organized within cells and thereafter released to form mucus gels are not understood. In particular, the initial rate of expansion of the mucins after release from their secretory granules is very rapid (seconds), but no clear mechanism for how it is achieved has emerged. Our major interest is in lung mucins, but most particularly in MUC5B, which is the major gel-forming mucin in mucus, and which provides its major protective matrix. In this study, using **OptiPrep** density gradient ultracentrifugation, we have isolated a small amount of a stable form of the recently secreted and expanding MUC5B mucin, which accounts for less than 2% of the total mucin present. It has an average mass of $\sim 150 \times 10^6$ Da and size R_g of 150 nm in radius of gyration. In transmission electron microscopy, this compact mucin has maintained a circular structure that is characterized by flexible chains connected around protein-rich nodes as determined by their ability to bind colloidal gold. The appearance indicates that the assembled mucins in a single granular form are organized around a number of nodes, each attached to four to eight subunits. The organization of the mucins in this manner is consistent with efficient packing of a number of large heavily glycosylated monomers while still permitting their rapid unfolding and hydration. For the first time, this provides some insight into how the carbohydrate regions might be organized around the NH_2 - and COOH -terminal globular protein domains within the granule and also explains how the mucin can expand so rapidly upon its release.

2.113 **Distribution of Chlorophyll- and Bacteriochlorophyll-derived Photosensitizers in Human Blood Plasma**

Dandler, J., Wilhelm, B. and Scheer, H.

Photochem. and Photobiol., **86**, 182-193 (2010)

Chlorophyll *a* and, in particular, bacteriochlorophyll *a* derivatives are promising candidates for photosensitizers in photodynamic therapy. The distribution of 21 (bacterio)chlorophyll derivatives among human blood plasma fractions was studied by iodixanol gradient ultracentrifugation and *in situ* absorption spectroscopy. Modifications of the natural pigments involved the central metal (Mg^{2+} , Zn^{2+} , Pd^{2+} , none), the isocyclic ring (closed, open and taurinated), substituents at C-3 (vinyl, acetyl, 1-hydroxyethyl) and C-17³ (phytyl ester, free acid). Cellular blood components bound only a small fraction of the pigments. Distribution among low-density lipoproteins (LDL), high-density lipoproteins (HDL) and high-density proteins (HDP) of the plasma was influenced as follows: (1) application in Cremophor[®] EL slightly altered pigment distribution by lipoprotein modification, (2) only very polar pigments with multiple hydrophilic substituents showed substantial HDP binding, (3) the presence of the esterifying alcohol at C-17³ caused enrichment in LDL, this was more pronounced with bacteriochlorophylls than with chlorophylls, (4) substituents at C-3 had only little influence on the distribution, (5) Zn^{2+} -complexes were enriched in HDL compared to Mg^{2+} and Pd^{2+} complexes, indicating specific binding of the former. Equilibration of pigments among the different fractions was largely complete within 3 h.

2.114 **Acrolein consumption induces systemic dyslipidemia and lipoprotein modification**

Conklin, D.J., Barski, O.A., Lesgards, J-F., Juvan, P., rezen, T., Rozman, D., Prough, R.A.,

Vladykovskaya, E., Liu, S., Srivastava, S. and Bhatnagar, A.

Tox. Appl. Pharmacol., **243(1)**, 1.12 (2010)

Aldehydes such as acrolein are ubiquitous pollutants present in automobile exhaust, cigarette, wood, and coal smoke. Such aldehydes are also constituents of several food substances and are present in drinking water, irrigation canals, and effluents from manufacturing plants. Oral intake represents the most significant source of exposure to acrolein and related aldehydes. To study the effects of short-term oral

exposure to acrolein on lipoprotein levels and metabolism, adult mice were gavage-fed 0.1 to 5 mg acrolein/kg bwt and changes in plasma lipoproteins were assessed. Changes in hepatic gene expression related to lipid metabolism and cytokines were examined by qRT-PCR analysis. Acrolein feeding did not affect body weight, blood urea nitrogen, plasma creatinine, electrolytes, cytokines or liver enzymes, but increased plasma cholesterol and triglycerides. Similar results were obtained with apoE-null mice. Plasma lipoproteins from acrolein-fed mice showed altered electrophoretic mobility on agarose gels. Chromatographic analysis revealed elevated VLDL cholesterol, phospholipids, and triglycerides levels with little change in LDL or HDL. NMR analysis indicated shifts from small to large VLDL and from large to medium-small LDL with no change in the size of HDL particles. Increased plasma VLDL was associated with a significant decrease in post-heparin plasma hepatic lipase activity and a decrease in hepatic expression of hepatic lipase. These observations suggest that oral exposure to acrolein could induce or exacerbate systemic dyslipidemia and thereby contribute to cardiovascular disease risk.

2.115 **Comparative proteomic profiling of plasma very-low-density and low-density lipoproteins**

Sun, H-Y., Chen, S-F., Lai, M-D., Chang, T-T., Chen, T-L., Li, P-Y., Shieh, D-B. and Young, K-C.
Clin. Chim. Acta., **411**, 336-344 (2010)

Background

Low-density lipoprotein (LDL) is a natural metabolite of very-low-density lipoprotein (VLDL) in the circulation. Systematic investigation of total protein components and dynamics might provide insights into this normal metabolic process.

Methods

VLDL and LDL were purified from normolipidemia pooled plasma by gradient ultracentrifugation with either ionic or non-ionic media. The protein contents were compared by liquid chromatography tandem mass analyses based on isobaric tag for relative and absolute quantitation and two-dimensional gel electrophoresis.

Results

Our comparative lipoproteomes revealed 21 associated proteins. Combined with Western blot analysis, and on the basis of the differential expression levels we classified them into 3 groups: (i) VLDL > LDL [apolipoprotein (apo) A-IV, apo(a), apoCs, apoE, apoJ and serum amyloid A-4]; (ii) VLDL < LDL [albumin, α -1-antitrypsin, apoD, apoF, apoM, and paraoxonase-1]; and (iii) VLDL = LDL [apoA-I, apoA-II, apoB-100, apoL-I and prenylcysteine oxidase-1]. The apoA-I level positively correlated with PCYOX1 but negatively with apoM in VLDL and LDL. Furthermore, the two-dimensional maps displayed 5 apoA-I isoforms in which phosphorylation at Ser55, Ser166, Thr185, Thr221 and Ser252 residues were identified.

Conclusions

This study revealed the VLDL- and LDL lipoproteomes and the full-spectrum protein changes during physiological VLDL-to-LDL transition. It provides a valuable dataset VLDL and LDL proteomes potentially applied to the development of diagnostics.

2.116 **Isolation and Characterization of Cytoplasmic Cofilin-Actin Rods**

Minamide, L.S., Maiti, S., Boyle, J.A., Davis, R.C., Coppinger, J.A., Bao, Y., Huang, T.Y., Yates, J., Bokoch, G.M. and Bamburg, J.R.
J. Biol. Chem., **285**(8), 5450-5460 (2010)

Cofilin-actin bundles (rods), which form in axons and dendrites of stressed neurons, lead to synaptic dysfunction and may mediate cognitive deficits in dementias. Rods form abundantly in the cytoplasm of non-neuronal cells in response to many treatments that induce rods in neurons. Rods in cell lysates are not stable in detergents or with added calcium. Rods induced by ATP-depletion and released from cells by mechanical lysis were first isolated from two cell lines expressing chimeric actin-depolymerizing factor (ADF)/cofilin fluorescent proteins by differential and equilibrium sedimentation on OptiPrep gradients and then from neuronal and non-neuronal cells expressing only endogenous proteins. Rods contain ADF/cofilin and actin in a 1:1 ratio. Isolated rods are stable in dithiothreitol, EGTA, Ca^{2+} , and ATP. Cofilin-GFP-containing rods are stable in 500 mM NaCl, whereas rods formed from endogenous proteins are significantly less stable in high salt. Proteomic analysis of rods formed from endogenous proteins identified other potential components whose presence in rods was examined by immunofluorescence staining of cells. Only actin and ADF/cofilin are in rods during all phases of their formation; furthermore, the rapid assembly of rods *in vitro* from these purified proteins at physiological concentration shows that they are the only proteins necessary for rod formation. Cytoplasmic rod formation is inhibited by cytochalasin D and jasplakinolide. Time lapse imaging of rod formation shows abundant small needle-shaped rods that coalesce over time. Rod filament lengths measured by ultrastructural tomography ranged

from 22 to 1480 nm. These results suggest rods form by assembly of cofilin-actin subunits, followed by self-association of ADF/cofilin-saturated F-actin.

2.117 Increased Glycation and Oxidative Damage to Apolipoprotein B100 of LDL Cholesterol in Patients With Type 2 Diabetes and Effect of Metformin

Rabbani, N., Chittari, M.V., Bopdmer, C.W., Zehnder, D., Ceriello, A. and Thornalley, P.J.
Diabetes, **59**, 1038-1045 (2010)

OBJECTIVE The aim of this study was to investigate whether apolipoprotein B100 of LDL suffers increased damage by glycation, oxidation, and nitration in patients with type 2 diabetes, including patients receiving metformin therapy.

RESEARCH DESIGN AND METHODS For this study, 32 type 2 diabetic patients and 21 healthy control subjects were recruited; 13 diabetic patients were receiving metformin therapy (median dose: 1.50 g/day). LDL was isolated from venous plasma by ultracentrifugation, delipidated, digested, and analyzed for protein glycation, oxidation, and nitration adducts by stable isotopic dilution analysis tandem mass spectrometry.

RESULTS Advanced glycation end product (AGE) content of apolipoprotein B100 of LDL from type 2 diabetic patients was higher than from healthy subjects: arginine-derived AGE, 15.8 vs. 5.3 mol% ($P < 0.001$); and lysine-derived AGE, 2.5 vs. 1.5 mol% ($P < 0.05$). Oxidative damage, mainly methionine sulfoxide residues, was also increased: 2.5 vs. 1.1 molar equivalents ($P < 0.001$). 3-Nitrotyrosine content was decreased: 0.04 vs. 0.12 mol% ($P < 0.05$). In diabetic patients receiving metformin therapy, arginine-derived AGE and methionine sulfoxide were lower than in patients not receiving metformin: 19.3 vs. 8.9 mol% ($P < 0.01$) and 2.9 vs. 1.9 mol% ($P < 0.05$), respectively; 3-nitrotyrosine content was higher: 0.10 vs. 0.03 mol% ($P < 0.05$). Fructosyl-lysine residue content correlated positively with fasting plasma glucose. Arginine-derived AGE residue contents were intercorrelated and also correlated positively with methionine sulfoxide.

CONCLUSIONS Patients with type 2 diabetes had increased arginine-derived AGEs and oxidative damage in apolipoprotein B100 of LDL. This was lower in patients receiving metformin therapy, which may contribute to decreased oxidative damage, atherogenicity, and cardiovascular disease.

2.118 The Physical Relationship between Infectivity and Prion Protein Aggregates Is Strain-Dependent

Tixador, P., Herzog, L., Reine, F., Jaumain, E., chapuis, J., Le Dur, A., Laude, H. and Beringue, V.
PloSPathogens, **6(4)**, e1000859 (2010)

Prions are unconventional infectious agents thought to be primarily composed of PrP^{Sc}, a multimeric misfolded conformer of the ubiquitously expressed host-encoded prion protein (PrP^C). They cause fatal neurodegenerative diseases in both animals and humans. The disease phenotype is not uniform within species, and stable, self-propagating variations in PrP^{Sc} conformation could encode this 'strain' diversity. However, much remains to be learned about the physical relationship between the infectious agent and PrP^{Sc} aggregation state, and how this varies according to the strain. We applied a sedimentation velocity technique to a panel of natural, biologically cloned strains obtained by propagation of classical and atypical sheep scrapie and BSE infectious sources in transgenic mice expressing ovine PrP. Detergent-solubilized, infected brain homogenates were used as starting material. Solubilization conditions were optimized to separate PrP^{Sc} aggregates from PrP^C. The distribution of PrP^{Sc} and infectivity in the gradient was determined by immunoblotting and mouse bioassay, respectively. As a general feature, a major proteinase K-resistant PrP^{Sc} peak was observed in the middle part of the gradient. This population approximately corresponds to multimers of 12–30 PrP molecules, if constituted of PrP only. For two strains, infectivity peaked in a markedly different region of the gradient. This most infectious component sedimented very slowly, suggesting small size oligomers and/or low density PrP^{Sc} aggregates. Extending this study to hamster prions passaged in hamster PrP transgenic mice revealed that the highly infectious, slowly sedimenting particles could be a feature of strains able to induce a rapidly lethal disease. Our findings suggest that prion infectious particles are subjected to marked strain-dependent variations, which in turn could influence the strain biological phenotype, in particular the replication dynamics.

2.119 The Conserved Bardet-Biedl Syndrome Proteins Assemble a Coat that Traffics Membrane Proteins to Cilia

Jin, H., White, S.R., Shida, T., Schulz, S., Aguiar, M., Gygi, S.P., Bazan, J.F. and Nachury, M.V.
Cell, **141**, 1208-1219 (2010)

The BBSome is a complex of Bardet-Biedl Syndrome (BBS) proteins that shares common structural

elements with COPI, COPII, and clathrin coats. Here, we show that the BBSome constitutes a coat complex that sorts membrane proteins to primary cilia. The BBSome is the major effector of the Arf-like GTPase Arl6/BBS3, and the BBSome and GTP-bound Arl6 colocalize at ciliary punctae in an interdependent manner. Strikingly, Arl6^{GTP}-mediated recruitment of the BBSome to synthetic liposomes produces distinct patches of polymerized coat apposed onto the lipid bilayer. Finally, the ciliary targeting signal of somatostatin receptor 3 needs to be directly recognized by the BBSome in order to mediate targeting of membrane proteins to cilia. Thus, we propose that trafficking of BBSome cargoes to cilia entails the coupling of BBSome coat polymerization to the recognition of sorting signals by the BBSome.

2.120 **Calcium-dependent Regulation of SNARE-mediated Membrane Fusion by Calmodulin**

Di Giovanni, J., Iborra, C., Maulet, Y., Leveque, C., El Far, O. and Seagar, M.
J. Biol. Chem., **285**(31), 23665-23675 (2010)

Neuroexocytosis requires SNARE proteins, which assemble into trans complexes at the synaptic vesicle/plasma membrane interface and mediate bilayer fusion. Ca²⁺ sensitivity is thought to be conferred by synaptotagmin, although the ubiquitous Ca²⁺-effector calmodulin has also been implicated in SNARE-dependent membrane fusion. To examine the molecular mechanisms involved, we examined the direct action of calmodulin and synaptotagmin *in vitro*, using fluorescence resonance energy transfer to assay lipid mixing between target- and vesicle-SNARE liposomes. Ca²⁺/calmodulin inhibited SNARE assembly and membrane fusion by binding to two distinct motifs located in the membrane-proximal regions of VAMP2 ($K_D = 500$ nM) and syntaxin 1 ($K_D = 2$ μ M). In contrast, fusion was increased by full-length synaptotagmin 1 anchored in vesicle-SNARE liposomes. When synaptotagmin and calmodulin were combined, synaptotagmin overcame the inhibitory effects of calmodulin. Furthermore, synaptotagmin displaced calmodulin binding to target-SNAREs. These findings suggest that two distinct Ca²⁺ sensors act antagonistically in SNARE-mediated fusion.

2.121 **Cigarette smoke and human plasma lycopene depletion**

Graham, D.L., Carail, M., Caris-Veyrat, C. and Lowe, G.M.
Food and Chem. Tox., **48**, 2413-2420 (2010)

It is known that smokers have a higher risk of developing cardiovascular disease and lung cancer. Plasma carotenoid concentrations in smokers are generally lower than in non-smokers and this may be due to modifications in diet or a direct or indirect action of cigarette smoke on carotenoids in the plasma. Recently it was reported that reactive nitrogen species derived from cigarette smoke could diffuse across the lung alveolar cell wall into the plasma. Such species may modify circulating low density lipoprotein (LDL) and in the process reduce circulating carotenoid concentrations. In an effort to address this rationale we have treated lycopene solutions, human plasma and isolated LDL with cigarette smoke and monitored all-(E)-lycopene, 5(Z)-lycopene and β -carotene depletion. In plasma, the depletion of all-(E)-lycopene ($15.0 \pm 11.0\%$, $n = 10$) was greater than 5(Z)-lycopene ($10.4 \pm 9.6\%$) or β -carotene ($12.4 \pm 10.5\%$). In LDL, both all-(E)- and 5(Z)-lycopene were more susceptible than β -carotene ($20.8 \pm 11.8\%$, $15.4 \pm 11.5\%$ and $11.5 \pm 12.5\%$, $n = 3$ respectively). The effects have been compared with Sin-1 reactions and isomerization of all-(E) lycopene is common to both treatments. The results clearly indicate that low plasma lycopene may be a direct consequence of smoke inhalation.

2.122 **Lipoprotein Particles Cross the Blood–Brain Barrier in *Drosophila***

Brankatschk, M. and Eaton, S.
J. Neurosci., **30**(31), 10441-10447 (2010)

The blood–brain barrier (BBB) regulates passage of nutrients and signaling molecules from the circulation into the brain. Whether lipoproteins cross the BBB *in vivo* has been controversial, and no clear requirement for circulating lipoproteins in brain development has been shown. We address these issues in *Drosophila*, which has a functionally conserved BBB, and lipoproteins that resemble those of vertebrates. We show that the *Drosophila* lipoprotein lipophorin exists in two isoforms. Both isoforms cross the BBB, but accumulate on distinct subsets of cells within the brain. In addition to acting as a lipid carrier, lipophorin carries both sterol-linked and GPI-linked proteins into the circulation and transports them across the BBB. Finally, lipophorin promotes neuroblast proliferation by a mechanism that does not depend on delivery of dietary lipids. Transport of lipophorin and its cargo across the BBB represents a novel mechanism by which peripherally synthesized proteins might enter the brain and influence its development. Furthermore, lipid-linkage may be an efficient method to transport therapeutic molecules across the BBB.

- 2.123 Tunable Leuko-polymerosomes That Adhere Specifically to Inflammatory Markers**
Robbins, G.P., Saunders, R.L., Haun, J.B., Rawson, J., Therien, M.J. and Hammer, D.A.
Langmuir, **26**(17), 14089-14096 (2010)

The polymerosome, a fully synthetic cell mimetic, is a tunable platform for drug delivery vehicles to detect and treat disease (theranostics). Here, we design a leuko-polymerosome, a polymerosome with the adhesive properties of leukocytes, which can effectively bind to inflammatory sites under flow. We hypothesize that optimal leukocyte adhesion can be recreated with ligands that mimic receptors of the two major leukocyte molecular adhesion pathways, the selectins and the integrins. Polymerosomes functionalized with sialyl Lewis X and an antibody against ICAM-1 adhere avidly and selectively to surfaces coated with inflammatory adhesion molecules P-selectin and ICAM-1 under flow. We find that maximal adhesion occurs at intermediate densities of both sialyl Lewis X and anti-ICAM-1, owing to synergistic binding effects between the two ligands. Leuko-polymerosomes bearing these two receptor mimetics adhere under physiological shear rates to inflamed endothelium in an *in vitro* flow chamber at a rate 7.5 times higher than those to uninfamed endothelium. This work clearly demonstrates that polymerosomes bearing only a single ligand bind less avidly and with lower selectivity, thus suggesting proper mimicry of leukocyte adhesion requires contributions from both pathways. This work establishes a basis for the design of polymerosomes for targeted drug delivery in inflammation.

- 2.124 Engineering Therapeutic Nanocarriers with Optimal Adhesion for Targeting**
Haun, J.B., Robbins, G.P. and Hammer, D.A.
J. Adhesion, **86**, 131-159 (2010)

There is considerable interest in developing therapeutic delivery carriers that can be targeted via receptor-ligand interactions to sites within the blood stream. The adhesion of carriers is determined by the combined effects of transport phenomena, hydrodynamic force, and the dynamics of multivalent receptor/ligand bonding. Optimizing the adhesion of carriers requires developing relationships between these factors and carrier properties such as size and receptor coating density. Recently, we developed canonical relationships for the binding of antibody-conjugated 200 nm particles to surfaces coated with a vascular adhesion molecule, intercellular adhesion molecule-1. Here we extend our previous studies of adhesion to particles of different size, including 40 nm and 1 μ m particles. Particle binding is assessed under fluid flow in a parallel plate flow chamber while varying particle receptor density, substrate ligand density, and flow rate. Using a stochastic simulation and transport-reaction model we then extract multivalent kinetic rate constants for particle attachment and detachment from the binding data. We demonstrate that particles go through a maximum in binding with particle size. For small particles, increasing size increases receptor-ligand encounter rates; for larger particles, fluid shear force begins to dominate, leading to higher forces and decreased adhesion. Our methods provide a means for optimizing particle size and receptor density for the selective binding of particles to vascular endothelium under flow.

- 2.125 Lack of effect of cold water prawns on plasma cholesterol and lipoproteins in normo-lipidaemic men**
Isherwood, C., Wong, M., Jones, W.S., Davies, I.G. and Griffin, B.A.
Cell. Mol. Biol., **56**(1), 52-58 (2010)

OBJECTIVE: Dietary guidelines for the prevention of coronary heart disease (CHD) have restricted the intake of foods rich in dietary cholesterol, on the grounds that the dietary cholesterol will increase blood cholesterol. In the case of shellfish, this recommendation may limit the intake of a valuable dietary source of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA). The objective of this study was to undertake a dietary intervention to determine the effects of cold water prawns on plasma lipids and lipoproteins.
METHODS: 23 healthy male subjects were randomised to receive either 225 g of cold water prawns or an equivalent weight of fish ('crab') sticks as a control for 12 weeks in a cross-over design. Blood samples were taken at the beginning and end of each intervention for the determination of plasma lipids and lipoproteins by routine enzymatic assays and iodixanol density gradient centrifugation respectively.
RESULTS: The diets were well matched for the intake of total energy and macronutrients, and body weight remained stable throughout the study. The prawn intervention increased the intake of dietary cholesterol to 750 mg/d against 200 mg/d on the control. The intake of LC n-3 PUFA from prawns was estimated to be between 0.5-0.7 g/d. The consumption of prawns produced no significant effects on the concentration of plasma total or LDL cholesterol, triacylglycerol, HDL cholesterol or apolipoproteins A-I and B relative to the control, or within each intervention group over time. There was also no significant effect on LDL density (particle size) relative to the control, or any difference between and within treatments in total plasma lipoprotein profiles by density gradient centrifugation.

CONCLUSION: These findings provide evidence to suggest that the consumption of cold water prawns, at least in healthy, male subjects, should not be restricted on the grounds of this seafood producing an adverse effect on plasma LDL cholesterol.

2.126 A Generalized System for Photoresponsive Membrane Rupture in Polymersomes

Kamat, N.P., Robbins, G.P., Rawson, J., Therien, M.J., Dmochowski, I.J. and Hammer, D.A.
Adv. Funct. Mater., **20**, 2588-2596 (2010)

Polymersomes are vesicles whose membranes comprise self-assembled block copolymers. It has recently been shown that co-encapsulating conjugated multiporphyrin dyes in a polymersome membrane with ferritin protein in the aqueous lumen confers photolability to the polymersome. In the present study, the photolability is shown to be extendable to vesicles containing dextran, an inert and inexpensive polysaccharide, as the luminal solute. How structural features of the polymersome/porphyrin/dextran composite affect its photoresponse is explored. Increasing dextran molecular weight, decreasing block copolymer molecular weight, and altering fluorophore-membrane interactions results in increasing the photoresponsiveness of the polymersomes. Amphiphilic interactions of the luminal encapsulant with the membrane coupled with localized heat production in the hydrophobic bilayer likely cause differential thermal expansion in the membrane and the subsequent membrane rupture. This study suggests a general approach to impart photoresponsiveness to any biomimetic vesicle system without chemical modification, as well as a simple, bio-inert method for constructing photosensitive carriers for controlled release of encapsulants.

2.127 Lipid accumulation and metabolism in polychaete spermatogenesis: Role of the large discoidal lipoprotein

Schenk, S. and Hoeger, U.
Mol. Reprod. Dev., **77**, 710-719 (2010)

In most oviparous animals, lipoprotein-mediated lipid transport plays an important role in the nutrient supply for the oocyte. In male gametes, lipids are used as energy substrates in spermatozoa but nothing is yet known about their origin and metabolism throughout spermatogenesis. The lipid profiles analyzed from different stages of male germ cell development in the marine annelid *Nereis virens* were found to undergo a dramatic change from primary triacylglycerides at the beginning of germ cell development to cholesterol and phospholipids at the end of development as demonstrated by HPLC with evaporative light scattering detection and mass spectrometry. The uptake of a large discoidal lipoprotein into the developing germ cells could be demonstrated by fluorescence labeling and electron microscopic techniques as well as by the presence of a lipoprotein receptor in the germ cells, thus establishing its role in lipid supply. The incorporated lipoprotein discs were found to be stored as intact complexes indicating that they are not readily degraded upon endocytotic uptake. The change in lipid composition during germ cell development reflects their metabolic activity, especially in spermatogonia. The high concentration of lipids maintained by spermatogonia during the early phase of gametogenesis seems to be required for the later rapid processes of meiosis and spermatocyte differentiation. At times when peak demand of lipids arises for membrane synthesis and increased metabolism, this may be met more efficiently by a rapid on-site mobilization of lipids instead of an external supply.

2.128 Functional roles of VASP phosphorylation in the regulation of chemotaxis and osmotic stress response

Lin, W-H., Nelson, S.E., Hollingsworth, R.J. and Chung, C.Y.
Cytoskeleton, **67**, 259-271 (2010)

Vasodilator-stimulated phosphoprotein (VASP) plays crucial roles in controlling F-actin-driven processes and growing evidence indicates that VASP function is modulated by phosphorylation at multiple sites. However, the complexity of mammalian system prevents the clear understanding of the role of VASP phosphorylation. In this study, we took advantage of *Dictyostelium* which possesses only one member of the Ena/VASP family to investigate the functional roles of VASP phosphorylation. Our results demonstrated that hyperosmotic stress and cAMP stimulation cause VASP phosphorylation. VASP phosphorylation plays a negative role for the early steps of filopodia/microspikes formation. VASP phosphorylation appears to modulate VASP localization at the membrane cortex and its interactions with WASP and WIPa. Analysis of chemotaxis of cells expressing VASP mutants showed that VASP phosphorylation is required for the establishment of cell polarity under a cAMP gradient.

2.129 Photochemistry of Bacteriochlorophylls in Human Blood Plasma: 1. Pigment Stability and Light-induced Modifications of Lipoproteins

Dandler, J., Wilhelm, B. and Scheer, H.

Photochem. Photobiol., **86**, 331-341 (2010)

Transmetalated derivatives of bacteriochlorophyll are promising sensitizers in photodynamic therapy. Protocols using short delay times between injection and irradiation cause interest in the photochemistry of these pigments in the blood. Using near-infrared irradiation where these pigments absorb strongly, we have studied the photochemistry of Zn- and Pd-bacteriopheophorbide (WST09), and of the highly polar taurinated Pd-derivative, WST11, in isolated fractions of human blood plasma. The stability of all pigments is increased in blood plasma, compared with monomeric solutions. Pd-bacteriopheophorbide is much more stable than the other two derivatives. It also has a higher capacity for inducing reactive oxygen species, yet the consumption of oxygen is comparable. There is furthermore evidence for photobleaching under anoxic conditions. The generation of hydroperoxides (ROOH) is faster with Pd- than with Zn-complexes; the formation of endoperoxides (ROOR'), measured as thiobarbituric acid reactive substances, is comparable with the two central metals. Formation of both ROOH and ROOR' is increased in low-density lipoproteins (LDL) compared with high-density lipoproteins (HDL), which is probably related to the higher concentration of target molecules in the former. In HDL, extensive cross-linking is induced among the apolipoproteins; judged from the electrophoretic mobility of LDL and HDL particles, there is also a gross structural change. Photosensitized cross-linking is much less pronounced with high-density proteins.

2.130 Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial

Jebb, S.A., Lovegrove, J.A., Griffin, B.A., Frost, G.S., Moore, C.S., Chatfield, M.D., Bluck, L.J., Williams, C.M. and Sanders, T.A.B.

Am. J. Clin. Nutr., **92**, 748-758 (2010)

Background: Insulin sensitivity (Si) is improved by weight loss and exercise, but the effects of the replacement of saturated fatty acids (SFAs) with monounsaturated fatty acids (MUFAs) or carbohydrates of high glycemic index (HGI) or low glycemic index (LGI) are uncertain.

Objective: We conducted a dietary intervention trial to study these effects in participants at risk of developing metabolic syndrome.

Design: We conducted a 5-center, parallel design, randomized controlled trial [RISCK (Reading, Imperial, Surrey, Cambridge, and Kings)]. The primary and secondary outcomes were changes in Si (measured by using an intravenous glucose tolerance test) and cardiovascular risk factors. Measurements were made after 4 wk of a high-SFA and HGI (HS/HGI) diet and after a 24-wk intervention with HS/HGI (reference), high-MUFA and HGI (HM/HGI), HM and LGI (HM/LGI), low-fat and HGI (LF/HGI), and LF and LGI (LF/LGI) diets.

Results: We analyzed data for 548 of 720 participants who were randomly assigned to treatment. The median Si was $2.7 \times 10^{-4} \text{ mL} \cdot \mu\text{U}^{-1} \cdot \text{min}^{-1}$ (interquartile range: 2.0, $4.2 \times 10^{-4} \text{ mL} \cdot \mu\text{U}^{-1} \cdot \text{min}^{-1}$), and unadjusted mean percentage changes (95% CIs) after 24 wk treatment ($P = 0.13$) were as follows: for the HS/HGI group, -4% (-12.7%, 5.3%); for the HM/HGI group, 2.1% (-5.8%, 10.7%); for the HM/LGI group, -3.5% (-10.6%, 4.3%); for the LF/HGI group, -8.6% (-15.4%, -1.1%); and for the LF/LGI group, 9.9% (2.4%, 18.0%). Total cholesterol (TC), LDL cholesterol, and apolipoprotein B concentrations decreased with SFA reduction. Decreases in TC and LDL-cholesterol concentrations were greater with LGI. Fat reduction lowered HDL cholesterol and apolipoprotein A1 and B concentrations.

Conclusions: This study did not support the hypothesis that isoenergetic replacement of SFAs with MUFAs or carbohydrates has a favorable effect on Si. Lowering GI enhanced reductions in TC and LDL-cholesterol concentrations in subjects, with tentative evidence of improvements in Si in the LF-treatment group. This trial was registered at clinicaltrials.gov as ISRCTN29111298.

2.131 Effects of antioxidants on postprandial oxidative stress and endothelial dysfunction in subjects with impaired glucose tolerance and Type 2 diabetes

Neri, S., Calvagno, S., Mauceri, B., Misseri, M., Tsami, A., Vecchio, C., Mastrosimone, G., Di Pino, A., Maiorca, D., Judica, A., Romano, G., Rizzotto, A. and Signorelli, S.S.

Eur. J. Nutr., **49**, 409-416 (2010)

Aim

To compare changes in the oxidation-reduction balance and endothelial function before and after meal in

patients with type 2 diabetes or impaired glucose tolerance and determine the effects of standard antioxidant supplementation.

Methods

Forty diabetics and 40 subjects with impaired glucose tolerance were compared with a control group. We assessed before and after a test meal (homogenized milkshake containing 80 g of saturated fat, amounting to 1,480 kcal), some reactive oxygen species, inflammation markers and flow-mediated vascular dilatation. These parameters were then reassessed after standard antioxidant treatment.

Results

After the meal, diabetics, subjects with impaired glucose tolerance and controls had higher levels of oxidant compounds compared to fasting levels. In subjects with diabetes and impaired glucose tolerance (IGT), Vascular Adhesion Molecule-1 and CRP were higher after the meal—diabetic subjects exhibited lower fasting flow-mediated dilatation, which deteriorated significantly after the meal. Antioxidant administration significantly improved the parameters investigated in all subjects.

Conclusions

In diabetic subjects, altered glycaemia and lipaemia are closely correlated with markers of systemic oxidative stress. Our results show that the abnormal changes in oxidative-reductive balance parameters are paralleled by similar changes in markers of endothelial dysfunction and inflammation at 4 h after ingestion of a fatty meal. Supplementation with a pool of antioxidants can reduce oxidative stress and inflammation in healthy subjects and, more importantly, in IGT patients. This previous aspect suggests that the timing of antioxidant supplementation has an important role in endothelium protection in healthy and pre-diabetic subjects, and along with prompt antioxidant treatment before irreversible endothelial damage has occurred, may have an important protective role in subjects with IGT—patients who require administration of adequate dietary antioxidants.

2.132 **Role of the Highly Conserved Middle Region of Prion Protein (PrP) in PrP–Lipid Interaction**

Wang, F., Yin, S., Wang, X., Zha, L., Sy, M-S. and Ma, J.

Biochemistry, **49**, 8169-8176 (2010)

Converting normal prion protein (PrP^C) to the pathogenic PrP^{Sc} isoform is central to prion disease. We previously showed that, in the presence of lipids, recombinant mouse PrP (rPrP) can be converted into the highly infectious conformation, suggesting a crucial role of lipid–rPrP interaction in PrP conversion. To understand the mechanism of lipid–rPrP interaction, we analyzed the ability of various rPrP mutants to bind anionic lipids and to gain lipid-induced proteinase K (PK) resistance. We found that the N-terminal positively charged region contributes to electrostatic rPrP–lipid binding but does not affect lipid-induced PK resistance. In contrast, the highly conserved middle region of PrP, consisting of a positively charged region and a hydrophobic domain, is essential for lipid-induced rPrP conversion. The hydrophobic domain deletion mutant significantly weakened the hydrophobic rPrP–lipid interaction and abolished the lipid-induced C-terminal PK resistance. The rPrP mutant without positive charges in the middle region reduced the amount of the lipid-induced PK-resistant rPrP form. Consistent with a critical role of the middle region in lipid-induced rPrP conversion, both disease-associated P105L and P102L mutations, localized between lysine residues in the positively charged region, significantly affected lipid-induced rPrP conversion. The hydrophobic domain-localized 129 polymorphism altered the strength of hydrophobic rPrP–lipid interaction. Collectively, our results suggest that the interaction between the middle region of PrP and lipids is essential for the formation of the PK-resistant conformation. Moreover, the influence of disease-associated PrP mutations and the 129 polymorphism on PrP–lipid interaction supports the relevance of PrP–lipid interaction to the pathogenesis of prion disease.

2.133 **Oral Treatment with the d-Enantiomeric Peptide D3 Improves the Pathology and Behavior of Alzheimer's Disease Transgenic Mice**

Funke, S.A., van Groen, T., Kadish, I., Bartnik, D., Nage-Steger, L., Brener, O., Sehl, T., Batra-Safferling, R., Moriscot, C., Schoehn, G., Horn, A.H.C., Müller-Schiffmann, A., Korth, C., Sticht, H. and Willbold, D. *ACS Chem. Neurosci.*, **1**(9), 639-648 (2010)

Several lines of evidence suggest that the amyloid- β -peptide (A β) plays a central role in the pathogenesis of Alzheimer's disease (AD). Not only A β fibrils but also small soluble A β oligomers in particular are suspected to be the major toxic species responsible for disease development and progression. The present study reports on in vitro and in vivo properties of the A β targeting D-enantiomeric amino acid peptide D3. We show that next to plaque load and inflammation reduction, oral application of the peptide improved the cognitive performance of AD transgenic mice. In addition, we provide in vitro data elucidating the potential mechanism underlying the observed in vivo activity of D3. These data suggest that D3

precipitates toxic A β species and converts them into nonamyloidogenic, nonfibrillar, and nontoxic aggregates without increasing the concentration of monomeric A β . Thus, D3 exerts an interesting and novel mechanism of action that abolishes toxic A β oligomers and thereby supports their decisive role in AD development and progression.

2.134 Phosphorylation of Aquaporin-2 Regulates Its Water Permeability

Eto, K., Noda, Y., Horikawa, S., Uchida, S. and Sasaki, S.
J. Biol. Chem., **285**(52), 40777-40784 (2010)

Vasopressin-regulated water reabsorption through the water channel aquaporin-2 (AQP2) in renal collecting ducts maintains body water homeostasis. Vasopressin activates PKA, which phosphorylates AQP2, and this phosphorylation event is required to increase the water permeability and water reabsorption of the collecting duct cells. It has been established that the phosphorylation of AQP2 induces its apical membrane insertion, rendering the cell water-permeable. However, whether this phosphorylation regulates the water permeability of this channel still remains unclear. To clarify the role of AQP2 phosphorylation in water permeability, we expressed recombinant human AQP2 in *Escherichia coli*, purified it, and reconstituted it into proteoliposomes. AQP2 proteins not reconstituted into liposomes were removed by fractionating on density step gradients. AQP2-reconstituted liposomes were then extruded through polycarbonate filters to obtain unilamellar vesicles. PKA phosphorylation significantly increased the osmotic water permeability of AQP2-reconstituted liposomes. We then examined the roles of AQP2 phosphorylation at Ser-256 and Ser-261 in the regulation of water permeability using phosphorylation mutants reconstituted into proteoliposomes. The water permeability of the non-phosphorylation-mimicking mutant S256A-AQP2 and non-phosphorylated WT-AQP2 was similar, and that of the phosphorylation-mimicking mutant S256D-AQP2 and phosphorylated WT-AQP2 was similar. The water permeability of S261A-AQP2 and S261D-AQP2 was similar to that of non-phosphorylated WT-AQP2. This study shows that PKA phosphorylation of AQP2 at Ser-256 enhances its water permeability.

2.135 The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout

Prindiville, J.S., Mennigen, J.A., Zamora, J.M., Moon, T.W. and Weber, J.M.
Tox. Appl. Pharmacol., **251**, 201-208 (2011)

Gemfibrozil (GEM) is a fibrate drug consistently found in effluents from sewage treatment plants. This study characterizes the pharmacological effects of GEM on the plasma lipoproteins of rainbow trout (*Oncorhynchus mykiss*). Our goals were to quantify the impact of the drug on: 1) lipid constituents of lipoproteins (phospholipids (PL), triacylglycerol (TAG), and cholesterol), 2) lipoprotein classes (high, low and very low density lipoproteins), and 3) fatty acid composition of lipoproteins. Potential mechanisms of GEM action were investigated by measuring lipoprotein lipase activity (LPL) and the hepatic gene expression of LPL and of the peroxisome proliferator-activated receptor (PPAR) α , β , and γ isoforms. GEM treatment resulted in decreased plasma lipoprotein levels (-29%) and a reduced size of all lipoprotein classes (lower PL:TAG ratios). However, the increase in HDL-cholesterol elicited by GEM in humans failed to be observed in trout. Therefore, HDL-cholesterol cannot be used to assess the impact of the drug on fish. GEM also modified lipoprotein composition by reducing the abundance of long-chain $n-3$ fatty acids, thereby potentially reducing the nutritional quality of exposed fish. The relative gene expression of LPL was increased, but the activity of the enzyme was not, and we found no evidence for the activation of PPAR pathways. The depressing effects of GEM on fish lipoproteins demonstrated here may be a concern in view of the widespread presence of fibrates in aquatic environments. Work is needed to test whether exposure to environmental concentrations of these drugs jeopardizes the capacity of fish for reproduction, temperature acclimation or migratory behaviors.

2.136 Small dense LDL particles - a predictor of coronary artery disease evaluated by invasive and CT-based techniques: a case-control study

Toft-Petersen, A.P., Tilsted, H.H., Aarøe, J., Rasmussen, K., Christensen, T., Griffin, B.A., Aardestrup, I.V., Andreasen, A. and Schmidt, E.B.
Lipids in Health and Disease, **10**, 21-27 (2011)

Background

Coronary angiography is the current standard method to evaluate coronary atherosclerosis in patients with suspected angina pectoris, but non-invasive CT scanning of the coronaries are increasingly used for the same purpose.

Low-density lipoprotein (LDL) cholesterol and other lipid and lipoprotein variables are major risk factors

for coronary artery disease. Small dense LDL particles may be of particular importance, but clinical studies evaluating their predictive value for coronary atherosclerosis are few.

Methods

We performed a study of 194 consecutive patients with chest pain, a priori considered of low to intermediate risk for significant coronary stenosis (>50% lumen obstruction) who were referred for elective coronary angiography. Plasma lipids and lipoproteins were measured including the subtype pattern of LDL particles, and all patients were examined by coronary CT scanning before coronary angiography.

Results

The proportion of small dense LDL was a strong univariate predictor of significant coronary artery stenosis evaluated by both methods. After adjustment for age, gender, smoking, and waist circumference only results obtained by traditional coronary angiography remained statistically significant.

Conclusion

Small dense LDL particles may add to risk stratification of patients with suspected angina pectoris.

2.137 LIPOPROTEIN SECRETION PROFILES AND VLDL PRODUCTION IN HEPATOCYTE CELL LINES

Jammart, B., Zoulin, F. and Durantel, D.

J. Hepatol., **54**, S318 (2011)

Background and Aims: Hepatitis C virus (HCV) is highly associated to apolipoprotein-B containing lipoproteins (LDL and VLDL) in infected patient sera, as most viral RNA is co-immunoprecipitated with anti-ApoB antibodies, but this association is barely seen in vitro (e.g. Huh7.5 cells infected with the HCV JFH-1 strain). Recent data suggested that these cells may be deficient for mature VLDL production. Thus, our aim was to: i. characterize lipoprotein secretion in Huh7.5 cells; ii. compare this secretion to natural VLDL production by primary human hepatocytes (PHH); iii. find other hepatocyte cell lines competent for VLDL production.

Methods: Cell culture supernatants were harvested, concentrated using an Amicon centrifugal filter unit with a cut-off of 100 kDa (Millipore™) and ultracentrifuged over an iodixanolsucrose gradient. Density distributions of apolipoproteins were determined using ELISA or western blot. The production of mature VLDL was further assessed by co-immunoprecipitation of different apolipoproteins.

Results: We found that Huh7.5 cells secrete a large amount of ApoB as compared to PHH. However, ApoB was detected at a density corresponding to LDL or IDL (sup. than 1.01 g/mL), but not VLDL, and was not associated with ApoE, as neither co-segregation nor co-immunoprecipitation were observed. Importantly, HCV infection increased ApoB secretion but did not affect the density of secreted particles. In contrast, secretion of ApoB/ApoE-containing lipoproteins with a density and composition comparable to that of PHH was observed in differentiated HepaRG cells, although the amount of secreted particles was lower. Finally, the hepatoblastoma cell line HepG2 was also able to secrete very-low-density particles containing ApoB and ApoE upon treatment with oleic acid (to stimulate lipoprotein production) and MEK/ERK inhibitors (to reduce the over-activation of MEK1 kinase in these cells).

Conclusion: We have characterized lipoprotein secretion profiles in 3 different cell lines (Huh7.5, HepG2 and HepaRG) as well as in PHH. Huh7.5 cell line, which is commonly used to study HCV replication, does not seem to produce mature VLDL and is therefore a poor model to study HCV particle secretion and its association to lipoproteins as observed in vivo. The other hepatocyte cell lines may therefore be more relevant study models of HCV morphogenesis.

2.138 The putative diabetic plasma marker, soluble CD36, is non-cleaved, non-soluble and entirely associated with microparticles

Alkhatatbeh, M.J., Mhaidat, N.M., Enjeti, A.K., Lincz, L.F. and Thorne, R.F.

J. Thrombosis and Haemostasis, **9**, 844-851 (2011)

Background: CD36 is a widely expressed cell surface receptor that binds lipoproteins, and its function has been implicated in many complications of the metabolic syndrome. A cell-free form of CD36, soluble CD36 (sCD36), has been reported in human plasma, found to be elevated in obesity and diabetes, and claimed as a marker of insulin resistance. **Objective:** To determine the nature of sCD36; in particular, whether sCD36 is truly soluble or, as hypothesized, is found as a component of circulating microparticles (MPs). **Methods:** Lipoproteins were fractionated by density gradient centrifugation, and plasma MPs were isolated by ultracentrifugation, size exclusion, and immunoprecipitation with CD36 detected by immunoblotting. MPs from plasma and activated platelets were analyzed by multicolor flow cytometry, with a DyLight-488 anti-CD36 conjugate in combination with antibodies against different cellular markers. **Results:** Cell-free plasma CD36 was not observed associated with lipoproteins and was not a proteolytic

fragment; rather, it was associated with the plasma MP fraction, suggesting that sCD36 in the plasma of normal subjects is a product of circulating MPs. Cytometric and immunoblotting analyses of plasma from normal donors showed that these MPs were derived mainly from platelets. Analysis of in vitro activated platelets also showed that CD36 to be secreted in the form of MPs. Conclusions: sCD36 is not a proteolytic product, but rather is associated with a specific subset of circulating MPs that can readily be analysed. This finding will enable more specific investigations into the cellular source of the increased levels of plasma CD36 found in subjects with diabetes.

2.139 Cell stress is related to re-localization of Argonaute 2 and to decreased RNA interference in human cells

Detzer, A., Engel, C., Wünsche, W. and Sczakiel, G.
Nucleic Acids Res., **39**(7), 2727-2741 (2011)

Various kinds of stress on human cells induce the formation of endogenous stress granules (SGs). Human Argonaute 2 (hAgo2), the catalytic core component of the RNA-induced silencing complex (RISC), can be recruited to SGs as well as P-bodies (PBs) indicating that the dynamic intracellular distribution of hAgo2 in SGs, in PBs or at other sub-cellular sites could be related to the efficiency of the RNA interference (RNAi) machinery. Here, we studied the influence of heat shock, sodium arsenite (NaAsO₂), cycloheximide (CHX) and LipofectamineTM 2000-mediated transfection of phosphorothioate (PS)-modified oligonucleotides (ON) on the intracellular localization of hAgo2 and the efficiency of RNAi. Fluorescence microscopy and sedimentation analysis of cell fractions indicate stress-induced accumulation of hAgo2 in SGs and the loss of distinctly composed complexes containing hAgo2 or their sub-cellular context. Transfection of cells with PS-ON induces cell stress that is phenotypically similar to the established inducers heat shock and NaAsO₂. The intracellular re-distribution of hAgo2 is related to its increased metabolic stability and to decreased RNAi directed by microRNA or by short interfering RNA. Here, we propose a functional model of the relationship between cell stress, translocation of hAgo2 to SGs providing a depot function, and loss of RNAi activity.

2.140 Forming giant vesicles with controlled membrane composition, asymmetry, and contents

Richmond, D.L., Schmidt, E.M., Martens, S., Stachowiak, J.C., Liska, N. and Fletcher, D.A.
PNAS, **108**(23), 9431-9436 (2011)

Growing knowledge of the key molecular components involved in biological processes such as endocytosis, exocytosis, and motility has enabled direct testing of proposed mechanistic models by reconstitution. However, current techniques for building increasingly complex cellular structures and functions from purified components are limited in their ability to create conditions that emulate the physical and biochemical constraints of real cells. Here we present an integrated method for forming giant unilamellar vesicles with simultaneous control over (i) lipid composition and asymmetry, (ii) oriented membrane protein incorporation, and (iii) internal contents. As an application of this method, we constructed a synthetic system in which membrane proteins were delivered to the outside of giant vesicles, mimicking aspects of exocytosis. Using confocal fluorescence microscopy, we visualized small encapsulated vesicles docking and mixing membrane components with the giant vesicle membrane, resulting in exposure of previously encapsulated membrane proteins to the external environment. This method for creating giant vesicles can be used to test models of biological processes that depend on confined volume and complex membrane composition, and it may be useful in constructing functional systems for therapeutic and biomaterials applications.

2.141 Actin and myosin contribute to mammalian mitochondrial DNA maintenance

Reyes, A. et al
Nucleic Acid Res., **39**(12), 5098-5108 (2011)

Mitochondrial DNA maintenance and segregation are dependent on the actin cytoskeleton in budding yeast. We found two cytoskeletal proteins among six proteins tightly associated with rat liver mitochondrial DNA: non-muscle myosin heavy chain IIA and β -actin. In human cells, transient gene silencing of *MYH9* (encoding non-muscle myosin heavy chain IIA), or the closely related *MYH10* gene (encoding non-muscle myosin heavy chain IIB), altered the topology and increased the copy number of mitochondrial DNA; and the latter effect was enhanced when both genes were targeted simultaneously. In contrast, genetic ablation of non-muscle myosin IIB was associated with a 60% decrease in mitochondrial DNA copy number in mouse embryonic fibroblasts, compared to control cells. Gene silencing of β -actin also affected mitochondrial DNA copy number and organization. Protease-protection experiments and iodixanol

gradient analysis suggest some β -actin and non-muscle myosin heavy chain IIA reside within human mitochondria and confirm that they are associated with mitochondrial DNA. Collectively, these results strongly implicate the actomyosin cytoskeleton in mammalian mitochondrial DNA maintenance.

2.142 Macromolecular organization of ATP synthase and complex I in whole mitochondria

Davies, K.M., Strauss, M., Daum, B., Kief, J.H., Osiewicz, H.D., Rycovska, A., Zickermann, V and Kühlbrandt, W.
PNAS, **108**(34), 14121-14126 (2011)

We used electron cryotomography to study the molecular arrangement of large respiratory chain complexes in mitochondria from bovine heart, potato, and three types of fungi. Long rows of ATP synthase dimers were observed in intact mitochondria and cristae membrane fragments of all species that were examined. The dimer rows were found exclusively on tightly curved cristae edges. The distance between dimers along the rows varied, but within the dimer the distance between F_1 heads was constant. The angle between monomers in the dimer was 70° or above. Complex I appeared as L-shaped densities in tomograms of reconstituted proteoliposomes. Similar densities were observed in flat membrane regions of mitochondrial membranes from all species except *Saccharomyces cerevisiae* and identified as complex I by quantum-dot labeling. The arrangement of respiratory chain proton pumps on flat cristae membranes and ATP synthase dimer rows along cristae edges was conserved in all species investigated. We propose that the supramolecular organization of respiratory chain complexes as proton sources and ATP synthase rows as proton sinks in the mitochondrial cristae ensures optimal conditions for efficient ATP synthesis.

2.143 Spongiform Encephalopathy in Transgenic Mice Expressing a Point Mutation in the $\beta 2$ - $\alpha 2$ Loop of the Prion Protein

Sigurdson, C., Joshi-Barr, S., Bett, C., Winson, O., Manco, G., Schwarz, P., Rüllicke, T., Nilsson, K.P.R., Margalith, I., Raeber, A., Peretz, D., Hornemann, S., Wüthrick, K. and Aguzzi, A.
J. Neurosci., **31**(39), 13840-13847 (2011)

Transmissible spongiform encephalopathies are fatal neurodegenerative diseases attributed to misfolding of the cellular prion protein, PrP^C, into a β -sheet-rich, aggregated isoform, PrP^{Sc}. We previously found that expression of mouse PrP with the two amino acid substitutions S170N and N174T, which result in high structural order of the $\beta 2$ - $\alpha 2$ loop in the NMR structure at pH 4.5 and 20°C , caused transmissible de novo prion disease in transgenic mice. Here we report that expression of mouse PrP with the single-residue substitution D167S, which also results in a structurally well ordered $\beta 2$ - $\alpha 2$ loop at 20°C , elicits spontaneous PrP aggregation in vivo. Transgenic mice expressing PrP^{D167S} developed a progressive encephalopathy characterized by abundant PrP plaque formation, spongiform change, and gliosis. These results add to the evidence that the $\beta 2$ - $\alpha 2$ loop has an important role in intermolecular interactions, including that it may be a key determinant of prion protein aggregation.

2.144 Fish Oil Supplementation During Late Pregnancy Does Not Influence Plasma Lipids or Lipoprotein Levels in Young Adult Offspring

Rytter, D., Schmidt, E.B., Bech, B.H., Christensen, J.H., Henriksen, T.B. and Olsen, S.F.
Lipids, **46**, 1091-1099 (2011)

Nutritional influences on cardiovascular disease operate throughout life. Studies in both experimental animals and humans have suggested that changes in the peri- and early post-natal nutrition can affect the development of the various components of the metabolic syndrome in adult life. This has led to the hypothesis that n-3 fatty acid supplementation in pregnancy may have a beneficial effect on lipid profile in the offspring. The aim of the present study was to investigate the effect of supplementation with n-3 fatty acids during the third trimester of pregnancy on lipids and lipoproteins in the 19-year-old offspring. The study was based on the follow-up of a randomized controlled trial from 1990 where 533 pregnant women were randomized to fish oil ($n = 266$), olive oil ($n = 136$) or no oil ($n = 131$). In 2009, the offspring were invited to a physical examination including blood sampling. A total of 243 of the offspring participated. Lipid values did not differ between the fish oil and olive oil groups. The relative adjusted difference (95% confidence intervals) in lipid concentrations was -3% ($-11; 7$) for LDL cholesterol, 3% ($-3; 10$) for HDL cholesterol, -1% ($-6; 5$) for total cholesterol, -4% ($-16; 10$) for TAG concentrations, 2% ($-2; 7$) for apolipoprotein A1, -1% ($-9; 7$) for apolipoprotein B and 3% ($-7; 15$) in relative abundance of small dense LDL. In conclusion, there was no effect of fish oil supplementation during the third trimester of pregnancy on offspring plasma lipids and lipoproteins in adolescence.

2.145 An albumin-associated PLA₂-like activity inactivates surfactant phosphatidylcholine secreted from fetal type II pneumocytes

Damas, J.E. and Cake, M.H.

Am. J. Physiol. Lung Cell. Mol. Physiol., **301(6)**, L966-L974 (2011)

Type II pneumocytes are responsible for the synthesis and secretion of pulmonary surfactant, which reduces surface tension in lung alveoli, thus decreasing their tendency to collapse during expiration. For this effect to be sustained, the integrity of the surface-active components of surfactant must be maintained. This study has shown that, when cultured type II pneumocytes are exposed to lipoprotein-free serum (LFS), the level of lyso-phosphatidylcholine (lyso-PC) in the secreted surfactant phospholipids is markedly elevated with a concomitant decline in the level of phosphatidylcholine (PC). This effect is the result of hydrolysis of surfactant PC by a phospholipase A₂ (PLA₂)-like activity present within serum. Anion-exchange chromatography, gel filtration chromatography and preparative electrophoresis of human LFS have shown that this PLA₂-like activity coelutes with albumin and is biochemically distinct from the secretory form of PLA₂. Furthermore, specific inhibitors of PLA₂ such as *p*-bromophenacyl bromide, aristolochic acid, and palmitoyl trifluoromethyl ketone do not inhibit this activity of serum. Commercially purified human serum albumin fraction V and recombinant human serum albumin (rHSA) are almost as effective as LFS in enhancing the level of lyso-PC in the media. The latter finding implies that rHSA directly generates lyso-PC from secreted PC and suggests that this PLA₂-like activity may be an intrinsic attribute of albumin.

2.146 BAP31 and BiP are essential for dislocation of SV40 from the endoplasmic reticulum to the cytosol

Geiger, R., Andritschke, D., Friebe, S., Herzog, F., Luisoni, S., heger, T. and Helenius, A.

Nature Cell Biol., **13(11)**, 1305-1314 (2011)

Non-enveloped viruses such as SV40 are transported from the extracellular space into the host cell nucleus through a pathway involving endocytosis, trafficking to the endoplasmic reticulum (ER) lumen, transport across the ER membrane to the cytoplasm, and subsequent nuclear import. Helenius and colleagues provide insight into how SV40 escapes from the ER by showing that viral proteins interact with components of the host ER-associated degradation machinery (ERAD). These interactions are crucial for translocation of SV40 into the cytoplasm and infectivity.

2.147 Does lycopene offer human LDL any protection against myeloperoxidase activity?

Chew, P.Y., Riley, L., Graham, D.L., Rahman, K. and Lowe, G.M.

Mol. Cell. Biochem., **361(1-2)**, 181-187 (2012)

Lycopene is a lipophilic antioxidant that is largely transported in human blood by Low Density Lipoproteins (LDL). One of the early events in the aetiology of atherosclerosis is thought to be the oxidation of LDL. Myeloperoxidase an enzyme secreted by neutrophils and macrophages is thought to oxidise human LDL particles. In this study, isolated human LDL was challenged with myeloperoxidase or copper, and the LDL was screened for lipoperoxidation and oxidation of apolipoprotein B100, depletion of lycopene and oxidation of cholesterol. Myeloperoxidase induced oxidation of LDL through direct interaction with apolipoprotein B100. No lipoperoxidation was observed following myeloperoxidase treatment; however, 7-ketocholesterol was detected indicating the products of myeloperoxidase interact with the surface of the LDL particles. Lycopene does react with the products of myeloperoxidase in solvent, but played no role in protecting against enzyme derived oxidation of human LDL.

2.148 Shedding of syndecan-1 from human hepatocytes alters very low density lipoprotein clearance

Deng, Y., Foley, E.M., Gonzales, J.C., Gordts, P.L., Li, Y. and Esko, J.D.

Hepatology, **55(1)**, 277-286 (2012)

We recently showed that the heparan sulfate proteoglycan syndecan-1 mediates hepatic clearance of triglyceride-rich lipoproteins in mice based on systemic deletion of syndecan-1 and hepatocyte-specific inactivation of sulfotransferases involved in heparan sulfate biosynthesis. Here, we show that syndecan-1 expressed on primary human hepatocytes and Hep3B human hepatoma cells can mediate binding and uptake of very low density lipoprotein (VLDL). Syndecan-1 also undergoes spontaneous shedding from primary human and murine hepatocytes and Hep3B cells. In human cells, phorbol myristic acid induces syndecan-1 shedding, resulting in accumulation of syndecan-1 ectodomains in the medium. Shedding occurs through a protein kinase C-dependent activation of ADAM17 (a disintegrin and metalloproteinase 17). Phorbol myristic acid stimulation significantly decreases DiD (1,1'-dioctadecyl-3,3,3',3'-

tetramethylindodicarbocyanine perchlorate)-VLDL binding to cells, and shed syndecan-1 ectodomains bind to VLDL. Although mouse hepatocytes appear resistant to induced shedding *in vitro*, injection of lipopolysaccharide into mice results in loss of hepatic syndecan-1, accumulation of ectodomains in the plasma, impaired VLDL catabolism, and hypertriglyceridemia. *Conclusion:* These findings suggest that syndecan-1 mediates hepatic VLDL turnover in humans as well as in mice and that shedding might contribute to hypertriglyceridemia in patients with sepsis.

2.149 Human telomerase acts as a hTR-independent reverse transcriptase in mitochondria

Sharma, N.K., Reyes, A., Green, P., Caron, M.J., Bonini, M.G., Gordon, D.M., Holt, I.J. and Hertzog Santos, J.

Nucleic Acids Res., **40**(2), 712-725 (2012)

Human telomerase reverse transcriptase (hTERT) is localized to mitochondria, as well as the nucleus, but details about its biology and function in the organelle remain largely unknown. Here we show, using multiple approaches, that mammalian TERT is mitochondrial, co-purifying with mitochondrial nucleoids and tRNAs. We demonstrate the canonical nuclear RNA [human telomerase RNA (hTR)] is not present in human mitochondria and not required for the mitochondrial effects of telomerase, which nevertheless rely on reverse transcriptase (RT) activity. Using RNA immunoprecipitations from whole cell and *in organello*, we show that hTERT binds various mitochondrial RNAs, suggesting that RT activity in the organelle is reconstituted with mitochondrial RNAs. In support of this conclusion, TERT drives first strand cDNA synthesis *in vitro* in the absence of hTR. Finally, we demonstrate that absence of hTERT specifically in mitochondria with maintenance of its nuclear function negatively impacts the organelle. Our data indicate that mitochondrial hTERT works as a hTR-independent reverse transcriptase, and highlight that nuclear and mitochondrial telomerases have different cellular functions. The implications of these findings to both the mitochondrial and telomerase fields are discussed.

2.150 Large Aggregates Are the Major Soluble A β Species in AD Brain Fractionated with Density Gradient Ultracentrifugation

Sehlin, D., Englund, H., Simu, B., Karlsson, M., Ingelsson, M., Nikolajeff, f., Lannfelt, L. and Pettersson, F.E.

PLoS One, **7**(2), e32014 (2012)

Soluble amyloid- β (A β) aggregates of various sizes, ranging from dimers to large protofibrils, have been associated with neurotoxicity and synaptic dysfunction in Alzheimer's Disease (AD). To investigate the properties of biologically relevant A β species, brain extracts from amyloid β protein precursor (A β PP) transgenic mice and AD patients as well as synthetic A β preparations were separated by size under native conditions with density gradient ultracentrifugation. The fractionated samples were then analyzed with atomic force microscopy (AFM), ELISA, and MTT cell viability assay. Based on AFM appearance and immunoreactivity to our protofibril selective antibody mAb158, synthetic A β 42 was divided in four fractions, with large aggregates in fraction 1 and the smallest species in fraction 4. Synthetic A β aggregates from fractions 2 and 3 proved to be most toxic in an MTT assay. In A β PP transgenic mouse brain, the most abundant soluble A β species were found in fraction 2 and consisted mainly of A β 40. Also in AD brains, A β was mainly found in fraction 2 but primarily as A β 42. All biologically derived A β from fraction 2 was immunologically discriminated from smaller species with mAb158. Thus, the predominant species of biologically derived soluble A β , natively separated by density gradient ultracentrifugation, were found to match the size of the neurotoxic, 80–500 kDa synthetic A β protofibrils and were equally detected with mAb158.

2.151 The small G protein Arf1 directs the trans-Golgi-specific targeting of the Arf1 exchange factors BIG1 and BIG2

Christis, C. and Munro, S.

J. Cell. Biol., **196**(3), 327-335 (2012)

The small G protein Arf1 regulates Golgi traffic and is activated by two related types of guanine nucleotide exchange factor (GEF). GBF1 acts at the cis-Golgi, whereas BIG1 and its close paralog BIG2 act at the trans-Golgi. Peripheral membrane proteins such as these GEFs are often recruited to membranes by small G proteins, but the basis for specific recruitment of Arf GEFs, and hence Arfs, to Golgi membranes is not understood. In this paper, we report a liposome-based affinity purification method to identify effectors for small G proteins of the Arf family. We validate this with the *Drosophila melanogaster* Arf1 orthologue (Arf79F) and the related class II Arf (Arf102F), which showed a similar pattern of effector binding.

Applying the method to the Arf-like G protein Arl1, we found that it binds directly to Sec71, the *Drosophila* ortholog of BIG1 and BIG2, via an N-terminal region. We show that in mammalian cells, Arl1 is necessary for Golgi recruitment of BIG1 and BIG2 but not GBF1. Thus, Arl1 acts to direct a trans-Golgi-specific Arf1 GEF, and hence active Arf1, to the trans side of the Golgi.

- 2.152 Crystal structure and biochemical analyses reveal Beclin 1 as a novel membrane binding protein**
Huang, W., Choi, W., Hu, W., Mi, N., Guo, Q., Ma, M., Liu, M., Tian, Y., Lu, P., Wang, F-L., Deng, H., Liu, L., Gao, N., Yu, L. and Shi, Y.
Cell Res., **22**, 473-489 (2012)

The *Beclin 1* gene is a haplo-insufficient tumor suppressor and plays an essential role in autophagy. However, the molecular mechanism by which Beclin 1 functions remains largely unknown. Here we report the crystal structure of the evolutionarily conserved domain (ECD) of Beclin 1 at 1.6 Å resolution. Beclin 1 ECD exhibits a previously unreported fold, with three structural repeats arranged symmetrically around a central axis. Beclin 1 ECD defines a novel class of membrane-binding domain, with a strong preference for lipid membrane enriched with cardiolipin. The tip of a surface loop in Beclin 1 ECD, comprising three aromatic amino acids, acts as a hydrophobic finger to associate with lipid membrane, consequently resulting in the deformation of membrane and liposomes. Mutation of these aromatic residues rendered Beclin 1 unable to stably associate with lipid membrane *in vitro* and unable to fully rescue autophagy in Beclin 1-knockdown cells *in vivo*. These observations form an important framework for deciphering the biological functions of Beclin 1.

- 2.153 Density Gradient Multilayer Polymerization for Creating Complex Tissue**
Karpiak, J.V., Ner, Y. and Almutairi, A.
Adv. Mater., **24(11)**, 1466-1470 (2012)

An adaptable density gradient multilayer polymerization (DGMP) method facilitates simple fabrication of complex multicompartments scaffolds with structurally continuous interfaces. Solvent density liquid-liquid phase segregation compartmentalizes varied mechanical and chemical cues independently. Bulk photopolymerization produces stratified three-dimensional and two-dimensional matrices. Cells attach to patterned adhesion peptides on biomimetic 2D substrates.

- 2.154 Distribution of perfluorooctanesulfonate and perfluorooctanoate into human plasma lipoprotein fractions**
Butenhoff, J.L., Pieterman, E., Ehresman, D.J., Gorman, G.S., Olsen, G.W., Chang, S-C. and Princen, H.M.G.
Toxicology Letters, **210**, 360-365 (2012)

Some cross-sectional epidemiological studies have reported positive associations of serum concentrations of non-high density lipoprotein cholesterol with serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). However, the strength of the reported associations is inconsistent for exposure-response across three orders of magnitude of serum PFOS and/or PFOA concentrations. These positive associations are unexpected based on toxicological/mechanistic studies, suggesting that the associations may have a biological, rather than a causal, basis. This study tested the hypothesis that PFOS and PFOA distribute into serum lipoprotein fractions such that increases in serum lipoproteins would result in corresponding increases in serum concentrations of PFOS and PFOA. Based on observed binding of PFOS and PFOA to isolated β -lipoproteins in physiological saline (96% and 40% bound, respectively) in preliminary experiments using ultrafiltration and LC-MS/MS methods, binding to human donor plasma lipoprotein fractions was investigated by two density gradient methods. The majority of PFOS and PFOA recovered masses were found in lipoprotein-depleted plasma. Plasma density gradient fractionation data suggested that maximally 9% of PFOS distributes to lipoprotein-containing fractions, yet only 1% or less of PFOA is so distributed. These data do not support a strong role for plasma lipoprotein fractions in explaining the inconsistent dose-response associations reported in cross-sectional epidemiological studies.

- 2.155 Fusion of single proteoliposomes with planar, cushioned bilayers in microfluidic flow cells**
Karatekin, E. and Ropthman, J.E.
Nature Protocols, **7(5)**, 903-920 (2012)

Many biological processes rely on membrane fusion, and therefore assays to study its mechanisms are necessary. Here we report an assay with sensitivity to single-vesicle, and even to single-molecule events using fluorescently labeled vesicle-associated v-SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) liposomes and target-membrane-associated t-SNARE-reconstituted planar, supported bilayers (t-SBLs). Docking and fusion events can be detected using conventional far-field epifluorescence or total internal reflection fluorescence microscopy. In this assay, fusion is dependent on SNAP-25, one of the t-SNARE subunits that is required for fusion *in vivo*. The success of the assay is due to the use of: (i) bilayers covered with a thin layer of poly(ethylene glycol) (PEG) to control bilayer-bilayer and bilayer-substrate interactions, and (ii) microfluidic flow channels that present many advantages, such as the removal of nonspecifically bound liposomes by flow. The protocol takes 6–8 d to complete. Analysis can take up to 2 weeks.

2.156 P52—Distribution of perfluorooctanesulfonate and perfluorooctanoate into human plasma lipoprotein fractions over a wide range of concentrations

Butenhoff, J.L., Pieterman, E.J., Ehresman, D.J., Olsen, G.W., Chang, S-C. and Princen, H.M.G. *Reprod. Toxicol.*, 33, 1-29, abstract P-52 (2012)

Certain observational epidemiological studies have been characterized as finding modest positive associations between serum concentrations of non-high density lipoprotein cholesterol (non-HDL-C) and serum concentrations of PFOS and/or PFOA. However, collectively, these primarily cross-sectional investigations of occupational, community-exposed, and general populations are remarkably inconsistent for any dose–response relationship across a range of PFOS and/or PFOA concentrations. These PFOS and PFOA concentrations span three orders of magnitude between the least and highest exposed populations. Contrary to the epidemiological positive associations, toxicological studies of PFOS and PFOA in laboratory animals, including cynomolgus monkeys, have observed either no change in serum lipids or decreases in serum cholesterol and/or triglycerides. These conflicting observations suggest that the reported epidemiologic associations of serum PFOS and PFOA with serum cholesterol may have biological, but not causal, significance. A possible non-causal hypothesis for the reported epidemiological associations between serum PFOS and/or PFOA and serum cholesterol is that PFOS and/or PFOA have affinity for and distribute into serum lipoprotein fractions. Thus, it can be postulated that, as serum lipoprotein concentration increases, distribution of PFOS and PFOA into serum lipoprotein fractions and total serum PFOS and/or PFOA concentrations would increase correspondingly. The study reported herein was undertaken to test the latter hypothesis through investigation of the effect of plasma concentration of PFOS and PFOA on the proportion of plasma PFOS and PFOA bound to the human plasma lipoprotein fractions VLDL, LDL, and HDL at background general population serum PFOS and PFOA concentrations and in serum spiked with either approximately 0.19 or 19 μM PFOS or PFOA. Plasma from a senior investigator was obtained and used to represent general population background concentrations of PFOS and PFOA, as well as to prepare approximately 0.19 and 19 μM concentrations of each fluorochemical. All experiments were performed in triplicate. Fractionation of 300 μL plasma into 25 fractions was accomplished by density gradient ultracentrifugation using both Redgrave and iodixanol density gradients. For each density gradient, fractions were pooled into very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and lipoprotein depleted plasma (LPDP) fractions as well as intermediate fractions based on determination of cholesterol concentrations in each of 25 fractions. Both density gradients were adjusted to give clean separations between the lipoprotein fractions and between HDL and albumin contained in LPDP. PFOS and PFOA concentrations in pooled fractions were determined by LC–MS/MS with lower limits of quantitation (LLOQ) ranging from 10 to 25 pg/mL. Total recovered mass of PFOS and PFOA based on duplicate determinations of initial concentrations ranged from 71 to 84% and 95 to 109% for PFOS and from 82 to 102% and 96 to 110% for PFOA for Redgrave and iodixanol density gradients, respectively. The majority of PFOS and PFOA recovered mass was found in LPDP (52–60% and 87–104% of PFOS mass and 81–98% and 94–109% of PFOA mass for Redgrave and iodixanol density gradients, respectively). Percent of mass recovered from fractions 1 to 19 (lipoprotein-containing fractions) ranged from 17 to 24% and <5 to 9% for PFOS and from <4 to <8% and <1 to <4% for PFOA for the Redgrave and iodixanol density gradients, respectively. PFOS concentrations in LDL and HDL were clearly higher than in VLDL and intermediate pooled lipoprotein fractions. For both PFOS and PFOA, there was minimal difference between concentrations tested and the resulting proportion distributed to the various pooled fractions. The results using the iodixanol method may be more representative physiologically, as iodixanol is a water soluble, non-ionic fluid. The iodixanol data suggest

that maximally 9% of PFOS may be distributed to lipoprotein-containing fractions in plasma, yet only 1% or less of PFOA is so distributed. Taken together, these data do not support a strong role for plasma lipoprotein fractions in explaining the inconsistent dose–response associations reported in observational epidemiological studies.

2.157 Identification and Characterization of an A β Oligomer Precipitating Peptide That May Be Useful to Explore Gene Therapeutic Approaches to Alzheimer Disease

Funke, S.A., Liu, H., Sehl, T., Bartnik, D., Brener, O., Nagel-Steger, L., Wiesehan, K. and Wilbold, D. *Rejuvenation Res.*, 15(2), 144-147 (2012)

A key feature of Alzheimer disease (AD) is the pathologic self-association of the amyloid- β (A β) peptide, leading to the formation of diffusible toxic A β oligomers and extracellular amyloid plaques. Next to extracellular A β , intraneuronal A β has important pathological functions in AD. Agents that specifically interfere with the oligomerization processes either outside or inside of neurons are highly desired for the elucidation of the pathologic mechanisms of AD and might even pave the way for new AD gene therapeutic approaches. Here, we characterize the A β binding peptide L3 and its influence on A β oligomerization in vitro. Preliminary studies in cell culture demonstrate that stably expressed L3 reduces cell toxicity of externally added A β in neuroblastoma cells.

2.158 A Dual Role for UVRAG in Maintaining Chromosomal Stability Independent of Autophagy

Zhao, Z., Oh, S., Li, D., Ni, D., Pirooz, S.D., Lee, J-H., yang, S., Lee, J-Y., Ghosalli, I., Costanzo, V., Stark, J.M. and Liang, C. *Developmental Cell*, 22(5), 1-16 (2012)

Autophagy defects have recently been associated with chromosomal instability, a hallmark of human cancer. However, the functional specificity and mechanism of action of autophagy-related factors in genome stability remain elusive. Here we report that UVRAG, an autophagic tumor suppressor, plays a dual role in chromosomal stability, surprisingly independent of autophagy. We establish that UVRAG promotes DNA double-strand-break repair by directly binding and activating DNA-PK in nonhomologous end joining. Disruption of UVRAG increases genetic instability and sensitivity of cells to irradiation. Furthermore, UVRAG was also found to be localized at centrosomes and physically associated with CEP63, an integral component of centrosomes. Disruption of the association of UVRAG with centrosomes causes centrosome instability and aneuploidy. UVRAG thus represents an autophagy-related molecular factor that also has a convergent role in patrolling both the structural integrity and proper segregation of chromosomes, which may confer autophagy-independent tumor suppressor activity.

2.159 Differential Effects of Grape (*Vitis vinifera*) Skin Polyphenolics on Human Platelet Aggregation and Low-Density Lipoprotein Oxidation

Shanmuganayagam, D., Beahm, M.R., Kuhns, M.A., Krueger, C.G., Reed, J.D. and Folts, J.D. *J. Agric. Food Chem.*, 60(23), 5787-5794 (2012)

Antioxidant and antiplatelet properties of grape products are thought to be responsible for observed antiatherosclerotic effects. Diverse classes of phenolics are derived from the seed and skin (GSK) of grapes. The relative contributions of the classes of phenolics to observed properties of grape products are unknown. In this paper, GSK fractions were used to examine effects on platelet aggregation, low-density lipoprotein (LDL) oxidation in vitro, and relative binding of phenolics to LDL. GSK was separated into six fractions (fractions 1–6), and primary phenolics were characterized using high-performance liquid chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Fractions 4, 5, and 6, enriched in polygalloyl polyflavan-3-ols (PGPFs) with 3–6, 4–8, and 6–15 degrees of polymerization, respectively, inhibited platelet aggregation. Fractions 1–3, containing various amounts of oligosaccharides, hydroxycinnamic acids, anthocyanins, flavanols, and low molecular weight PGPFs, significantly increased platelet aggregation. Fractions 4–6 were most effective in binding LDL and inhibiting LDL oxidation. Fractions 5 and 6 exhibited the greatest inhibition of platelet aggregation and LDL oxidation, suggesting that polymeric PGPFs are responsible for the beneficial effects of grape products. Conversely, phenolics in fractions 1–3 may reduce the net biological potency of the grape products and have undesirable effects on cardiovascular disease risk factors.

2.160 , 58-63 (2012)small mitochondrial ribosomal subunit

He, J., Cooper, H.M., Reyes, A., Di Re, M., Kazak, L., Wood, S.R., Mao, C.C., Fearnley, I.M., Walker, J.E.

and Holt, I.J.
Nucleic Acids Res., **40**(13), 6097-6108 (2012)

The bacterial homologue of C4orf14, YqeH, has been linked to assembly of the small ribosomal subunit. Here, recombinant C4orf14 isolated from human cells, co-purified with the small, 28S subunit of the mitochondrial ribosome and the endogenous protein co-fractionated with the 28S subunit in sucrose gradients. Gene silencing of C4orf14 specifically affected components of the small subunit, leading to decreased protein synthesis in the organelle. The GTPase of C4orf14 was critical to its interaction with the 28S subunit, as was GTP. Therefore, we propose that C4orf14, with bound GTP, binds to components of the 28S subunit facilitating its assembly, and GTP hydrolysis acts as the release mechanism. C4orf14 was also found to be associated with human mitochondrial nucleoids, and C4orf14 gene silencing caused mitochondrial DNA depletion. *In vitro* C4orf14 is capable of binding to DNA. The association of C4orf14 with mitochondrial translation factors and the mitochondrial nucleoid suggests that the 28S subunit is assembled at the mitochondrial nucleoid, enabling the direct transfer of messenger RNA from the nucleoid to the ribosome in the organelle.

2.161 Mitochondrial nucleoid interacting proteins support mitochondrial protein synthesis

He, J., Cooper, H.M., Reyes, A., Di Re, M., Sembongi, H., Litwin, T.R., Gao, J., Neuman, K.C., Fearnley, I.M., Spinazzola, A., Walker, J.E. and Holt, I.J.
Nucleic Acids Res., **40**(13), 6109-6121 (2012)

Mitochondrial ribosomes and translation factors co-purify with mitochondrial nucleoids of human cells, based on affinity protein purification of tagged mitochondrial DNA binding proteins. Among the most frequently identified proteins were ATAD3 and prohibitin, which have been identified previously as nucleoid components, using a variety of methods. Both proteins are demonstrated to be required for mitochondrial protein synthesis in human cultured cells, and the major binding partner of ATAD3 is the mitochondrial ribosome. Altered ATAD3 expression also perturbs mtDNA maintenance and replication. These findings suggest an intimate association between nucleoids and the machinery of protein synthesis in mitochondria. ATAD3 and prohibitin are tightly associated with the mitochondrial membranes and so we propose that they support nucleic acid complexes at the inner membrane of the mitochondrion.

2.162 Benzophenones and xanthenes from *Garcinia cantleyana* var. *cantleyana* and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation

Jantan, I. and Saputri, C.
Phytochemistry, **80**, 58-63 (2012)

Three benzophenones, 2,6,3',5'-tetrahydroxybenzophenone (**1**), 3,4,5,3',5'-pentahydroxybenzophenone (**3**) and 3,5,3',5'-tetrahydroxy-4-methoxybenzophenone (**4**), as well as a xanthone, 1,3,6-trihydroxy-5-methoxy-7-(3'-methyl-2'-oxo-but-3'-enyl)xanthone (**9**), were isolated from the twigs of *Garcinia cantleyana* var. *cantleyana*. Eight known compounds, 3,4,5,3'-tetrahydroxy benzophenone (**2**), 1,3,5-trihydroxyxanthone (**5**), 1,3,8-trihydroxyxanthone (**6**), 2,4,7-trihydroxyxanthone (**7**), 1,3,5,7-tetrahydroxyxanthone (**8**), quercetin, glutin-5-en-3 β -ol and friedelin were also isolated. The structures of the compounds were elucidated by spectroscopic methods. The compounds were investigated for their ability to inhibit low-density lipoprotein (LDL) oxidation and platelet aggregation in human whole blood *in vitro*. Most of the compounds showed strong antioxidant activity with compound **8** showing the highest inhibition with an IC₅₀ value of 0.5 μ M, comparable to that of probucol. Among the compounds tested, only compound **4** exhibited strong inhibitory activity against platelet aggregation induced by arachidonic acid (AA), adenosine diphosphate (ADP) and collagen. Compounds **3**, **5** and **8** showed selective inhibitory activity on platelet aggregation induced by ADP.

2.163 Purified and synthetic Alzheimer's amyloid beta (A β) prions

Stöhr, J., Watts, J.C., Mensinger, Z.L., Oehler, A., Grillo, S.K., DeArmond, S.J., Prusiner, S.B. and Giles, K.
PNAS, **109**(27), 11025-11030 (2012)

The aggregation and deposition of amyloid- β (A β) peptides are believed to be central events in the pathogenesis of Alzheimer's disease (AD). Inoculation of brain homogenates containing A β aggregates

into susceptible transgenic mice accelerated A β deposition, suggesting that A β aggregates are capable of self-propagation and hence might be prions. Recently, we demonstrated that A β deposition can be monitored in live mice using bioluminescence imaging (BLI). Here, we use BLI to probe the ability of A β aggregates to self-propagate following inoculation into bigenic mice. We report compelling evidence that A β aggregates are prions by demonstrating widespread cerebral β -amyloidosis induced by inoculation of either purified A β aggregates derived from brain or aggregates composed of synthetic A β . Although synthetic A β aggregates were sufficient to induce A β deposition in vivo, they exhibited lower specific biological activity compared with brain-derived A β aggregates. Our results create an experimental paradigm that should lead to identification of self-propagating A β conformations, which could represent novel targets for interrupting the spread of A β deposition in AD patients.

2.164 Feedback Regulation of Transcriptional Termination by the Mammalian Circadian Clock PERIOD Complex

Padmanabhan, K. et al
Science, **337**, 599-602 (2012)

Eukaryotic circadian clocks are built on transcriptional feedback loops. In mammals, the PERIOD (PER) and CRYPTOCHROME (CRY) proteins accumulate, form a large nuclear complex (PER complex), and repress their own transcription. We found that mouse PER complexes included RNA helicases DDX5 and DHX9, active RNA polymerase II large subunit, *Per* and *Cry* pre-mRNAs, and SETX, a helicase that promotes transcriptional termination. During circadian negative feedback, RNA polymerase II accumulated near termination sites on *Per* and *Cry* genes but not on control genes. Recruitment of PER complexes to the elongating polymerase at *Per* and *Cry* termination sites inhibited SETX action, impeding RNA polymerase II release and thereby repressing transcriptional reinitiation. Circadian clock negative feedback thus includes direct control of transcriptional termination.

2.165 The dual PH domain protein Opy1 functions as a sensor and modulator of PtdIns(4,5)P₂ synthesis

Ling, Y., Stefan, C.J., MacGurn, J.A., Audhya, A. and Emr, S.D.
EMBO J., **31**(13), 2882-2894 (2012)

Phosphatidylinositol-4,5-bisphosphate, PtdIns(4,5)P₂, is an essential signalling lipid that regulates key processes such as endocytosis, exocytosis, actin cytoskeletal organization and calcium signalling. Maintaining proper levels of PtdIns(4,5)P₂ at the plasma membrane (PM) is crucial for cell survival and growth. We show that the conserved PtdIns(4)P 5-kinase, Mss4, forms dynamic, oligomeric structures at the PM that we term PIK patches. The dynamic assembly and disassembly of Mss4 PIK patches may provide a mechanism to precisely modulate Mss4 kinase activity, as needed, for localized regulation of PtdIns(4,5)P₂ synthesis. Furthermore, we identify a tandem PH domain-containing protein, Opy1, as a novel Mss4-interacting protein that partially colocalizes with PIK patches. Based upon genetic, cell biological, and biochemical data, we propose that Opy1 functions as a coincidence detector of the Mss4 PtdIns(4)P 5-kinase and PtdIns(4,5)P₂ and serves as a negative regulator of PtdIns(4,5)P₂ synthesis at the PM. Our results also suggest that additional conserved tandem PH domain-containing proteins may play important roles in regulating phosphoinositide signalling.

2.166 Lipoproteins in *Drosophila melanogaster*—Assembly, Function, and Influence on Tissue Lipid Composition

Palm, W., Sampaio, J.L., Brankatschk, M., Carvalho, M., Mahmoud, A., Shevchenko, A. and Eaton, S.
PLoS One, **8**(7), e1002828 (2012)

Interorgan lipid transport occurs via lipoproteins, and altered lipoprotein levels correlate with metabolic disease. However, precisely how lipoproteins affect tissue lipid composition has not been comprehensively analyzed. Here, we identify the major lipoproteins of *Drosophila melanogaster* and use genetics and mass spectrometry to study their assembly, interorgan trafficking, and influence on tissue lipids. The apoB-family lipoprotein Lipophorin (Lpp) is the major hemolymph lipid carrier. It is produced as a phospholipid-rich particle by the fat body, and its secretion requires Microsomal Triglyceride Transfer Protein (MTP). Lpp acquires sterols and most diacylglycerol (DAG) at the gut via Lipid Transfer Particle (LTP), another fat body-derived apoB-family lipoprotein. The gut, like the fat body, is a lipogenic organ, incorporating both *de novo*-synthesized and dietary fatty acids into DAG for export. We identify distinct requirements for LTP and Lpp-dependent lipid mobilization in contributing to the neutral and polar lipid composition of the brain and wing imaginal disc. These studies define major routes of interorgan lipid transport in *Drosophila* and uncover surprising tissue-specific differences in lipoprotein lipid utilization.

2.167 **Functions of Nonmuscle Myosin II in Assembly of the Cellular Contractile System**

Shutova, M., Yang, C., Vasiliev, J.M. and Svitkina, T.
PLoS One, 7(7), e40814 (2012)

The contractile system of nonmuscle cells consists of interconnected actomyosin networks and bundles anchored to focal adhesions. The initiation of the contractile system assembly is poorly understood structurally and mechanistically, whereas system's maturation heavily depends on nonmuscle myosin II (NMII). Using platinum replica electron microscopy in combination with fluorescence microscopy, we characterized the structural mechanisms of the contractile system assembly and roles of NMII at early stages of this process. We show that inhibition of NMII by a specific inhibitor, blebbistatin, in addition to known effects, such as disassembly of stress fibers and mature focal adhesions, also causes transformation of lamellipodia into unattached ruffles, loss of immature focal complexes, loss of cytoskeleton-associated NMII filaments and peripheral accumulation of activated, but unpolymerized NMII. After blebbistatin washout, assembly of the contractile system begins with quick and coordinated recovery of lamellipodia and focal complexes that occurs before reappearance of NMII bipolar filaments. The initial formation of focal complexes and subsequent assembly of NMII filaments preferentially occurred in association with filopodial bundles and concave actin bundles formed by filopodial roots at the lamellipodial base. Over time, accumulating NMII filaments help to transform the precursor structures, focal complexes and associated thin bundles, into stress fibers and mature focal adhesions. However, semi-sarcomeric organization of stress fibers develops at much slower rate. Together, our data suggest that activation of NMII motor activity by light chain phosphorylation occurs at the cell edge and is uncoupled from NMII assembly into bipolar filaments. We propose that activated, but unpolymerized NMII initiates focal complexes, thus providing traction for lamellipodial protrusion. Subsequently, the mechanical resistance of focal complexes activates a load-dependent mechanism of NMII polymerization in association with attached bundles, leading to assembly of stress fibers and maturation of focal adhesions.

2.168 **Structural and genetic basis for development of broadly neutralizing influenza antibodies**

Lingwood, D., McTamney, P., Yassine, H.M., Whittle, J.R., Guo, X., Boyington, J.C., Wei, C.-J. and Nabel, G.J.
Nature, 487, 566-570 (2012)

Influenza viruses take a yearly toll on human life despite efforts to contain them with seasonal vaccines. These viruses evade human immunity through the evolution of variants that resist neutralization. The identification of antibodies that recognize invariant structures on the influenza haemagglutinin (HA) protein have invigorated efforts to develop universal influenza vaccines. Specifically, antibodies to the highly conserved stem region of HA neutralize diverse viral subtypes. These antibodies largely derive from a specific antibody gene, heavy-chain variable region *IGHV1-69*, after limited affinity maturation from their germline ancestors^{1,2}, but how HA stimulates naive B cells to mature and induce protective immunity is unknown. To address this question, we analysed the structural and genetic basis for their engagement and maturation into broadly neutralizing antibodies. Here we show that the germline-encoded precursors of these antibodies act as functional B-cell antigen receptors (BCRs) that initiate subsequent affinity maturation. Neither the germline precursor of a prototypic antibody, CR6261 (ref. 3), nor those of two other natural human *IGHV1-69* antibodies, bound HA as soluble immunoglobulin-G (IgG). However, all three *IGHV1-69* precursors engaged HA when the antibody was expressed as cell surface IgM. HA triggered BCR-associated tyrosine kinase signalling by germline transmembrane IgM. Recognition and virus neutralization was dependent solely on the heavy chain, and affinity maturation of CR6261 required only seven amino acids in the complementarity-determining region (CDR) H1 and framework region 3 (FR3) to restore full activity. These findings provide insight into the initial events that lead to the generation of broadly neutralizing antibodies to influenza, informing the rational design of vaccines to elicit such antibodies and providing a model relevant to other infectious diseases, including human immunodeficiency virus/AIDS. The data further suggest that selected immunoglobulin genes recognize specific protein structural 'patterns' that provide a substrate for further affinity maturation.

2.169 **Small dense LDL: An emerging risk factor for cardiovascular disease**

Hirayama, S. and Miida, T.
Clinica Chimica Acta, 414, 215-224 (2012)

Although low-density lipoprotein cholesterol (LDL-C) is a strong risk factor for coronary artery disease

(CAD), LDL-C levels are not always elevated in CAD patients. LDL consists of several subclasses with distinct sizes, densities, and physicochemical compositions. Thus, LDL subclasses can be separated by various laboratory procedures. Among them, ultracentrifugation and electrophoresis have been used most frequently for determining LDL subclasses. Accumulating evidence has shown that a predominance of small dense LDL (sd-LDL) is closely associated with CAD. Moreover, sd-LDL-cholesterol (sd-LDL-C) concentrations are elevated in groups at a high risk for CAD, such as patients with type 2 diabetes and metabolic syndrome. Therefore, sd-LDL concentration is recognized as a surrogate marker for CAD. However, some studies failed to show therapeutic modulation of sd-LDL, likely because separating methods and sd-LDL particle definitions have not yet been standardized. Recently, a detergent-based homogenous assay for sd-LDL-C has been developed. This method does not require any pretreatment, and the measured values are highly reproducible with an automated analyzer. These features are suitable for large-scale clinical studies. This homogeneous assay is a useful tool for clarifying whether sd-LDL-C is a superior marker to LDL-C, and whether sd-LDL-C lipid-lowering therapies decrease the incidence of CAD.

2.170 **Inhibitory Activities of Compounds from the Twigs of *Garcinia hombroniana* Pierre on Human Low-density Lipoprotein (LDL) Oxidation and Platelet Aggregation**

Saputri, F.C. and Jantan, I.

Phytother. Res., **26**(12), 1845-1850 (2012)

The methanol extract of the twigs of *Garcinia hombroniana*, which showed strong LDL antioxidation and antiplatelet aggregation activities, was subjected to column chromatography to obtain 3,5,3',5'-tetrahydroxy-4-methoxybenzophenone, 1,7-dihydroxyxanthone and eight triterpenoids, garcihombronane B, D, E and F, friedelin, glutin-5-en-3 β -ol, stigmasterol and lupeol. The structures of the compounds were elucidated by spectroscopic methods. The compounds were evaluated for their ability to inhibit copper-mediated LDL oxidation and arachidonic acid (AA)-, adenosine diphosphate (ADP)-, collagen-induced platelet aggregation *in vitro*. Among the compounds tested, 3,5,3',5'-tetrahydroxy-4-methoxybenzophenone and 1,7-dihydroxyxanthone showed strong inhibitory activity on LDL oxidation with half-maximal inhibitory concentration (IC₅₀) values of 6.6 and 1.7 μ m, respectively. 3,5,3',5'-Tetrahydroxy-4-methoxybenzophenone exhibited strong activity on AA-, ADP- and collagen-induced platelet aggregation with IC₅₀ values of 53.6, 125.7 and 178.6 μ m, respectively, while 1,7-dihydroxyxanthone showed significant and selective inhibitory activity against ADP-induced aggregation with IC₅₀ value of 5.7 μ m. Of the triterpenoids tested, garcihombronane B showed moderate activity against LDL oxidation and garcihombronane D and F showed selective inhibition on ADP-induced platelet aggregation.

2.171 Why working with porcine circulating serum amyloid A is a pig of a job

Soler, L., Molenaar, A., Merola, N., Eckersall, P.D., Butierrez, A., Ceron, J.J., Mulero, V. and Niewold, T.A:

J. Theoretical Biol., 317, 119-125 (2012)

Serum amyloid A (SAA) is a major acute phase protein in most species, and is widely employed as a health marker. Systemic SAA isoforms (SAA1, and SAA2) are apolipoproteins synthesized by the liver which associate with high density lipoproteins (HDL). Local SAA (SAA3) isoforms are synthesized in other tissues and are present in colostrums, mastitic milk and mammary dry secretions. Of systemic SAA the bulk is monomeric and bound to HDL, and a small proportion is found in serum in a multimeric form with a buried HDL binding site. In most species, systemic SAA could easily be studied by purifying it from serum of diseased individuals by hydrophobic interaction chromatography methods. For years, we were not able to isolate systemic pig SAA using the latter methods, and found that the bulk of pig SAA did not reside in the HDL-rich serum fractions but in the soluble protein fraction mainly as a multimeric protein.

Based on these surprising results, we analysed *in silico* the theoretical properties and predicted the secondary structure of pig SAA by using the published pig primary SAA amino acid sequence. Results of the analysis confirmed that systemic pig SAA had the highest homology with local SAA3 which in other species is the isoform associated with non-hepatic production in tissues such as mammary gland and intestinal epithelium. Furthermore, the primary sequence of the pig SAA N-terminal HDL binding site did differ considerably from SAA1/2. Secondary structure analysis of the predicted alpha-helical structure of this HDL binding site showed a considerable reduction in hydrophobicity compared to SAA1/2. Based on these results, it is argued that systemic acute phase SAA in the pig has the structural properties of locally produced SAA (SAA3). It is proposed that in pig SAA multimers the charged N-terminal sequence is buried, which would explain their different properties.

It is concluded that pig systemic SAA is unique compared to other species, which raises questions about the proposed importance of acute phase SAA in HDL metabolism during inflammation in this species.

2.172 Arenavirus Infection Induces Discrete Cytosolic Structures for RNA Replication

Baird, N.L., York, J. and Nunberg, J.H.

J. Virol., 86(20), 11301-11310 (2012)

Arenaviruses are responsible for acute hemorrhagic fevers with high mortality and pose significant threats to public health and biodefense. These enveloped negative-sense RNA viruses replicate in the cell cytoplasm and express four proteins. To better understand how these proteins insinuate themselves into cellular processes to orchestrate productive viral replication, we have identified and characterized novel cytosolic structures involved in arenavirus replication and transcription. In cells infected with the nonpathogenic Tacaribe virus or the attenuated Candid#1 strain of Junín virus, we find that newly synthesized viral RNAs localize to cytosolic puncta containing the nucleoprotein (N) of the virus. Density gradient centrifugation studies reveal that these replication-transcription complexes (RTCs) are associated with cellular membranes and contain full-length genomic- and antigenomic-sense RNAs. Viral mRNAs segregate at a higher buoyant density and are likewise scant in immunopurified RTCs, consistent with their translation on bulk cellular ribosomes. In addition, confocal microscopy analysis reveals that RTCs contain the lipid phosphatidylinositol-4-phosphate and proteins involved in cellular mRNA metabolism, including the large and small ribosomal subunit proteins L10a and S6, the stress granule protein G3BP1, and a subset of translation initiation factors. Elucidating the structure and function of RTCs will enhance our understanding of virus-cell interactions that promote arenavirus replication and mitigate against host cell immunity. This knowledge may lead to novel intervention strategies to limit viral virulence and pathogenesis.

2.173 Alternative translation initiation augments the human mitochondrial proteome

Kazak, L., Reyes, A., Duncan, A.L., Rorbach, J., Wood, S.R., Brea-Calvo, g., Gammage, P.A., Robinson, A.J., Minczuk, M. and Holt, I.J.

Nucleic Acids Res., 41(4), 2354-2369 (2013)

Alternative translation initiation (ATI) is a mechanism of producing multiple proteins from a single transcript, which in some cases regulates trafficking of proteins to different cellular compartments, including mitochondria. Application of a genome-wide computational screen predicts a cryptic mitochondrial targeting signal for 126 proteins in mouse and man that is revealed when an AUG codon located downstream from the canonical initiator methionine codon is used as a translation start site, which

we term downstream ATI (dATI). Experimental evidence in support of dATI is provided by immunoblotting of endogenous truncated proteins enriched in mitochondrial cell fractions or of co-localization with mitochondria using immunocytochemistry. More detailed cellular localization studies establish mitochondrial targeting of a member of the cytosolic poly(A) binding protein family, PABPC5, and of the RNA/DNA helicase PIF1 α . The mitochondrial isoform of PABPC5 co-immunoprecipitates with the mitochondrial poly(A) polymerase, and is markedly reduced in abundance when mitochondrial DNA and RNA are depleted, suggesting it plays a role in RNA metabolism in the organelle. Like PABPC5 and PIF1 α , most of the candidates identified by the screen are not currently annotated as mitochondrial proteins, and so dATI expands the human mitochondrial proteome.

2.174 FisB mediates membrane fission during sporulation in *Bacillus subtilis*

Doan, T., Coleman, J., Marquis, K.A. et al
Genes Dev., **27**(3), 322-334 (2013)

How bacteria catalyze membrane fission during growth and differentiation is an outstanding question in prokaryotic cell biology. Here, we describe a protein (FisB, for fission protein B) that mediates membrane fission during the morphological process of spore formation in *Bacillus subtilis*. Sporulating cells divide asymmetrically, generating a large mother cell and smaller forespore. After division, the mother cell membranes migrate around the forespore in a phagocytic-like process called engulfment. Membrane fission releases the forespore into the mother cell cytoplasm. Cells lacking FisB are severely and specifically impaired in the fission reaction. Moreover, GFP-FisB forms dynamic foci that become immobilized at the site of fission. Purified FisB catalyzes lipid mixing *in vitro* and is only required in one of the fusing membranes, suggesting that FisB–lipid interactions drive membrane remodeling. Consistent with this idea, the extracytoplasmic domain of FisB binds with remarkable specificity to cardiolipin, a lipid enriched in the engulfing membranes and regions of negative curvature. We propose that membrane topology at the final stage of engulfment and FisB–cardiolipin interactions ensure that the mother cell membranes are severed at the right time and place. The unique properties of FisB set it apart from the known fission machineries in eukaryotes, suggesting that it represents a new class of fission proteins.

2.175 Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains

Volmer, R., van der Ploeg, K. and Ron, D.
PNAS, **110**(12), 4628-4633 (2013)

Endoplasmic reticulum (ER) stress sensors use a related luminal domain to monitor the unfolded protein load and convey the signal to downstream effectors, signaling an unfolded protein response (UPR) that maintains compartment-specific protein folding homeostasis. Surprisingly, perturbation of cellular lipid composition also activates the UPR, with important consequences in obesity and diabetes. However, it is unclear if direct sensing of the lipid perturbation contributes to UPR activation. We found that mutant mammalian ER stress sensors, IRE1 α and PERK, lacking their luminal unfolded protein stress-sensing domain, nonetheless retained responsiveness to increased lipid saturation. Lipid saturation-mediated activation in cells required an ER-spanning transmembrane domain and was positively regulated *in vitro* by acyl-chain saturation in reconstituted liposomes. These observations suggest that direct sensing of the lipid composition of the ER membrane contributes to the UPR.

2.176 Low Density Lipoprotein Binds to Proprotein Convertase Subtilisin/Kexin Type-9 (PCSK9) in Human Plasma and Inhibits PCSK9-mediated Low Density Lipoprotein Receptor Degradation

Kosenko, T., Golder, M., Leblond, G., Weng, W. and Lagace, T.A.
J. Biol. Chem., **288**(12), 8279-8288 (2013)

Proprotein convertase subtilisin/kexin type-9 (PCSK9) is a secreted protein that binds to the epidermal growth factor-like-A domain of the low density lipoprotein receptor (LDLR) and mediates LDLR degradation in liver. Gain-of-function mutations in *PCSK9* are associated with autosomal dominant hypercholesterolemia in humans. Size-exclusion chromatography of human plasma has shown PCSK9 to be partly associated with undefined high molecular weight complexes within the LDL size range. We used density gradient centrifugation to isolate LDL in plasma pooled from 5 normolipidemic subjects and report that >40% of total PCSK9 was associated with LDL. Binding of fluorophore-labeled recombinant PCSK9 to isolated LDL *in vitro* was saturable with a $K_D \sim 325$ nm. This interaction was competed >95% by excess unlabeled PCSK9, and competition binding curves were consistent with a one-site binding model. An N-terminal region of the PCSK9 prodomain (amino acids 31–52) was required for binding to LDL *in*

vitro. LDL dose-dependently inhibited binding and degradation of cell surface LDLRs by exogenous PCSK9 in Huh7 cells. LDL also inhibited PCSK9 binding to mutant LDLRs defective at binding LDL. These data suggest that association of PCSK9 with LDL particles in plasma lowers the ability of PCSK9 to bind to cell surface LDLRs, thereby blunting PCSK9-mediated LDLR degradation.

2.177 Secretion and Signaling Activities of Lipoprotein-Associated Hedgehog and Non-Sterol-Modified Hedgehog in Flies and Mammals

Palm, W., Swierczynska, M.M., Kumari, V., Ehrhart-Bornstein, M., Bornstein, S.R. and Eaton, S.
PLoS Biology, **11**(3), e1001505 (2013)

Hedgehog (Hh) proteins control animal development and tissue homeostasis. They activate gene expression by regulating processing, stability, and activation of Gli/Cubitus interruptus (Ci) transcription factors. Hh proteins are secreted and spread through tissue, despite becoming covalently linked to sterol during processing. Multiple mechanisms have been proposed to release Hh proteins in distinct forms; in *Drosophila*, lipoproteins facilitate long-range Hh mobilization but also contain lipids that repress the pathway. Here, we show that mammalian lipoproteins have conserved roles in Sonic Hedgehog (Shh) release and pathway repression. We demonstrate that lipoprotein-associated forms of Hh and Shh specifically block lipoprotein-mediated pathway inhibition. We also identify a second conserved release form that is not sterol-modified and can be released independently of lipoproteins (Hh-N*/Shh-N*). Lipoprotein-associated Hh/Shh and Hh-N*/Shh-N* have complementary and synergistic functions. In *Drosophila* wing imaginal discs, lipoprotein-associated Hh increases the amount of full-length Ci, but is insufficient for target gene activation. However, small amounts of non-sterol-modified Hh synergize with lipoprotein-associated Hh to fully activate the pathway and allow target gene expression. The existence of Hh secretion forms with distinct signaling activities suggests a novel mechanism for generating a diversity of Hh responses

2.178 Very-Low-Density Lipoprotein (VLDL)-Producing and Hepatitis C Virus-Replicating HepG2 Cells Secrete No More Lipoviroparticles than VLDL-Deficient Huh7.5 Cells

Jammart, B., Michelet, M., Pecheur, E.-I., Parent, R., Bartosch, B., Zoulim, F. and Durantel, D.
J. Virol., **87**(9), 5065-5080 (2013)

In the plasma samples of hepatitis C virus (HCV)-infected patients, lipoviroparticles (LVPs), defined as (very-) low-density viral particles immunoprecipitated with anti- β -lipoproteins antibodies are observed. This HCV-lipoprotein association has major implications with respect to our understanding of HCV assembly, secretion, and entry. However, cell culture-grown HCV (HCVcc) virions produced in Huh7 cells, which are deficient for very-low-density lipoprotein (VLDL) secretion, are only associated with and dependent on apolipoprotein E (apoE), not apolipoprotein B (apoB), for assembly and infectivity. In contrast to Huh7, HepG2 cells can be stimulated to produce VLDL by both oleic acid treatment and inhibition of the MEK/extracellular signal-regulated kinase (ERK) pathway but are not permissive for persistent HCV replication. Here, we developed a new HCV cell culture model to study the interaction between HCV and lipoproteins, based on engineered HepG2 cells stably replicating a blasticidin-tagged HCV JFH1 strain (JB). Control Huh7.5-JB as well as HepG2-JB cell lines persistently replicated viral RNA and expressed viral proteins with a subcellular colocalization of double-stranded RNA (dsRNA), core, gpE2, and NS5A compatible with virion assembly. The intracellular RNA replication level was increased in HepG2-JB cells upon dimethyl sulfoxide (DMSO) treatment, MEK/ERK inhibition, and NS5A overexpression to a level similar to that observed in Huh7.5-JB cells. Both cell culture systems produced infectious virions, which were surprisingly biophysically and biochemically similar. They floated at similar densities on gradients, contained mainly apoE but not apoB, and were not neutralized by anti-apoB antibodies. This suggests that there is no correlation between the ability of cells to simultaneously replicate HCV as well as secrete VLDL and their capacity to produce LVPs.

2.179 Production, Purification and Characterization of Recombinant, Full-Length Human Claudin-1

Bonander, N., Jamshad, M., Oberthür, D., Clare, M., Barwell, J., Hu, K., Farquhar, M.J., Stamatakis, Z., Harris, H.J., Dierks, K., Daffron, T.R., Betzel, C., McKeating, J.A. and Bill, R.M.
PLoS One, **8**(5), e64517 (2013)

The transmembrane domain proteins of the claudin superfamily are the major structural components of cellular tight junctions. One family member, claudin-1, also associates with tetraspanin CD81 as part of a receptor complex that is essential for hepatitis C virus (HCV) infection of the liver. To understand the molecular basis of claudin-1/CD81 association we previously produced and purified milligram quantities

of functional, full-length CD81, which binds a soluble form of HCV E2 glycoprotein (sE2). Here we report the production, purification and characterization of claudin-1. Both yeast membrane-bound and detergent-extracted, purified claudin-1 were antigenic and recognized by specific antibodies. Analytical ultracentrifugation demonstrated that extraction with n-octyl- β -d-glucopyranoside yielded monodispersed, dimeric pools of claudin-1 while extraction with profoldin-8 or n-decylphosphocholine yielded a dynamic mixture of claudin-1 oligomers. Neither form bound sE2 in line with literature expectations, while further functional analysis was hampered by the finding that incorporation of claudin-1 into proteoliposomes rendered them intractable to study. Dynamic light scattering demonstrated that claudin-1 oligomers associate with CD81 *in vitro* in a defined molar ratio of 1:2 and that complex formation was enhanced by the presence of cholesteryl hemisuccinate. Attempts to assay the complex biologically were limited by our finding that claudin-1 affects the properties of proteoliposomes. We conclude that recombinant, correctly-folded, full-length claudin-1 can be produced in yeast membranes, that it can be extracted in different oligomeric forms that do not bind sE2 and that a dynamic preparation can form a specific complex with CD81 *in vitro* in the absence of any other cellular components. These findings pave the way for the structural characterization of claudin-1 alone and in complex with CD81.

2.180 A Cryptic Targeting Signal Creates a Mitochondrial FEN1 Isoform with Tailed R-Loop Binding Properties

Kazak, L., Reyes, A., He, J., Wood, S.R., Brea-Calvo, G., Holen, T.T. and Holt, I.J.
PLoS One, 8(5), e62340 (2013)

A growing number of DNA transacting proteins is found in the nucleus and in mitochondria, including the DNA repair and replication protein Flap endonuclease 1, FEN1. Here we show a truncated FEN1 isoform is generated by alternative translation initiation, exposing a mitochondrial targeting signal. The shortened form of FEN1, which we term FENMIT, localizes to mitochondria, based on import into isolated organelles, immunocytochemistry and subcellular fractionation. *In vitro* FENMIT binds to flap structures containing a 5' RNA flap, and prefers such substrates to single-stranded RNA. FENMIT can also bind to R-loops, and to a lesser extent to D-loops. Exposing human cells to ethidium bromide results in the generation of RNA/DNA hybrids near the origin of mitochondrial DNA replication. FENMIT is recruited to the DNA under these conditions, and is released by RNase treatment. Moreover, high levels of recombinant FENMIT expression inhibit mtDNA replication, following ethidium bromide treatment. These findings suggest FENMIT interacts with RNA/DNA hybrids in mitochondrial DNA, such as those found at the origin of replication.

2.181 Separation of the principal HDL subclasses by iodixanol ultracentrifugation

Harman, N.L., Griffin, B.A. and Davies, I.G.
J. Lipid Res., 54, 2273-2281 (2013)

HDL subclasses detection, in cardiovascular risk, has been limited due to the time-consuming nature of current techniques. We have developed a time-saving and reliable separation of the principal HDL subclasses employing iodixanol density gradient ultracentrifugation (IxDGUC) combined with digital photography. HDL subclasses were separated in 2.5 h from prestained plasma on a three-step iodixanol gradient. HDL subclass profiles were generated by digital photography and gel scan software. Plasma samples (n = 46) were used to optimize the gradient for the resolution of HDL heterogeneity and to compare profiles generated by IxDGUC with gradient gel electrophoresis (GGE); further characterization from participants (n = 548) with a range of lipid profiles was also performed. HDL subclass profiles generated by IxDGUC were comparable to those separated by GGE as indicated by a significant association between areas under the curve for both HDL₂ and HDL₃ (HDL₂, $r = 0.896$, $P < 0.01$; HDL₃, $r = 0.894$, $P < 0.01$). The method was highly reproducible, with intra- and interassay coefficient of variation percentage < 5 for percentage area under the curve HDL₂ and HDL₃, and < 1% for peak R_f and peak density. The method provides time-saving and cost-effective detection and preparation of the principal HDL subclasses.

2.182 Galectin-3 mediates oligomerization of secreted hensin using its carbohydrate-recognition domain

Vijayakumar, S., Peng, H. and Schwartz, G.J.
Am. J. Physiol. Renal Physiol., 305, F90-F99 (2013)

A multidomain, multifunctional 230-kDa extracellular matrix (ECM) protein, hensin, regulates the adaptation of rabbit kidney to metabolic acidosis by remodeling collecting duct intercalated cells. Conditional deletion of hensin in intercalated cells of the mouse kidney leads to distal renal tubular

acidosis and to a significant reduction in the number of cells expressing the basolateral chloride-bicarbonate exchanger kAE1, a characteristic marker of α -intercalated cells. Although hensin is secreted as a monomer, its polymerization and ECM assembly are essential for its role in the adaptation of the kidney to metabolic acidosis. Galectin-3, a unique lectin with specific affinity for β -galactoside glycoconjugates, directly interacts with hensin. Acidotic rabbits had a significant increase in the number of cells expressing galectin-3 in the collecting duct and exhibited colocalization of galectin-3 with hensin in the ECM of microdissected tubules. In this study, we confirmed the increased expression of galectin-3 in acidotic rabbit kidneys by real-time RT-PCR. Galectin-3 interacted with hensin in vitro via its carbohydrate-binding COOH-terminal domain, and the interaction was competitively inhibited by lactose, removal of the COOH-terminal domain of galectin-3, and deglycosylation of hensin. Galectin-9, a lectin with two carbohydrate-recognition domains, is also present in the rabbit kidney; galectin-9 partially oligomerized hensin in vitro. Our results demonstrate that galectin-3 plays a critical role in hensin ECM assembly by oligomerizing secreted monomeric hensin. Both the NH₂-terminal and COOH-terminal domains are required for this function. We suggest that in the case of galectin-3-null mice galectin-9 may partially substitute for the function of galectin-3.

2.183 Serum Proprotein Convertase Subtilisin/Kexin Type 9 and Cell Surface Low-Density Lipoprotein Receptor: Evidence for a Reciprocal Regulation

Tavori, H., Fan, D., Blakemore, J.L., Yancey, P.G., Ding, L., MacRae, F.-L. and Fazio, S.
Circulation, **127**, 2403-2413 (2013)

Background—Proprotein convertase subtilisin/kexin type 9 (PCSK9) modulates low-density lipoprotein (LDL) receptor (LDLR) degradation, thus influencing serum cholesterol levels. However, dysfunctional LDLR causes hypercholesterolemia without affecting PCSK9 clearance from the circulation.

Methods and Results—To study the reciprocal effects of PCSK9 and LDLR and the resultant effects on serum cholesterol, we produced transgenic mice expressing human (h) PCSK9. Although hPCSK9 was expressed mainly in the kidney, LDLR degradation was more evident in the liver. Adrenal LDLR levels were not affected, likely because of the impaired PCSK9 retention in this tissue. In addition, hPCSK9 expression increased hepatic secretion of apolipoprotein B-containing lipoproteins in an LDLR-independent fashion. Expression of hPCSK9 raised serum murine PCSK9 levels by 4.3-fold in wild-type mice and not at all in LDLR^{-/-} mice, in which murine PCSK9 levels were already 10-fold higher than in wild-type mice. In addition, LDLR^{+/-} mice had a 2.7-fold elevation in murine PCSK9 levels and no elevation in cholesterol levels. Conversely, acute expression of human LDLR in transgenic mice caused a 70% decrease in serum murine PCSK9 levels. Turnover studies using physiological levels of hPCSK9 showed rapid clearance in wild-type mice (half-life, 5.2 minutes), faster clearance in human LDLR transgenics (2.9 minutes), and much slower clearance in LDLR^{-/-} recipients (50.5 minutes). Supportive results were obtained with an in vitro system. Finally, up to 30% of serum hPCSK9 was associated with LDL regardless of LDLR expression.

Conclusions—Our results support a scenario in which LDLR represents the main route of elimination of PCSK9 and a reciprocal regulation between these 2 proteins controls serum PCSK9 levels, hepatic LDLR expression, and serum LDL levels.

2.184 Very low-density lipoprotein/lipo-viro particles reverse lipoprotein lipase-mediated inhibition of hepatitis C virus infection via apolipoprotein C-III

Sun, H-Y., Lin, C-C., Lee, J-C., Wang, S-W., Cheng, P-N., Wu, I-C., Chang, T-T., Lai, M-D., Shieh, D.B. and Young, K-C.
Gut, **62**(8), 1193-1203 (2013)

Objective Circulating hepatitis C virus (HCV) virions are associated with triglyceride-rich lipoproteins, including very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), designated as lipo-viro-particles (LVPs). Previous studies showed that lipoprotein lipase (LPL), a key enzyme for hydrolysing the triglyceride in VLDL to finally become LDL, may suppress HCV infection. This investigation considers the regulation of LPL by lipoproteins and LVPs, and their roles in the LPL-mediated anti-HCV function.

Design The lipoproteins were fractionated from normolipidemic blood samples using iodixanol gradients. Subsequent immunoglobulin-affinity purification from the canonical VLDL and LDL yielded the corresponding VLDL-LVP and LDL-LVP. Apolipoprotein (apo) Cs, LPL activity and HCV infection were quantified.

Results A higher triglyceride/cholesterol ratio of LDL was found more in HCV-infected donors than in healthy volunteers, and the triglyceride/cholesterol ratio of LDL-LVP was much increased, suggesting that

the LPL hydrolysis of triglyceride may be impaired. VLDL, VLDL-LVP, LDL-LVP, but not LDL, suppressed LPL lipolytic activity, which was restored by antibodies that recognised apoC-III/-IV and correlated with the steadily abundant apoC-III/-IV quantities in those particles. In a cell-based system, treatment with VLDL and LVPs reversed the LPL-mediated inhibition of HCV infection in apoC-III/-IV-dependent manners. A multivariate logistic regression revealed that plasma HCV viral loads correlated negatively with LPL lipolytic activity, but positively with the apoC-III content of VLDL. Additionally, apoC-III in VLDL was associated with a higher proportion of HCV-RNA than was IgG.

Conclusion This study reveals that LPL is an anti-HCV factor, and that apoC-III in VLDL and LVPs reduces the LPL-mediated inhibition of HCV infection.

2.185 **Quaternary Structure of Pathological Prion Protein as a Determining Factor of Strain-Specific Prion Replication Dynamics**

Laferriere, F., Tixador, P., Moudjou, M., Chapuis, J., Sibille, P., Herzog, L., Reine, F., Jaumain, E., Laude, H., Rezaei, H. and Beringue, V.

PloS Pathogens, **9(10)**, e1003702 (2013)

Prions are proteinaceous infectious agents responsible for fatal neurodegenerative diseases in animals and humans. They are essentially composed of PrP^{Sc}, an aggregated, misfolded conformer of the ubiquitously expressed host-encoded prion protein (PrP^C). Stable variations in PrP^{Sc} conformation are assumed to encode the phenotypically tangible prion strains diversity. However the direct contribution of PrP^{Sc} quaternary structure to the strain biological information remains mostly unknown. Applying a sedimentation velocity fractionation technique to a panel of ovine prion strains, classified as *fast* and *slow* according to their incubation time in ovine PrP transgenic mice, has previously led to the observation that the relationship between prion infectivity and PrP^{Sc} quaternary structure was not univocal. For the *fast* strains specifically, infectivity sedimented slowly and segregated from the bulk of proteinase-K resistant PrP^{Sc}. To carefully separate the respective contributions of size and density to this hydrodynamic behavior, we performed sedimentation at the equilibrium and varied the solubilization conditions. The density profile of prion infectivity and proteinase-K resistant PrP^{Sc} tended to overlap whatever the strain, *fast* or *slow*, leaving only size as the main responsible factor for the specific velocity properties of the *fast* strain most infectious component. We further show that this velocity-isolable population of discrete assemblies perfectly resists limited proteolysis and that its templating activity, as assessed by protein misfolding cyclic amplification outcompetes by several orders of magnitude that of the bulk of larger size PrP^{Sc} aggregates. Together, the tight correlation between small size, conversion efficiency and duration of disease establishes PrP^{Sc} quaternary structure as a determining factor of prion replication dynamics. For certain strains, a subset of PrP assemblies appears to be the best template for prion replication. This has important implications for fundamental studies on prions.

2.186 **Mitochondrial Ribosomal RNA (rRNA) Methyltransferase Family Members Are Positioned to Modify Nascent rRNA in Foci near the Mitochondrial DNA Nucleoid**

Lee, K-W., Okot-Kotber, C., LaComb, J.F. and Bogenhagen, D.F.

J. Biol. Chem., **288(43)**, 31386-31399 (2013)

We have identified RNMTL1, MRM1, and MRM2 (FtsJ2) as members of the RNA methyltransferase family that may be responsible for the three known 2'-O-ribose modifications of the 16 S rRNA core of the large mitochondrial ribosome subunit. These proteins are confined to foci located in the vicinity of mtDNA nucleoids. They show distinct patterns of association with mtDNA nucleoids and/or mitochondrial ribosomes in cell fractionation studies. We focused on the role of the least studied protein in this set, RNMTL1, to show that this protein interacts with the large ribosomal subunit as well as with a series of non-ribosomal proteins that may be involved in coupling of the rate of rRNA transcription and ribosome assembly in mitochondria. siRNA-directed silencing of RNMTL1 resulted in a significant inhibition of translation on mitochondrial ribosomes. Our results are consistent with a role for RNMTL1 in methylation of G¹³⁷⁰ of human 16 S rRNA.

2.187 **Infrared Microspectroscopy Detects Protein Misfolding Cyclic Amplification (PMCA)-induced Conformational Alterations in Hamster Scrapie Progeny Seeds**

Daus, M.L., Wagenführ, K., Thomzig, A., Boerner, S., Hermann, P., Hermelink, A., Beekes, M. and Lasch, P.

J. Biol. Chem., **288(49)**, 35068-35080 (2013)

The self-replicative conformation of misfolded prion proteins (PrP) is considered a major determinant for the seeding activity, infectiousness, and strain characteristics of prions in different host species. Prion-associated seeding activity, which converts cellular prion protein (PrP^C) into Proteinase K-resistant, infectious PrP particles (PrP^{TSE}), can be monitored *in vitro* by protein misfolding cyclic amplification (PMCA). Thus, PMCA has been established as a valuable analytical tool in prion research. Currently, however, it is under discussion whether prion strain characteristics are preserved during PMCA when parent seeds are amplified in PrP^C substrate from the identical host species. Here, we report on the comparative structural analysis of parent and progeny (PMCA-derived) PrP seeds by an improved approach of sensitive infrared microspectroscopy. Infrared microspectroscopy revealed that PMCA of native hamster 263K scrapie seeds in hamster PrP^C substrate caused conformational alterations in progeny seeds that were accompanied by an altered resistance to Proteinase K, higher sedimentation velocities in gradient ultracentrifugations, and a longer incubation time in animal bioassays. When these progeny seeds were propagated in hamsters, misfolded PrP from brain extracts of these animals showed mixed spectroscopic and biochemical properties from both parental and progeny seeds. Thus, strain modifications of 263K prions induced by PMCA seem to have been partially reversed when PMCA products were reinoculated into the original host species.

2.188 **Loss of Plasma Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) After Lipoprotein Apheresis**

Tavori, H., Giunzioni, I., MacRae, F.L. and Fazio, S.
Circ. Res., **113**, 1290-1295 (2013)

Rationale: Lipoprotein apheresis (LA) reduces low-density lipoprotein (LDL) levels in patients with severe familial hypercholesterolemia (FH). We have recently reported that >30% of plasma proprotein convertase subtilisin/kexin 9 (PCSK9) is bound to LDL, thus we predicted that LA would also reduce plasma PCSK9 levels by removing LDL.

Objective: Pre- and post-apheresis plasma from 6 patients with familial hypercholesterolemia on 3 consecutive treatment cycles was used to determine changes in PCSK9 levels.

Methods and Results: LA drastically reduced plasma LDL (by 77±4%). Concomitantly, PCSK9 levels fell by 52±5%, strongly correlating with the LDL drop ($P=0.0322$; $r^2=0.26$), but not with decreases in triglyceride (49±13%) or high-density lipoprotein levels (18±2%). Levels of albumin, creatinine, and CK-MB did not show significant changes after LA. Similar to LDL, PCSK9 levels returned to pretreatment values between cycles (2-week intervals). Fractionation of pre- and post-apheresis plasma showed that 81±11% of LDL-bound PCSK9 and 48±14% of apolipoprotein B-free PCSK9 were removed. Separation of whole plasma, purified LDL, or the apolipoprotein B-free fraction through a scaled-down, experimental dextran sulfate cellulose beads column produced similar results.

Conclusions: Our results show, for the first time, that modulation of LDL levels by LA directly affects plasma PCSK9 levels, and suggest that PCSK9 reduction is an additional benefit of LA. Because the loss of PCSK9 could contribute to the LDL-lowering effect of LA, then (1) anti-PCSK9 therapies may reduce frequency of LA in patients currently approved for therapy, and (2) LA and anti-PCSK9 therapies may be used synergistically to reduce treatment burden.

2.189 **PrimPol, an Archaic Primase/Polymerase Operating in Human Cells**

Garcia-Gomez, S., Reyes, A., Martinez-Jimenez, M.I., Chocron, E.S., Mouron, S., Terrados, G., Powell, C., Salido, E., Mendez, J., Holt, I.J. and Blanco, L.
Molecular Cell, **52**, 541-553 (2013)

We describe a second primase in human cells, PrimPol, which has the ability to start DNA chains with deoxynucleotides unlike regular primases, which use exclusively ribonucleotides. Moreover, PrimPol is also a DNA polymerase tailored to bypass the most common oxidative lesions in DNA, such as abasic sites and 8-oxoguanine. Subcellular fractionation and immunodetection studies indicated that PrimPol is present in both nuclear and mitochondrial DNA compartments. PrimPol activity is detectable in mitochondrial lysates from human and mouse cells but is absent from mitochondria derived from PRIMPOL knockout mice. PRIMPOL gene silencing or ablation in human and mouse cells impaired mitochondrial DNA replication. On the basis of the synergy observed with replicative DNA polymerases Pol γ and Pol ϵ , PrimPol is proposed to facilitate replication fork progression by acting as a translesion DNA polymerase or as a specific DNA primase reinitiating downstream of lesions that block synthesis during both mitochondrial and nuclear DNA replication.

2.190 Interactome of Two Diverse RNA Granules Links mRNA Localization to Translational Repression in Neurons

Fritzsche, R. et al

Cell Reports, 5, 1749-1762 (2013)

Transport of RNAs to dendrites occurs in neuronal RNA granules, which allows local synthesis of specific proteins at active synapses on demand, thereby contributing to learning and memory. To gain insight into the machinery controlling dendritic mRNA localization and translation, we established a stringent protocol to biochemically purify RNA granules from rat brain. Here, we identified a specific set of interactors for two RNA-binding proteins that are known components of neuronal RNA granules, Barentsz and Staufen2. First, neuronal RNA granules are much more heterogeneous than previously anticipated, sharing only a third of the identified proteins. Second, dendritically localized mRNAs, e.g., *Arc* and *CaMKII α* , associate selectively with distinct RNA granules. Third, our work identifies a series of factors with known roles in RNA localization, translational control, and RNA quality control that are likely to keep localized transcripts in a translationally repressed state, often in distinct types of RNPs.

2.191 Hepatic trans-Golgi action coordinated by the GTPase ARFRP1 is crucial for lipoprotein lipidation and assembly

Hesse, D., Radloff, K., Jaschke, A., Lagerpusch, M., Chung, B., Tailleux, A., Staels, B. and Schürmann, A. *J. Lipid Res.*, 55, 41-52 (2014)

The liver is a major organ in whole body lipid metabolism and malfunctioning can lead to various diseases including dyslipidemia, fatty liver disease, and type 2 diabetes. Triglycerides and cholesteryl esters are packed in the liver as very low density lipoproteins (VLDLs). Generation of these lipoproteins is initiated in the endoplasmic reticulum and further maturation likely occurs in the Golgi. ADP-ribosylation factor-related protein 1 (ARFRP1) is a small *trans*-Golgi-associated guanosine triphosphatase (GTPase) that regulates protein sorting and is required for chylomicron lipidation and assembly in the intestine. Here we show that the hepatocyte-specific deletion of *Arfrp1* (*Arfrp1^{liv-/-}*) results in impaired VLDL lipidation leading to reduced plasma triglyceride levels in the fasted state as well as after inhibition of lipoprotein lipase activity by Triton WR-1339. In addition, the concentration of ApoC3 that comprises 40% of protein mass of secreted VLDLs is markedly reduced in the plasma of *Arfrp1^{liv-/-}* mice but accumulates in the liver accompanied by elevated triglycerides. Fractionation of *Arfrp1^{liv-/-}* liver homogenates reveals more ApoB48 and a lower concentration of triglycerides in the Golgi compartments than in the corresponding fractions from control livers. In conclusion, ARFRP1 and the Golgi apparatus play an important role in lipoprotein maturation in the liver by influencing lipidation and assembly of proteins to the lipid particles.

2.192 The 51 kDa FADS3 is Secreted in the ECM of Hepatocytes and Blood in Rat

Blanchard, H., Boulier-Monthean, N., Legrand, P. and Pedrono, F.

J. Cell. Biochem., 115(1), 199-207 (2014)

The fatty acid desaturase (*Fads*) cluster is composed of three genes encoding for the Δ 5- and Δ 6-desaturases and FADS3. The two former proteins are involved in the fatty acid biosynthesis; the latter one shares a high sequence identity but has still no attributed function. In a previous work performed in rat, we described three isoforms of FADS3 expressed in a tissue-dependent manner. In the present study, we demonstrated a specific subcellular targeting depending on the isoform. In cultured hepatocytes, which mainly expressed the 51 kDa protein, FADS3 was unexpectedly present in the cytosolic fraction, but was also secreted in the extracellular matrix on fibronectin-containing fibers. The secretion pathway was investigated and we determined the presence of exosome-like vesicles on the FADS3-stained fibers. In parallel, FADS3 was detected in blood of hepatic vessel, and particularly in serum. In conclusion, this study demonstrated a very specific intra- and extracellular location of FADS3 in comparison with the Δ 5- and Δ 6-desaturases, suggesting a unique function for this putative desaturase, even if no activity has been yet identified neither in the extracellular matrix of hepatocytes nor in serum.

2.193 Pathogenic Mutations within the Hydrophobic Domain of the Prion Protein Lead to the Formation of Protease-Sensitive Prion Species with Increased Lethality

Coleman, B.M., Harrison, C.F., Guo, B., Masters, C.L., Barnham, K.J., Lawson, V.A. and Hill, A.F.

J. Virol., 88(5), 2690-2703 (2014)

Prion diseases are a group of fatal and incurable neurodegenerative diseases affecting both humans and animals. The principal mechanism of these diseases involves the misfolding the host-encoded cellular

prion protein, PrP^C, into the disease-associated isoform, PrP^{Sc}. Familial forms of human prion disease include those associated with the mutations G114V and A117V, which lie in the hydrophobic domain of PrP. Here we have studied the murine homologues (G113V and A116V) of these mutations using cell-based and animal models of prion infection. Under normal circumstances, the mutant forms of PrP^C share similar processing, cellular localization, and physicochemical properties with wild-type mouse PrP (MoPrP). However, upon exposure of susceptible cell lines expressing these mutants to infectious prions, very low levels of protease-resistant aggregated PrP^{Sc} are formed. Subsequent mouse bioassay revealed high levels of infectivity present in these cells. Thus, these mutations appear to limit the formation of aggregated PrP^{Sc}, giving rise to the accumulation of a relatively soluble, protease sensitive, prion species that is highly neurotoxic. Given that these mutations lie next to the glycine-rich region of PrP that can abrogate prion infection, these findings provide further support for small, protease-sensitive prion species having a significant role in the progression of prion disease and that the hydrophobic domain is an important determinant of PrP conversion.

2.194 Immobilization of Homogeneous Monomeric, Oligomeric and Fibrillar A β Species for Reliable SPR Measurements

Frenzel, D., Glück, J.M., Brener, O., Oesterhelt, F., Nagel-Steger, L. and Willbold, D.
PLoS One, **9**(3), e89490 (2014)

There is strong evidence that the amyloid-beta peptide (A β) plays a central role in the pathogenesis of Alzheimer's disease (AD). In this context, a detailed quantitative description of the interactions with different A β species is essential for characterization of physiological and artificial ligands. However, the high aggregation propensity of A β in concert with its susceptibility to structural changes due to even slight changes in solution conditions has impeded surface plasmon resonance (SPR) studies with homogeneous A β conformer species. Here, we have adapted the experimental procedures to state-of-the-art techniques and established novel approaches to reliably overcome the aforementioned challenges. We show that the application of density gradient centrifugation (DGC) for sample purification and the use of a single chain variable fragment (scFv) of a monoclonal antibody directed against the amino-terminus of A β allows reliable SPR measurements and quality control of the immobilized A β aggregate species at any step throughout the experiment.

2.195 Replication factors transiently associate with mtDNA at the mitochondrial inner membrane to facilitate replication

Rajala, N., Gerhold, J.M., Martinsson, P., Klymov, A. and Spelbrink, J.N.
Nucleic Acids Res., **42**(2), 952-967 (2014)

Mitochondrial DNA (mtDNA) is organized in discrete protein-DNA complexes, nucleoids, that are usually considered to be mitochondrial-inner-membrane associated. Here we addressed the association of replication factors with nucleoids and show that endogenous mtDNA helicase Twinkle and single-stranded DNA-binding protein, mtSSB, co-localize only with a subset of nucleoids. Using nucleotide analogs to identify replicating mtDNA in situ, the fraction of label-positive nucleoids that is Twinkle/mtSSB positive, is highest with the shortest labeling-pulse. In addition, the recruitment of mtSSB is shown to be Twinkle dependent. These proteins thus transiently associate with mtDNA in an ordered manner to facilitate replication. To understand the nature of mtDNA replication complexes, we examined nucleoid protein membrane association and show that endogenous Twinkle is firmly membrane associated even in the absence of mtDNA, whereas mtSSB and other nucleoid-associated proteins are found in both membrane-bound and soluble fractions. Likewise, a substantial amount of mtDNA is found as soluble or loosely membrane bound. We show that, by manipulation of Twinkle levels, mtDNA membrane association is partially dependent on Twinkle. Our results thus show that Twinkle recruits or is assembled with mtDNA at the inner membrane to form a replication platform and amount to the first clear demonstration that nucleoids are dynamic both in composition and concurrent activity.

2.196 Initial Steps in RNA Processing and Ribosome Assembly Occur at Mitochondrial DNA Nucleoids

Borgenhagen, D.F., Martin, D.W. and Koller, A.
Cell Metabolism, **19**, 618-629 (2014)

Mammalian mitochondrial DNA (mtDNA) resides in compact nucleoids, where it is replicated and transcribed into long primary transcripts processed to generate rRNAs, tRNAs, and mRNAs encoding 13 proteins. This situation differs from bacteria and eukaryotic nucleoli, which have dedicated rRNA transcription units. The assembly of rRNAs into mitoribosomes has received little study. We show that

mitochondrial RNA processing enzymes involved in tRNA excision, ribonuclease P (RNase P) and ELAC2, as well as a subset of nascent mitochondrial ribosomal proteins (MRPs) associate with nucleoids to initiate RNA processing and ribosome assembly. SILAC pulse-chase labeling experiments show that nascent MRPs recruited to the nucleoid fraction were highly labeled after the pulse in a transcription-dependent manner and decreased in labeling intensity during the chase. These results provide insight into the landscape of binding events required for mitochondrial ribosome assembly and firmly establish the mtDNA nucleoid as a control center for mitochondrial biogenesis.

2.197 Regulated Oligomerization Induces Uptake of a Membrane Protein into COPII Vesicles Independent of Its Cytosolic Tail

Springer, S., Malkus, P., Borchert, B., Wellbrock, U., Duden, R. and Schekman, R.
Traffic, **15**, 531-545 (2014)

Export of transmembrane proteins from the endoplasmic reticulum (ER) is driven by directed incorporation into coat protein complex II (COPII)-coated vesicles. The sorting of some cargo proteins into COPII vesicles was shown to be mediated by specific interactions between transmembrane and COPII-coat-forming proteins. But even though some signals for ER exit have been identified on the cytosolic domains of membrane proteins, the general signaling and sorting mechanisms of ER export are still poorly understood. To investigate the role of cargo protein oligomer formation in the export process, we have created a transmembrane fusion protein that – owing to its FK506-binding protein domains – can be oligomerized in isolated membranes by addition of a small-molecule dimerizer. Packaging of the fusion protein into COPII vesicles is strongly enhanced in the presence of the dimerizer, demonstrating that the oligomeric state is an ER export signal for this membrane protein. Surprisingly, the cytosolic tail is not required for this oligomerization-dependent effect on protein sorting. Thus, an alternative mechanism, such as membrane bending, must account for ER export of the fusion protein.

2.198 The metabolic inter-relationships between changes in waist circumference, triglycerides, insulin sensitivity and small, dense low-density lipoprotein particles with acute weight loss in clinically obese children and adolescents

Hobkirk, J.P., King, R.E., Davies, I., Harman, N., Gately, P., Pemberton, P., Smith, A., Barth, J.H. and Carroll, S.
Pediatric Obesity, **9**(3), 209-217 (2014)

Objective

Small, dense low-density lipoprotein (LDL) particles are highly atherogenic and strongly associated with obesity-related dyslipidemia. The metabolic inter-relationships between weight loss induced changes in waist circumference, triglycerides, insulin sensitivity and small-dense LDL particles in clinically obese children and adolescents have not been studied.

Methods

Seventy-five clinically obese boys and girls (standardized body mass index 3.07 ± 0.59 , aged 8–18 years) were recruited. Anthropometric, body composition and cardiometabolic risk factors were measured pre- and post-weight loss.

Results

There were highly significant reductions in anthropometric, body composition and cardiometabolic risk factors. Triglyceride change was positively correlated with LDL peak particle density and percentage LDL pattern B changes (relative abundance of small, dense LDL particles). Multiple regression analyses showed that changes in triglyceride concentration accounted for between 24 and 18% of the variance in LDL peak particle density and percentage LDL pattern B change, respectively. Changes in waist circumference and insulin sensitivity did not predict these changes in LDL characteristics.

Conclusion

Acute and highly significant weight loss significantly decreased LDL peak particle density and percentage LDL pattern B. The change in triglycerides was a strong predictor of LDL peak particle density and percentage LDL pattern B change.

2.199 Efficient replication of a paramyxovirus independent of full zippering of the fusion protein six-helix bundle domain

Brindley, M.A., Plattet, P. and Plemper, R.K.
PNAS, **111**(36), E3795-E3804 (2014)

Enveloped viruses such as HIV and members of the paramyxovirus family use metastable, proteinaceous fusion machineries to merge the viral envelope with cellular membranes for infection. A hallmark of the fusogenic glycoproteins of these pathogens is refolding into a thermodynamically highly stable fusion core structure composed of six antiparallel α -helices, and this structure is considered instrumental for pore opening and/or enlargement. Using a paramyxovirus fusion (F) protein, we tested this paradigm by engineering covalently restricted F proteins that are predicted to be unable to close the six-helix bundle core structure fully. Several candidate bonds formed efficiently, resulting in F trimers and higher-order complexes containing covalently linked dimers. The engineered F complexes were incorporated into recombinant virions efficiently and were capable of refolding into a postfusion conformation without temporary or permanent disruption of the disulfide bonds. They efficiently formed fusion pores based on virus replication and quantitative cell-to-cell and virus-to-cell fusion assays. Complementation of these F mutants with a monomeric, fusion-inactive F variant enriched the F oligomers for heterotrimers containing a single disulfide bond, without affecting fusion complementation profiles compared with standard F protein. Our demonstration that complete closure of the fusion core does not drive paramyxovirus entry may aid the design of strategies for inhibiting virus entry.

2.200 **Virus-Inspired Membrane Encapsulation of DNA Nanostructures To Achieve In Vivo Stability**

Perrault, S.D. and Shih, W.M.

ACSNano, 8(5), 5132-5140 (2014)

DNA nanotechnology enables engineering of molecular-scale devices with exquisite control over geometry and site-specific functionalization. This capability promises compelling advantages in advancing nanomedicine; nevertheless, instability in biological environments and innate immune activation remain as obstacles for *in vivo* application. Natural particle systems (*i.e.*, viruses) have evolved mechanisms to maintain structural integrity and avoid immune recognition during infection, including encapsulation of their genome and protein capsid shell in a lipid envelope. Here we introduce virus-inspired enveloped DNA nanostructures as a design strategy for biomedical applications. Achieving a high yield of tightly wrapped unilamellar nanostructures, mimicking the morphology of enveloped virus particles, required precise control over the density of attached lipid conjugates and was achieved at 1 per $\sim 180 \text{ nm}^2$. Envelopment of DNA nanostructures in PEGylated lipid bilayers conferred protection against nuclease digestion. Immune activation was decreased 2 orders of magnitude below controls, and pharmacokinetic bioavailability improved by a factor of 17. By establishing a design strategy suitable for biomedical applications, we have provided a platform for the engineering of sophisticated, translation-ready DNA nanodevices.

2.201 **Abstract 433: Examination of Factors Affecting the Association of PCSK9 With Low-Density Lipoprotein Particles in Human Plasma**

Golder, M., Sarkar, S., Kosenko, T., McPherson, R. and Lagace, T.A.

Arterioscler. Thromb. Vasc. Biol., 34:A433 (2014)

Rationale: We have previously shown that a substantial proportion of plasma PCSK9 (30-40%) is associated with LDL particles in normolipidemic subjects. Cellular assays show that LDL-bound PCSK9 is less active for binding to cell surface LDLRs. Therefore, the ability of circulating PCSK9 to direct LDLR degradation in liver could be regulated by plasma LDL levels. In addition, LDL subspecies may have altered abilities in binding PCSK9. We have mapped the LDL binding region to a short stretch of amino acids (aa 31-52) in the PCSK9 prodomain. It is unknown whether a common loss-of-function PCSK9 mutation (R46L) within this region affects LDL binding.

Objective: To determine whether plasma PCSK9 distribution (LDL-bound versus unbound) is affected in hypercholesterolemic subjects. To further characterize the interaction of PCSK9 and LDL, we investigated the interaction of PCSK9 with two subspecies of LDL - large, buoyant LDL (LBLDL; $d=1.019-1.044 \text{ g/ml}$) and small, dense LDL (SDLDL; $d=1.044-1.063 \text{ g/ml}$). Additionally, we investigated the effect of the R46L PCSK9 mutation on the LDL binding affinity of PCSK9.

Methods and Results: We used flotation ultracentrifugation in Optiprep density gradients to fractionate human plasma samples followed by immunoprecipitation and western blot to quantify PCSK9 distribution in LDL and non-LDL fractions. In a pilot study, the proportion of total plasma PCSK9 in the LDL fraction was increased from $38 \pm 5\%$ to $57 \pm 3\%$ ($N=6$) in hypercholesterolemic subjects ($\text{LDL} > 4.9 \text{ mM}$, $\text{TG} < 2.3 \text{ mM}$) versus normal controls ($\text{LDL} < 3 \text{ mM}$, $\text{TG} < 2.3 \text{ mM}$). Saturation binding assays showed that SDLDL bound PCSK9 with lower affinity ($K_d = 361.9 \text{ nM}$) than LBLDL ($K_d = 263.9 \text{ nM}$). Competition binding assays determined that recombinant purified PCSK9-R46L secreted from HEK293 cells did not bind to isolated

LDL with significantly altered affinity compared to wild-type PCSK9.

Conclusion: Our preliminary results indicate that plasma PCSK9 distribution is altered in hypercholesterolemia, with an increased proportion of total PCSK9 bound to LDL particles. Our in vitro results suggest that circulating small, dense LDL may bind more poorly to PCSK9 than larger LDL subspecies.

2.202 Absence of an effect of vitamin E on protein and lipid radical formation during lipoperoxidation of LDL by lipoxygenase

Ganini, D. and Mason, R.P.

Free Radical Biology and Medicine, **76**, 61-68 (2014)

Low-density lipoprotein (LDL) oxidation is the primary event in atherosclerosis, and LDL lipoperoxidation leads to modifications in apolipoprotein B-100 (apo B-100) and lipids. Intermediate species of lipoperoxidation are known to be able to generate amino acid-centered radicals. Thus, we hypothesized that lipoperoxidation intermediates induce protein-derived free radical formation during LDL oxidation. Using DMPO and immuno-spin trapping, we detected the formation of protein free radicals on LDL incubated with Cu^{2+} or the soybean lipoxygenase (LPOx)/phospholipase A_2 (PLA_2). With low concentrations of DMPO (1 mM), Cu^{2+} dose-dependently induced oxidation of LDL and easily detected apo B-100 radicals. Protein radical formation in LDL incubated with Cu^{2+} showed maximum yields after 30 min. In contrast, the yields of apo B-100 radicals formed by LPOx/ PLA_2 followed a typical enzyme-catalyzed kinetics that was unaffected by DMPO concentrations of up to 50 mM. Furthermore, when we analyzed the effect of antioxidants on protein radical formation during LDL oxidation, we found that ascorbate, urate, and Trolox dose-dependently reduced apo B-100 free radical formation in LDL exposed to Cu^{2+} . In contrast, Trolox was the only antioxidant that even partially protected LDL from LPOx/ PLA_2 . We also examined the kinetics of lipid radical formation and protein radical formation induced by Cu^{2+} or LPOx/ PLA_2 for LDL supplemented with α -tocopherol. In contrast to the potent antioxidant effect of α -tocopherol on the delay of LDL oxidation induced by Cu^{2+} , when we used the oxidizing system LPOx/ PLA_2 , no significant protection was detected. The lack of protection of α -tocopherol on the apo B-100 and lipid free radical formation by LPOx may explain the failure of vitamin E as a cardiovascular protective agent for humans.

2.203 MPV17L2 is required for ribosome assembly in mitochondria

Rosa, I.D., Durigon, R., Pearce, S.F., Rorbach, J., Hirst, E.M.A., Vidoni, S., Reyes, A., Brea-Calvo, G., Minczuk, M., Woellhaf, M.W., Herrmann, J.M., Huynen, M.A., Holt, I.J. and Spinazzola, A.
Nucleic Acids Res., **42(13)**, 8500-8515 (2014)

MPV17 is a mitochondrial protein of unknown function, and mutations in MPV17 are associated with mitochondrial deoxyribonucleic acid (DNA) maintenance disorders. Here we investigated its most similar relative, MPV17L2, which is also annotated as a mitochondrial protein. Mitochondrial fractionation analyses demonstrate MPV17L2 is an integral inner membrane protein, like MPV17. However, unlike MPV17, MPV17L2 is dependent on mitochondrial DNA, as it is absent from $\rho 0$ cells, and co-sediments on sucrose gradients with the large subunit of the mitochondrial ribosome and the monosome. Gene silencing of MPV17L2 results in marked decreases in the monosome and both subunits of the mitochondrial ribosome, leading to impaired protein synthesis in the mitochondria. Depletion of MPV17L2 also induces mitochondrial DNA aggregation. The DNA and ribosome phenotypes are linked, as in the absence of MPV17L2 proteins of the small subunit of the mitochondrial ribosome are trapped in the enlarged nucleoids, in contrast to a component of the large subunit. These findings suggest MPV17L2 contributes to the biogenesis of the mitochondrial ribosome, uniting the two subunits to create the translationally competent monosome, and provide evidence that assembly of the small subunit of the mitochondrial ribosome occurs at the nucleoid.

2.204 Transcriptomic characterization of short duration endoplasmic reticulum stress on cultured human proximal tubule cells

Zhang, Y., barati, M., Munoz, I., Li, M., Wilkey, D., Rouchka, E. and Merchant, M.
BMC Bioinformatics, **15 (Suppl 10)** P5 (2014)

Stress granules (SG) are formed as collections of protein and RNA (ribonucleoprotein structures) and continuously assembled/disassembled in response to stresses such as heat, osmotic, or oxidant stress; representing an attempt to survive the stress through salvage of important proteins and RNA. Recent

research suggests diabetic nephropathy (DN) may change or alter SG biology and in conditions that model DN may involve the receptor for activated C-kinases (RACK1). The incorporation of RACK1 into stress granules may down-regulate programmed cell death and further may impart the ability to scaffold to and sequester key signaling proteins to affect cell survival or death. We hypothesized that the inappropriate or dysregulated scaffolding of proteins or RNA into stress granules may be of importance in the development of diabetic nephropathy. The goal of this study is to qualitatively and semi-quantitatively characterize the effects of cell culture conditions modeling diabetes and ER stress in conjunction with over-expression studies of SG stabilizing proteins on RNA transcripts.

- 2.205 A C-terminal Membrane Anchor Affects the Interactions of Prion Proteins with Lipid Membranes**
Chu, N.K., Shabbir, W., Bove-Fwenderson, E., Araman, C., Lemmens-Gruber, R., Harris, D.A. and Becker, C.F.W.
J. Biol. Chem., **289**, 30144-30160 (2014)

Membrane attachment via a C-terminal glycosylphosphatidylinositol anchor is critical for conversion of PrP^C into pathogenic PrP^{Sc}. Therefore the effects of the anchor on PrP structure and function need to be deciphered. Three PrP variants, including full-length PrP (residues 23–231, FL_PrP), N-terminally truncated PrP (residues 90–231, T_PrP), and PrP missing its central hydrophobic region (Δ 105–125, Δ CR_PrP), were equipped with a C-terminal membrane anchor via a semisynthesis strategy. Analyses of the interactions of lipidated PrPs with phospholipid membranes demonstrated that C-terminal membrane attachment induces a different binding mode of PrP to membranes, distinct from that of non-lipidated PrPs, and influences the biochemical and conformational properties of PrPs. Additionally, fluorescence-based assays indicated pore formation by lipidated Δ CR_PrP, a variant that is known to be highly neurotoxic in transgenic mice. This finding was supported by using patch clamp electrophysiological measurements of cultured cells. These results provide new evidence for the role of the membrane anchor in PrP-lipid interactions, highlighting the importance of the N-terminal and the central hydrophobic domain in these interactions.

- 2.206 RuvB-like ATPases Function in Chromatin Decondensation at the End of Mitosis**
Magalska, A., Schellhaus, A.K., Moreno-Andres, D., Zanini, F., Schooley, A., Sachdev, R., Schwarz, H., Madlung, J. and Antonin, W.
Developmental Cell, **31**(3), 305-318 (2014)

Chromatin undergoes extensive structural changes during the cell cycle. Upon mitotic entry, metazoan chromatin undergoes tremendous condensation, creating mitotic chromosomes with 50-fold greater compaction relative to interphase chromosomes. At the end of mitosis, chromosomes reestablish functional interphase chromatin competent for replication and transcription through a decondensation process that is cytologically well described. However, the underlying molecular events and factors remain unidentified. We describe a cell-free system that recapitulates chromatin decondensation based on purified mitotic chromatin and *Xenopus* egg extracts. Using biochemical fractionation, we identify RuvB-like ATPases as chromatin decondensation factors and demonstrate that their ATPase activity is essential for decondensation. Our results show that decompaction of metaphase chromosomes is not merely an inactivation of known chromatin condensation factors but rather an active process requiring specific molecular machinery. Our cell-free system provides an important tool for further molecular characterization of chromatin decondensation and its coordination with concomitant processes.

- 2.207 Amphipathic α -Helices in Apolipoproteins Are Crucial to the Formation of Infectious Hepatitis C Virus Particles**
Fukuhara, T., Wada, M., Nakamura, S., Ono, C., Shiokawa, M., Yamamoto, S., Motomura, T., Okamoto, T., Okuzaki, D., Yamamoto, M., Saito, I., Wakita, T., Koike, K. and Matsuura, Y.
PLoS Pathogens, **10**(12), e1004534 (2014)

Apolipoprotein B (ApoB) and ApoE have been shown to participate in the particle formation and the tissue tropism of hepatitis C virus (HCV), but their precise roles remain uncertain. Here we show that amphipathic α -helices in the apolipoproteins participate in the HCV particle formation by using zinc finger nucleases-mediated apolipoprotein B (ApoB) and/or ApoE gene knockout Huh7 cells. Although Huh7 cells deficient in either ApoB or ApoE gene exhibited slight reduction of particles formation, knockout of both ApoB and ApoE genes in Huh7 (DKO) cells severely impaired the formation of infectious HCV particles, suggesting that ApoB and ApoE have redundant roles in the formation of infectious HCV particles. cDNA microarray analyses revealed that ApoB and ApoE are dominantly expressed in Huh7

cells, in contrast to the high level expression of all of the exchangeable apolipoproteins, including ApoA1, ApoA2, ApoC1, ApoC2 and ApoC3 in human liver tissues. The exogenous expression of not only ApoE, but also other exchangeable apolipoproteins rescued the infectious particle formation of HCV in DKO cells. In addition, expression of these apolipoproteins facilitated the formation of infectious particles of genotype 1b and 3a chimeric viruses. Furthermore, expression of amphipathic α -helices in the exchangeable apolipoproteins facilitated the particle formation in DKO cells through an interaction with viral particles. These results suggest that amphipathic α -helices in the exchangeable apolipoproteins play crucial roles in the infectious particle formation of HCV and provide clues to the understanding of life cycle of HCV and the development of novel anti-HCV therapeutics targeting for viral assembly.

2.208 **Cytotoxicity of Human Endogenous Retrovirus K-Specific T Cells toward Autologous Ovarian Cancer Cells**

Rycaj, K., Plummer, J.B., Yin, B., Li, M., Garza, J., Radvanyi, L., Ramondetta, L.M., Lin, K., Johanning, G.L., Tang, D.G. and Wang-Johanning, F.
Clin. Cancer Res., 21(2), 471-483 (2015)

Purpose: To determine whether HERV-K envelope (ENV) protein could function as a tumor-associated antigen and elicit specific T-cell responses against autologous ovarian cancer cells.

Experimental Design: The expression of HERV-K transcripts and ENV protein, the presence of serum antibodies against HERV-K, reverse transcriptase (RT) activities, and cellular immune responses in primary ovarian cancer tissues and patient blood samples were analyzed and compared with samples from patients with benign ovarian diseases and normal female donors.

Results: Ovarian cancer cells in primary tumors and ascites expressed markers of cancer stem cells and markers of both mesenchymal and epithelial cells. Expression of HERV transcripts and HERV-K ENV protein and reverse transcriptase activities were higher in ovarian cancer compared with adjacent normal and benign tissues. The ovarian cancer patient plasma also had high reverse transcriptase activities and the ovarian cancer patient sera contained HERV-K immunoreactive antibodies. HERV-K-specific T cells generated from autologous dendritic cells pulsed with HERV-K ENV antigens exhibited phenotypes and functions consistent with a cellular immune response including T-cell proliferation, IFN γ production, and HERV-K-specific cytotoxic T lymphocyte (CTL) activity. Significantly higher CTL lysis of autologous tumor cells than of uninvolved normal cells was demonstrated in patients with ovarian cancer than patients with benign diseases and further enhanced lysis was observed if T regulatory cells were depleted.

Conclusion: Endogenous retroviral gene products in ovarian cancer may represent a potentially valuable new pool of tumor-associated antigens for targeting of therapeutic vaccines to ovarian cancer.

2.209 **Atomic Structure of T6SS Reveals Interlaced Array Essential to Function**

Clemens, D.L., Ge, P., Horwitz, M.A. and Zhou, Z.H.
Cell, 160, 940-951 (2015)

Type VI secretion systems (T6SSs) are newly identified contractile nanomachines that translocate effector proteins across bacterial membranes. The *Francisella* pathogenicity island, required for bacterial phagosome escape, intracellular replication, and virulence, was presumed to encode a T6SS-like apparatus. Here, we experimentally confirm the identity of this T6SS and, by cryo electron microscopy (cryoEM), show the structure of its post-contraction sheath at 3.7 Å resolution. We demonstrate the assembly of this T6SS by IglA/IglB and secretion of its putative effector proteins in response to environmental stimuli. The sheath has a quaternary structure with handedness opposite that of contracted sheath of T4 phage tail and is organized in an interlaced two-dimensional array by means of β sheet augmentation. By structure-based mutagenesis, we show that this interlacing is essential to secretion, phagosomal escape, and intracellular replication. Our atomic model of the T6SS will facilitate design of drugs targeting this highly prevalent secretion apparatus.

2.210 **MinD-like ATPase FlhG effects location and number of bacterial flagella during C-ring assembly**

Schuhmacher, J.S., Rossmann, F., Dempwolff, F., Knauer, C., Altegoer, f., Steinchen, W., Dörrich, A.K., Klingl, A., Stephen, M., Linne, U., Thormann, K.M. and Bange, G.
PNAS, 7(10), 3092-3097 (2015)

The number and location of flagella, bacterial organelles of locomotion, are species specific and appear in regular patterns that represent one of the earliest taxonomic criteria in microbiology. However, the mechanisms that reproducibly establish these patterns during each round of cell division are poorly understood. FlhG (previously YlxH) is a major determinant for a variety of flagellation patterns. Here, we

show that FlhG is a structural homolog of the ATPase MinD, which serves in cell-division site determination. Like MinD, FlhG forms homodimers that are dependent on ATP and lipids. It interacts with a complex of the flagellar C-ring proteins FliM and FliY (also FliN) in the Gram-positive, peritrichous-flagellated *Bacillus subtilis* and the Gram-negative, polar-flagellated *Shewanella putrefaciens*. FlhG interacts with FliM/FliY in a nucleotide-independent manner and activates FliM/FliY to assemble with the C-ring protein FliG in vitro. FlhG-driven assembly of the FliM/FliY/FliG complex is strongly enhanced by ATP and lipids. The protein shows a highly dynamic subcellular distribution between cytoplasm and flagellar basal bodies, suggesting that FlhG effects flagellar location and number during assembly of the C-ring. We describe the molecular evolution of a MinD-like ATPase into a flagellation pattern effector and suggest that the underappreciated structural diversity of the C-ring proteins might contribute to the formation of different flagellation patterns.

2.211 **Control of lipid organization and actin assembly during clathrin-mediated endocytosis by the cytoplasmic tail of the rhomboid protein Rbd2**

Cortesio, C.L., Lewellyn, E.B. and Drubin, D.G.

Mol. Biol. Cell, 26, 1509-1522 (2015)

Clathrin-mediated endocytosis (CME) is facilitated by a precisely regulated burst of actin assembly. PtdIns(4,5)P₂ is an important signaling lipid with conserved roles in CME and actin assembly regulation. Rhomboid family multipass transmembrane proteins regulate diverse cellular processes; however, rhomboid-mediated CME regulation has not been described. We report that yeast lacking the rhomboid protein Rbd2 exhibit accelerated endocytic-site dynamics and premature actin assembly during CME through a PtdIns(4,5)P₂-dependent mechanism. Combined genetic and biochemical studies showed that the cytoplasmic tail of Rbd2 binds directly to PtdIns(4,5)P₂ and is sufficient for Rbd2's role in actin regulation. Analysis of an Rbd2 mutant with diminished PtdIns(4,5)P₂-binding capacity indicates that this interaction is necessary for the temporal regulation of actin assembly during CME. The cytoplasmic tail of Rbd2 appears to modulate PtdIns(4,5)P₂ distribution on the cell cortex. The syndapin-like F-BAR protein Bzz1 functions in a pathway with Rbd2 to control the timing of type 1 myosin recruitment and actin polymerization onset during CME. This work reveals that the previously unstudied rhomboid protein Rbd2 functions in vivo at the nexus of three highly conserved processes: lipid regulation, endocytic regulation, and cytoskeletal function.

2.212 **Prevalence of plasma small dense LDL is increased in obesity in a Thai population**

Kulanuwar, S., Tungrongchitr, R., Billington, D. and Davies, I.G.

Lipids in Health and Disease, 14:30 (2015)

Background

Plasma low density lipoprotein (LDL) particles vary in size, density, electrical charge and chemical composition. An increased presence of small dense LDL (sdLDL), along with raised triglyceride concentrations and decreased high density lipoprotein (HDL) cholesterol concentrations is commonly known as the atherogenic triad and has been observed in some cases of obesity, principally in Europe and America. This study examines the prevalence of sdLDL in the plasma of an obese (BMI \geq 25 kg/m²) Thai population.

Methods

Plasma from fasted obese (n = 48) and non-obese (n = 16) Thai participants was subjected to density gradient ultracentrifugation in iodixanol to separate lipoproteins. Gradients were unloaded top-to-bottom into 20 fractions which were assayed for cholesterol, triglyceride, apo B and apo A-1 to identify lipoprotein types and subtypes.

Results

LDL cholesterol was subfractionated into LDL I + II (fractions 3–6, ρ = 1.021-1.033 g/ml) which was considered to represent large buoyant LDL (IbLDL), LDL III (fractions 7–9, ρ = 1.036-1.039 g/ml) which was considered to represent sdLDL, and, LDL IV (fractions 10–12, ρ = 1.044-1.051 g/ml) which was considered to represent very sdLDL. Concentrations of LDL III and IV were increased by 15-20% in obese participants whilst that of LDL I + II was concomitantly decreased by 10%. This was accompanied by a 50% increase in plasma triglyceride concentrations and 15% decrease in HDL cholesterol concentrations. Only 3/16 (19%) non-obese participants had a pattern B LDL cholesterol profile (peak density of $>$ 1.033 g/ml), whilst 28/48 (58%) obese participants were pattern B. When expressed as a fraction of the LDL concentration, total sdLDL (i.e. LDL III + IV) showed highly significant correlations to plasma triglyceride concentrations and the triglyceride/HDL cholesterol ratio.

Conclusions

The prevalence of sdLDL is increased in obesity in a Thai population such that they demonstrate a similar atherogenic triad to that previously observed in European and American populations.

2.213 **Cry Protein Crystals: A Novel Platform for Protein Delivery**

Nair, M.S., Lee, M.M., Bonnegarde-Bernard, A., Wallace, J.A., Dean, D.H., Ostrowski, M.C., Burry, R.W., Boyaka, P.N. and Chan, K.
PLoS One, **10**(6), e0127669 (2015)

Protein delivery platforms are important tools in the development of novel protein therapeutics and biotechnologies. We have developed a new class of protein delivery agent based on sub-micrometer-sized Cry3Aa protein crystals that naturally form within the bacterium *Bacillus thuringiensis*. We demonstrate that fusion of the *cry3Aa* gene to that of various reporter proteins allows for the facile production of Cry3Aa fusion protein crystals for use in subsequent applications. These Cry3Aa fusion protein crystals are efficiently taken up and retained by macrophages and other cell lines *in vitro*, and can be delivered to mice *in vivo* via multiple modes of administration. Oral delivery of Cry3Aa fusion protein crystals to C57BL/6 mice leads to their uptake by MHC class II cells, including macrophages in the Peyer's patches, supporting the notion that the Cry3Aa framework can be used to stabilize cargo protein against degradation for delivery to gastrointestinal lymphoid tissues.

2.214 **Effects of impaired membrane interactions on α -synuclein aggregation and neurotoxicity**

Ysselstein, Joshi, M., Mishra, V., Grigg, A.M., Asiago, J.M., McCabe, G.P., Stanciu, L.A., Post, C.B. and Rochet, J-C.
Neurobiology of Disease, **79**, 150-163 (2015)

The post-mortem brains of individuals with Parkinson's disease (PD) and other synucleinopathy disorders are characterized by the presence of aggregated forms of the presynaptic protein α -synuclein (aSyn). Understanding the molecular mechanism of aSyn aggregation is essential for the development of neuroprotective strategies to treat these diseases. In this study, we examined how interactions between aSyn and phospholipid vesicles influence the protein's aggregation and toxicity to dopaminergic neurons. Two-dimensional NMR data revealed that two familial aSyn mutants, A30P and G51D, populated an exposed, membrane-bound conformer in which the central hydrophobic region was dissociated from the bilayer to a greater extent than in the case of wild-type aSyn. A30P and G51D had a greater propensity to undergo membrane-induced aggregation and elicited greater toxicity to primary dopaminergic neurons compared to the wild-type protein. In contrast, the non-familial aSyn mutant A29E exhibited a weak propensity to aggregate in the presence of phospholipid vesicles or to elicit neurotoxicity, despite adopting a relatively exposed membrane-bound conformation. Our findings suggest that the aggregation of exposed, membrane-bound aSyn conformers plays a key role in the protein's neurotoxicity in PD and other synucleinopathy disorders.

2.215 **A novel and rapid method for obtaining high titre intact prion strains from mammalian brain**

Wenborn, A., Terry, C., Gros, N., Joiner, S., D'Castro, L., Panico, S., Sells, J., Cronier, S., Linehan, J.M., Brandner, S., Saibil, H.R., Collinge, J. and Wadsworth, J.D.F.
Scientific Reports, **5**:10062 (2015)

Mammalian prions exist as multiple strains which produce characteristic and highly reproducible phenotypes in defined hosts. How this strain diversity is encoded by a protein-only agent remains one of the most interesting and challenging questions in biology with wide relevance to understanding other diseases involving the aggregation or polymerisation of misfolded host proteins. Progress in understanding mammalian prion strains has however been severely limited by the complexity and variability of the methods used for their isolation from infected tissue and no high resolution structures have yet been reported. Using high-throughput cell-based prion bioassay to re-examine prion purification from first principles we now report the isolation of prion strains to exceptional levels of purity from small quantities of infected brain and demonstrate faithful retention of biological and biochemical strain properties. The method's effectiveness and simplicity should facilitate its wide application and expedite structural studies of prions.

2.216 **The Effect of n-3 Fatty Acids on Small Dense Low-Density Lipoproteins in Patients With End-Stage Renal Disease: A Randomized Placebo-Controlled Intervention Study**

Sørensen, G.V.B., Svensson, M., Strandhave, C., Schmidt, E.B., Jørgensen, K.A. and Christensen, J.H.
J. Renal Nutrition, **25**(4), 376-380 (2015)

Objective

Patients with end-stage renal disease (ESRD) have a high risk of cardiovascular disease. Small dense low-density lipoprotein (sdLDL) particles are particularly atherogenic. Marine n-3 polyunsaturated fatty acids (PUFA) may have a beneficial effect on numbers of sdLDL particles, and the aim of this study was to investigate the effect of n-3 PUFA on plasma levels of sdLDL in patients with ESRD.

Methods

ESRD patients with cardiovascular disease (n = 161) on chronic hemodialysis were randomized to treatment with 1.7 g of n-3 PUFA (n = 81) or 2 g of placebo (olive oil; n = 80) for 3 months. The study was double-blinded. Densities of LDL and percentages of sdLDL (sdLDL%) of total LDL were measured before and after intervention. On the basis of sdLDL%, patients were classified as having lipid pattern A, I (intermediate), or B defined by a successive increase in sdLDL concentration and decrease in lipid particle size.

Results

n-3 PUFAs significantly reduced triglycerides. However, LDL cholesterol remained unchanged. In the n-3 group, the LDL density did not change significantly during follow-up. Similarly, the LDL density remained unchanged in the placebo group. In the n-3 group, the sdLDL% was 34% at baseline and unchanged at follow-up. At baseline 71% had LDL pattern A, 9% had pattern I, and 20% had pattern B, and none of these patterns were significantly changed by n-3 PUFA supplementation.

Conclusion

Dietary supplementation with 1.7 g of n-3 PUFA had no effect on LDL density or sdLDL levels in patients with ESRD.

2.217 Co-option of Membrane Wounding Enables Virus Penetration into Cells

Luisoni, S., Suomalainen, M., Boucke, K., Grzybek, M., Coskun, U. and Greber, U.F.

Cell Host & Microbe, 18, 75-85 (2011)

During cell entry, non-enveloped viruses undergo partial uncoating to expose membrane lytic proteins for gaining access to the cytoplasm. We report that adenovirus uses membrane piercing to induce and hijack cellular wound removal processes that facilitate further membrane disruption and infection. Incoming adenovirus stimulates calcium influx and lysosomal exocytosis, a membrane repair mechanism resulting in release of acid sphingomyelinase (ASMase) and degradation of sphingomyelin to ceramide lipids in the plasma membrane. Lysosomal exocytosis is triggered by small plasma membrane lesions induced by the viral membrane lytic protein-VI, which is exposed upon mechanical cues from virus receptors, followed by virus endocytosis into leaky endosomes. Chemical inhibition or RNA interference of ASMase slows virus endocytosis, inhibits virus escape to the cytosol, and reduces infection. Ceramide enhances binding of protein-VI to lipid membranes and protein-VI-induced membrane rupture. Thus, adenovirus uses a positive feedback loop between virus uncoating and lipid signaling for efficient membrane penetration.

2.218 Hybrid pulmonary surfactant-coated nanogels mediate efficient in vivo delivery of siRNA to murine alveolar macrophages

De Backer, L., Naessens, t., De Koker, S., Zagato, E., Demeester, J., Grooten, J., De Smedt, S.C. and Raemdonck, K.

J. Controlled Release, 217, 53-63 (2015)

The local delivery of small interfering RNA (siRNA) to the lungs may provide a therapeutic solution to a range of pulmonary disorders. Resident alveolar macrophages (rAM) in the bronchoalveolar lumen play a critical role in lung inflammatory responses and therefore constitute a particularly attractive target for siRNA therapeutics. However, achieving efficient gene silencing in the lung while avoiding pulmonary toxicity requires appropriate formulation of siRNA in functional nanocarriers. In this study, we evaluated pulmonary surfactant-coated dextran nanogels for the delivery of siRNA to rAM upon pharyngeal aspiration in BALB/c mice. Both the surfactant-coated and uncoated nanogels achieved high levels of siRNA uptake in rAM, yet only the surfactant-coated formulation could significantly reduce gene expression on the protein level. Surfactant-coated nanogels induced a profound downregulation of target mRNA levels, reaching 70% knockdown with $\sim 1 \text{ mg kg}^{-1}$ siRNA dose. In addition, only mild acute pro-inflammatory cytokine and chemokine responses were detected one day after nanoparticle aspiration, accompanied by a moderate neutrophil infiltration in the bronchoalveolar lumen. The latter could be substantially reduced by removal of excess surfactant from the formulation. Overall, our hybrid core-shell nanoparticles have demonstrated safe and effective siRNA delivery to rAM, providing a new therapeutic approach for treatment of inflammatory pathologies in the lung.

2.219 QIAD assay for quantitating a compound's efficacy in elimination of toxic A β oligomers

Brener, O. et al

Scientific Reports, 5:13222 (2015)

Strong evidence exists for a central role of amyloid β -protein (A β) oligomers in the pathogenesis of Alzheimer's disease. We have developed a fast, reliable and robust in vitro assay, termed QIAD, to quantify the effect of any compound on the A β aggregate size distribution. Applying QIAD, we studied the effect of homotaurine, scyllo-inositol, EGCG, the benzofuran derivative KMS88009, ZA β 3W, the D-enantiomeric peptide D3 and its tandem version D3D3 on A β aggregation. The predictive power of the assay for in vivo efficacy is demonstrated by comparing the oligomer elimination efficiency of D3 and D3D3 with their treatment effects in animal models of Alzheimer's disease.

2.220 Platelet binding sites for factor VIII in relation to fibrin and phosphatidylserine

Gilbert, G.E., Novakovic, V.A., Shi, J., Rasmussen, J. and Pipe, S.W.

Blood, 126(10), 1237-1244 (2015)

Thrombin-stimulated platelets expose very little phosphatidylserine (PS) but express binding sites for factor VIII (fVIII), casting doubt on the role of exposed PS as the determinant of binding sites. We previously reported that fVIII binding sites are increased three- to sixfold when soluble fibrin (SF) binds the $\alpha_{IIb}\beta_3$ integrin. This study focuses on the hypothesis that platelet-bound SF is the major source of fVIII binding sites. Less than 10% of fVIII was displaced from thrombin-stimulated platelets by lactadherin, a PS-binding protein, and an fVIII mutant defective in PS-dependent binding retained platelet affinity. Therefore, PS is not the determinant of most binding sites. FVIII bound immobilized SF and paralleled platelet binding in affinity, dependence on separation from von Willebrand factor, and mediation by the C2 domain. SF also enhanced activity of fVIII in the factor Xase complex by two- to fourfold. Monoclonal antibody (mAb) ESH8, against the fVIII C2 domain, inhibited binding of fVIII to SF and platelets but not to PS-containing vesicles. Similarly, mAb ESH4 against the C2 domain, inhibited >90% of platelet-dependent fVIII activity vs 35% of vesicle-supported activity. These results imply that platelet-bound SF is a component of functional fVIII binding sites.

2.221 Structure-based drug design identifies polythiophenes as antiprion compounds

Herrmann, U.S. et al

Science Translational Medicine, 7(299), 299ra123 (2015)

In a mouse model of prion disease, Herrmann *et al.* evaluated the therapeutic efficacy of luminescent conjugated polythiophenes (LCPs), which are molecules with a high affinity for ordered protein aggregates. Intracerebral administration of LCPs into prion-infected mice using osmotic pumps increased survival. Solid-state nuclear magnetic resonance and in silico binding studies of LCPs to simplified model fibrils allowed the authors to define structural rules, which they then used for the design of LCPs with superior prophylactic and therapeutic potency. The new work demonstrates the feasibility of rational drug design for developing therapeutics to treat prion diseases.

2.222 Nutritional values and bioactive components of under-utilised vegetables consumed by indigenous people in Malaysia

Wahab, N.A., Ahdan, R., Afa, Z.A., Kong, K.W., Johar, M.H., Shariff, Z.M. and ismail, A.

J. Sci. Food Agric., 95(13), 2704-2711 (2015)

BACKGROUND

Diverse plants species in the forest remain under-utilised and they are mainly consumed only by local people. However, increasing issues in food security prompted the present study, which explores the nutritional and antioxidant aspects of Malaysian under-utilised vegetables. The studied vegetables were Paku Nyai (*Stenochlaena palustris*), Cemperai (*Champerea manillana*), Maman Pasir (*Cleome viscosa*), Dudung (*Erechtites valerianifolia*) and Semambuk (*Ardisia pendula*).

RESULTS

Overall, these vegetables exhibited a low proximal content but they were high in vitamin C [7.07–1263 mg kg⁻¹ edible fresh sample (EFS)] and β -carotene content (18.4–43.9 mg kg⁻¹ kg⁻¹ EFS). Cemperai had the highest calcium content (565 mg kg⁻¹ EFS), whereas Semambuk had the highest total phenolic content [28.21 g gallic acid equivalents kg⁻¹ edible dried sample (EDS)] and antioxidant activity (86.1%) measured using β -carotene bleaching assay. Maman Pasir contained the highest total flavonoid content (39.99 g CE

kg⁻¹ EDS) and 1,1-diphenyl-2-picryl hydrazyl radical scavenging activity (82.2%). The extracts of these vegetables had significantly prevented the oxidation of haemoglobin and low-density lipoprotein, which yielded a reduced production of malondialdehyde.

CONCLUSION

Semambuk and Maman Pasir are potent to be used as new food and functional food sources as they are rich in nutrients and antioxidants. © 2014 Society of Chemical Industry.

- 2.223 **Human Mitochondrial DNA-Protein Complexes Attach to a Cholesterol-Rich Membrane Structure**
Gerhold, J.M., Cansiz-Arda, S., Löhmus, M., Engberg, O., Reyes, A., van Rennes, H., Sanz, A., Holt, I.J., Cooper, H.M. and Spelbrink, J.N.
Scientific Reports, 5:15292 (2015)

The helicase Twinkle is indispensable for mtDNA replication in nucleoids. Previously, we showed that Twinkle is tightly membrane-associated even in the absence of mtDNA, which suggests that Twinkle is part of a membrane-attached replication platform. Here we show that this platform is a cholesterol-rich membrane structure. We fractionated mitochondrial membrane preparations on flotation gradients and show that membrane-associated nucleoids accumulate at the top of the gradient. This fraction was shown to be highly enriched in cholesterol, a lipid that is otherwise low abundant in mitochondria. In contrast, more common mitochondrial lipids, and abundant inner-membrane associated proteins concentrated in the bottom-half of these gradients. Gene silencing of ATAD3, a protein with proposed functions related to nucleoid and mitochondrial cholesterol homeostasis, modified the distribution of cholesterol and nucleoids in the gradient in an identical fashion. Both cholesterol and ATAD3 were previously shown to be enriched in ER-mitochondrial junctions, and we detect nucleoid components in biochemical isolates of these structures. Our data suggest an uncommon membrane composition that accommodates platforms for replicating mtDNA, and reconcile apparently disparate functions of ATAD3. We suggest that mtDNA replication platforms are organized in connection with ER-mitochondrial junctions, facilitated by a specialized membrane architecture involving mitochondrial cholesterol.

- 2.224 **α -Tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: a randomized, double-blind, crossover trial**
Mah, E., Sapper, T., Chitchumroonchokchai, C., Failla, M.L., Schill, K.E., Clinton, S.K., Bobe, G., Traber, M.G. and Bruno, R.S.
Am. J. Clin. Nutr., 102(5), 1070-1080 (2015)

Background: Increasing dietary fat intake is expected to improve α -tocopherol bioavailability, which could be beneficial for improving α -tocopherol status, especially in cohorts at high cardiometabolic risk who fail to meet dietary α -tocopherol requirements.

Objective: Our objective was to assess dose-dependent effects of dairy fat and metabolic syndrome (MetS) health status on α -tocopherol pharmacokinetics in plasma and lipoproteins.

Design: A randomized, crossover, double-blind study was conducted in healthy and MetS adults ($n = 10$ /group) who ingested encapsulated hexadeuterium-labeled (d_6)-*RRR*- α -tocopherol (15 mg) with 240 mL nonfat (0.2 g fat), reduced-fat (4.8 g fat), or whole (7.9 g fat) milk before blood collection at regular intervals for 72 h.

Results: Compared with healthy participants, those with MetS had lower ($P < 0.05$) baseline plasma α -tocopherol ($\mu\text{mol}/\text{mmol}$ lipid) and greater oxidized low-density lipoprotein (LDL), interleukin (IL)-6, IL-10, and C-reactive protein. Regardless of health status, d_6 - α -tocopherol bioavailability was unaffected by increasing amounts of dairy fat provided by milk beverages, but MetS participants had lower estimated d_6 - α -tocopherol absorption ($\pm\text{SEM}$) than did healthy participants ($26.1\% \pm 1.0\%$ compared with $29.5\% \pm 1.1\%$). They also had lower plasma d_6 - α -tocopherol AUC from 0 to 72 h, as well as maximal concentrations (C_{max} : 2.04 ± 0.14 compared with 2.73 ± 0.18 $\mu\text{mol}/\text{L}$) and slower rates of plasma disappearance but similar times to C_{max} . MetS participants had lower d_6 - α -tocopherol AUC from $t = 0$ –12 h ($\text{AUC}_{0-t_{\text{final}}}$) in lipoprotein fractions [chylomicron, very-low-density lipoprotein (VLDL), LDL, high-density lipoprotein]. Percentages of d_6 - α -tocopherol $\text{AUC}_{0-t_{\text{final}}}$ in both the chylomicron ($r = -0.46$ to -0.52) and VLDL ($r = -0.49$ to -0.68) fractions were inversely correlated with oxidized LDL, IL-10, IL-6, and C-reactive protein.

Conclusions: At dietary intakes equivalent to the Recommended Dietary Allowance, α -tocopherol bioavailability is unaffected by dairy fat quantity but is lower in MetS adults, potentially because of greater inflammation and oxidative stress that limits small intestinal α -tocopherol absorption and/or impairs hepatic α -tocopherol trafficking. These findings support higher dietary α -tocopherol requirements for MetS adults. This trial was registered at www.clinicaltrials.gov as [NCT01787591](https://clinicaltrials.gov/ct2/show/study/NCT01787591).

2.225 Surface acoustic wave controlled integrated band-pass filter

Skowronek, V., Rambach, R.W. and Franke, T.
Microfluid. Nanofluid., **19**(2), 335-341 (2015)

We introduce a microfluidic band-pass filter for particles that is fully integrated in a polydimethylsiloxane-based microchannel device. This acoustic filter allows a continuous and label-free separation of particles. To demonstrate the functionality, mixtures of particles with different sizes are exposed to propagating surface acoustic waves generated by two laterally displaced interdigitated transducers, one on each side of the microchannel. Dependent on the frequency used, a specific size or even a size range of particles can be extracted. We sort particles of sizes of ~1–10 μm and estimate the size resolution to be smaller than $\Delta r < 0.88 \mu\text{m}$. We examine the performance of the device and achieve a throughput of $\sim 10^5$ particles/s with an efficiency as high as 99 %.

2.226 Hitchhiking nanoparticles: Reversible coupling of lipid-based nanoparticles to cytotoxic T lymphocytes

Wayteck, L., Dewitte, H., De Backer, L., Breckpot, K., Demeester, J., De Smedt, S.C. and Raemdonck, K.
Biomaterials, **77**, 143-154 (2016)

Following intravenous injection of anti-cancer nanomedicines, many barriers need to be overcome en route to the tumor. Cell-mediated delivery of nanoparticles (NPs) is promising in terms of overcoming several of these barriers based on the tumor-tropic migratory properties of particular cell types. This guided transport aims to enhance the NP accumulation in the tumor and moreover enhance the infiltration of regions that are typically inaccessible for free NPs. Within this study, cytotoxic CD8⁺ T cells were selected as carriers based on both their ability to migrate to the tumor and their intrinsic cytolytic activity against tumor cells. Many anti-cancer nanomedicines require tumor cell internalization to mediate cytosolic drug delivery and enhance the anti-cancer effect. This proof-of-concept therefore reports on the reversible attachment of liposomes to the surface of cytotoxic T lymphocytes via a reduction sensitive coupling. The activation status of the T cells and the liposome composition are shown to strongly influence the loading efficiency. Loading the cells with liposomes does not compromise T cell functionalities like proliferation and cytolytic function. Additionally, the triggered liposome release is demonstrated upon the addition of glutathione. Based on this optimization using liposomes as model NPs, a small interfering RNA (siRNA)-loaded NP was developed that can be coupled to the surface of CD8⁺ T cells.

2.227 In vitro reconstitution of B cell receptor–antigen interactions to evaluate potential vaccine candidates

Weaver, G.C., Villar, R.F., Kanekiyo, M., Nabel, G.J., Mascola, J.R. and Lingwood, D.
Nature Protocols, **11**(2), 193-213 (2016)

Predicting immune responses before vaccination is challenging because of the complexity of the governing parameters. Nevertheless, recent work has shown that B cell receptor (BCR)-antigen engagement *in vitro* can prove a powerful means of informing the design of antibody-based vaccines. We have developed this principle into a two-phased immunogen evaluation pipeline to rank-order vaccine candidates. In phase 1, recombinant antigens are screened for reactivity to the germline precursors that produce the antibody responses of interest. To both mimic the architecture of initial antigen engagement and facilitate rapid immunogen screening, these antibodies are expressed as membrane-anchored IgM (mIgM) in 293F indicator cells. In phase 2, the binding hits are multimerized by nanoparticle or proteoliposome display, and they are evaluated for BCR triggering in an engineered B cell line displaying the IgM sequences of interest. Key developments that complement existing methodology in this area include the following: (i) introduction of a high-throughput screening step before evaluation of more time-intensive BCR-triggering analyses; (ii) generalizable multivalent antigen-display platforms needed for BCR activation; and (iii) engineered use of a human B cell line that does not display endogenous antibody, but only ectopically expressed BCR sequences of interest. Through this pipeline, the capacity to initiate favorable antibody responses is evaluated. The entire protocol can be completed within 2.5 months.

2.228 Association of mitotane with chylomicrons and serum lipoproteins: practical implications for treatment of adrenocortical carcinoma

Kroiss, M., Plonne, D., Kendl, S., Schirmer, D., Ronchi, C.L., Schirbel, A., Zink, M., Lapa, C., Klinker, H., Fassnacht, M., Heinz, W and Sbiera, S.
Eur. J. Endocrinol., **174**(3), 343-353 (2016)

Objective Oral mitotane (o,p'-DDD) is a cornerstone of medical treatment for adrenocortical carcinoma (ACC).

Aim Serum mitotane concentrations >14mg/l are targeted for improved efficacy but not achieved in about half of patients. Here we aimed at a better understanding of intestinal absorption and lipoprotein association of mitotane and metabolites o,p'-dichlorodiphenylacetic acid (o,p'-DDA) and o,p'-dichlorodiphenyldichloroethane (o,p'-DDE).

Design Lipoproteins were isolated by ultracentrifugation from the chyle of a 29-year-old patient and serum from additional 14 ACC patients treated with mitotane. HPLC was applied for quantification of mitotane and metabolites. We assessed NCI-H295 cell viability, cortisol production, and expression of endoplasmic reticulum (ER) stress marker genes to study the functional consequences of mitotane binding to lipoproteins.

Results Chyle of the index patient contained 197mg/ml mitotane, 53mg/ml o,p'-DDA, and 51mg/l o,p'-DDE. Of the total mitotane in serum, lipoprotein fractions contained 21.7±21.4% (VLDL), 1.9±0.8% (IDL), 8.9±5.5% (LDL1), 18.9±9.6% (LDL2), 10.1±4.0% (LDL3), and 26.3±13.0% (HDL2). Only 12.3±5.5% were in the lipoprotein-depleted fraction.

Discussion Mitotane content of lipoproteins directly correlated with their triglyceride and cholesterol content. O,p'-DDE was similarly distributed, but 87.9±4.2% of o,p'-DDA found in the HDL2 and lipoprotein-depleted fractions. Binding of mitotane to human lipoproteins blunted its anti-proliferative and anti-hormonal effects on NCI-H295 cells and reduced ER stress marker gene expression.

Conclusion Mitotane absorption involves chylomicron binding. High concentrations of o,p'-DDA and o,p'-DDE in chyle suggest intestinal mitotane metabolism. In serum, the majority of mitotane is bound to lipoproteins. *In vitro*, lipoprotein binding inhibits activity of mitotane suggesting that lipoprotein-free mitotane is the therapeutically active fraction.

2.229 Competitive Mirror Image Phage Display Derived Peptide Modulates Amyloid Beta Aggregation and Toxicity

Rudolph, S., Klein, A.N., Tusche, M., Schlosser, C., Elfgen, A., Brener, O, Teunissen, C., Gremer, L., Funke, S.A., Kutzsche, J. and Willbold, D.
PLoS One, **11**(2), e0147470 (2016)

Alzheimer's disease is the most prominent type of dementia and currently no causative treatment is available. According to recent studies, oligomeric species of the amyloid beta (A β) peptide appear to be the most toxic A β assemblies. A β monomers, however, may be not toxic per se and may even have a neuroprotective role. Here we describe a competitive mirror image phage display procedure that allowed us to identify preferentially A β ₁₋₄₂ monomer binding and thereby stabilizing peptides, which destabilize and thereby eliminate toxic oligomer species. One of the peptides, called Mosd1 (monomer specific d-peptide 1), was characterized in more detail. Mosd1 abolished oligomers from a mixture of A β ₁₋₄₂ species, reduced A β ₁₋₄₂ toxicity in cell culture, and restored the physiological phenotype in neuronal cells stably transfected with the gene coding for human amyloid precursor protein.

2.230 Simvastatin Efficiently Lowers Small LDL-IgG Immune Complex Levels: A Therapeutic Quality beyond the Lipid-Lowering Effect

Hörl, G., Froehlich, H., Fersti, U., Iedinski, G., Binder, J., Cvirm, G., Stojakovic, T., Trauner, M., Koidl, C., Tafel, E., Amrein, K., Scharnagi, H., Jürgens, G. and Hallström, S.
PLoS One, **11**(2), e0148210 (2016)

We investigated a polyethylene glycol non-precipitable low-density lipoprotein (LDL) subfraction targeted by IgG and the influence of statin therapy on plasma levels of these small LDL-IgG-immune complexes (LDL-IgG-IC). LDL-subfractions were isolated from 6 atherosclerotic subjects and 3 healthy individuals utilizing iodixanol density gradient ultracentrifugation. Cholesterol, apoB and malondialdehyde (MDA) levels were determined in each fraction by enzymatic testing, dissociation-enhanced lanthanide fluorescence immunoassay and high-performance liquid chromatography, respectively. The levels of LDL-IgG-IC were quantified densitometrically following lipid electrophoresis, particle size distribution was assessed with dynamic light scattering and size exclusion chromatography. The influence of simvastatin (40 mg/day for three months) on small LDL-IgG-IC levels and their distribution among LDL-subfractions (salt gradient separation) were investigated in 11 patients with confirmed coronary artery disease (CAD). We demonstrate that the investigated LDL-IgG-IC are small particles present in atherosclerotic patients and healthy subjects. *In vitro* assembly of LDL-IgG-IC resulted in particle density shifts indicating a

composition of one single molecule of IgG per LDL particle. Normalization on cholesterol levels revealed MDA values twice as high for LDL-subfractions rich in small LDL-IgG-IC if compared to dominant LDL-subfractions. Reactivity of affinity purified small LDL-IgG-IC to monoclonal antibody OB/04 indicates a high degree of modified apoB and oxidative modification. Simvastatin therapy studied in the CAD patients significantly lowered LDL levels and to an even higher extent, small LDL-IgG-IC levels without affecting their distribution. In conclusion simvastatin lowers levels of small LDL-IgG-IC more effectively than LDL-cholesterol and LDL-apoB levels in atherosclerotic patients. This antiatherogenic effect may additionally contribute to the known beneficial effects of this drug in the treatment of atherosclerosis.

2.231 Scalable Isolation of Mammalian Mitochondria for Nucleic Acid and Nucleoid Analysis

Lee, K-W. and Bogenhagen, D.F.

Methods in Mol. Biol., **1351**, 67-79 (2016)

Isolation of mitochondria from cultured cells and animal tissues for analysis of nucleic acids and bona fide mitochondrial nucleic acid binding proteins and enzymes is complicated by contamination with cellular nucleic acids and their adherent proteins. Protocols presented here allow for quick isolation of mitochondria from a small number of cells and for preparation of highly purified mitochondria from a larger number of cells using nuclease treatment and high salt washing of mitochondria to reduce contamination. We further describe a method for the isolation of mitochondrial DNA–protein complexes known as nucleoids from these highly purified mitochondria using a combination of glycerol gradient sedimentation followed by isopycnic centrifugation in a non-ionic iodixanol gradient.

2.232 Immunogenicity of Leishmania-derived hepatitis B small surface antigen particles exposing highly conserved E2 epitope of hepatitis C virus

Czarnota, A., Tyborowska, J., Peszynska-Sularz, G., Growatzka, B., Bienkowska-Szewczyk, K. and Grzyb, K.

Microb. Cell Fact., **15**:62 (2016)

Background

Hepatitis C virus (HCV) infection is a major health problem worldwide, affecting an estimated 2–3 % of human population. An HCV vaccine, however, remains unavailable. High viral diversity poses a challenge in developing a vaccine capable of eliciting a broad neutralizing antibody response against all HCV genotypes. The small surface antigen (sHBsAg) of hepatitis B virus (HBV) has the ability to form highly immunogenic subviral particles which are currently used as an efficient anti-HBV vaccine. It also represents an attractive antigen carrier for the delivery of foreign sequences. In the present study, we propose a bivalent vaccine candidate based on novel chimeric particles in which highly conserved epitope of HCV E2 glycoprotein (residues 412–425) was inserted into the hydrophilic loop of sHBsAg.

Results

The expression of chimeric protein was performed in an unconventional, *Leishmania tarentolae* expression system resulting in an assembly of particles which retained immunogenicity of both HCV epitope and sHBsAg protein. Direct transmission electron microscopy observation and immunogold staining confirmed the formation of spherical particles approximately 22 nm in diameter, and proper foreign epitope exposition. Furthermore, the sera of mice immunized with chimeric particles proved reactive not only to purified yeast-derived sHBsAg proteins but also HCV E2 412–425 synthetic peptide. Most importantly, they were also able to cross-react with E1E2 complexes from different HCV genotypes.

Conclusions

For the first time, we confirmed successful assembly of chimeric sHBsAg virus-like particles (VLPs) in the *L. tarentolae* expression system which has the potential to produce high-yields of properly N-glycosylated mammalian proteins. We also proved that chimeric *Leishmania*-derived VLPs are highly immunogenic and able to elicit cross-reactive antibody response against HCV. This approach may prove useful in the development of a bivalent prophylactic vaccine against HBV and HCV and opens up a new and low-cost opportunity for the production of chimeric sHBsAg VLPs requiring N-glycosylation process for their proper functionality and immunogenicity.

2.233 Hepatitis C Virus-Induced Degradation of Cell Death-Inducing DFFA-Like Effector B Leads to Hepatic Lipid Dysregulation

Lee, E.M., Alsagheir, A., Wu, X., Hammack, C., Mclaughlan, J., Watanabe, N., Wakita, T., Kneteman, N.M., Douglas, D.N. and Tang, H.

Individuals chronically infected with hepatitis C virus (HCV) commonly exhibit hepatic intracellular lipid accumulation, termed steatosis. HCV infection perturbs host lipid metabolism through both cellular and virus-induced mechanisms, with the viral core protein playing an important role in steatosis development. We have recently identified a liver protein, the cell death-inducing DFFA-like effector B (CIDEB), as an HCV entry host dependence factor that is downregulated by HCV infection in a cell culture model. In this study, we investigated the biological significance and molecular mechanism of this downregulation. HCV infection in a mouse model downregulated CIDEB in the liver tissue, and knockout of the CIDEB gene in a hepatoma cell line results in multiple aspects of lipid dysregulation that can contribute to hepatic steatosis, including reduced triglyceride secretion, lower lipidation of very-low-density lipoproteins, and increased lipid droplet (LD) stability. The potential link between CIDEB downregulation and steatosis is further supported by the requirement of the HCV core and its LD localization for CIDEB downregulation, which utilize a proteolytic cleavage event that is independent of the cellular proteasomal degradation of CIDEB.

2.234 Increased Presence of Remnant Lipoprotein Cholesterol in The Hdl of Diabetic Subjects

Gonzalez, M., Heras, M., Rosales, R., Guardiola, M., Plana, N., Vallve, J.C., Masana, L. and Ribalta, J. *Ann. Clin. Lab. Sci.*, **46**(2), 229 (2016)

The atherogenic dyslipidemia associated with type II diabetes (T2DM) is characterized by elevated fasting triglycerides (TG) and remnant lipoproteins (RL) as well as small and dense low-density lipoprotein (sdLDL) particles and low high-density lipoprotein cholesterol (HDLc) levels [1]. Epidemiological studies have demonstrated that both fasting and non-fasting hyperlipidemia are important risk factors for atherosclerosis and cardiovascular events [2]. In the non-fasting state, the lipid profile is characterized by the accumulation of RL, a heterogeneous group of catabolized hepatic and intestinal lipoproteins, which differ in size, density, electrophoretic mobility, composition, and receptor affinity [3]. RL are considered an independent risk factor in cardiovascular disease [4–6]. The analytical determination of RL is not straightforward. Measurements of circulating apolipoprotein (apo) B48 estimate postprandial remnant lipoproteins [7]. On the other hand, the remnant-like particle cholesterol (RLPc) fraction obtained by immunochemistry identifies a wider spectrum of lipoproteins, ranging from VLDL to HDL, that cannot effectively bind to antibodies against apoA1 and apoB100 [8]. The RLPs density range varies according to the population studied; for instance, in normolipidemic subjects, RLPc is normally undetectable in the IDL fraction [5]. However, in diabetic or dysbetalipoproteinemic patients, the IDL can be found in the RLPc fraction [9].

The aim of this study is two-fold: 1) To analyze which lipoproteins have RLPs and how this varies between normolipidemic and T2DM patients; 2) To test how this distribution varies postprandially in normolipidemic subjects with iodixanol gradient ultracentrifugation, a method that allows for more accurate subfraction separation permitting the quantification of up to 21 lipoprotein subclasses [10].

2.235 Optimization of the All-D Peptide D3 for A β Oligomer Elimination

Klein, A.-N., Ziehm, T., Tusche, M., Buitenhuis, J., bartnik, D., Boeddrich, A., Wiglenda, T., Wanker, E., Funke, S.A., Brener, O., Gremer, L., Kutzsche, J. and Willbold, D. *PLoS One*, **11**(4), e0153035 (2016)

The aggregation of amyloid- β (A β) is postulated to be the crucial event in Alzheimer's disease (AD). In particular, small neurotoxic A β oligomers are considered to be responsible for the development and progression of AD. Therefore, elimination of these oligomers represents a potential causal therapy of AD. Starting from the well-characterized d-enantiomeric peptide D3, we identified D3 derivatives that bind monomeric A β . The underlying hypothesis is that ligands bind monomeric A β and stabilize these species within the various equilibria with A β assemblies, leading ultimately to the elimination of A β oligomers. One of the hereby identified d-peptides, DB3, and a head-to-tail tandem of DB3, DB3DB3, were studied in detail. Both peptides were found to: (i) inhibit the formation of Thioflavin T-positive fibrils; (ii) bind to A β monomers with micromolar affinities; (iii) eliminate A β oligomers; (iv) reduce A β -induced cytotoxicity; and (v) disassemble preformed A β aggregates. The beneficial effects of DB3 were improved by DB3DB3, which showed highly enhanced efficacy. Our approach yielded A β monomer-stabilizing ligands that can be investigated as a suitable therapeutic strategy against AD.

2.236 A Programmable DNA Origami Platform to Organize SNAREs for Membrane Fusion

Xu, W., Nathwani, B., Lin, C., Wang, J., Karatekin, E., Pincet, F., Shih, W. and Rothman, J.E.
J. Am. Chem. Soc., **138**(13), 4439-4447 (2016)

Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes are the core molecular machinery of membrane fusion, a fundamental process that drives inter- and intracellular communication and trafficking. One of the questions that remains controversial has been whether and how SNAREs cooperate. Here we show the use of self-assembled DNA-nanostructure rings to template uniform-sized small unilamellar vesicles containing predetermined maximal number of externally facing SNAREs to study the membrane-fusion process. We also incorporated lipid-conjugated complementary ssDNA as tethers into vesicle and target membranes, which enabled bypass of the rate-limiting docking step of fusion reactions and allowed direct observation of individual membrane-fusion events at SNARE densities as low as one pair per vesicle. With this platform, we confirmed at the single event level that, after docking of the templated-SUVs to supported lipid bilayers (SBL), one to two pairs of SNAREs are sufficient to drive fast lipid mixing. Modularity and programmability of this platform makes it readily amenable to studying more complicated systems where auxiliary proteins are involved.

2.237 Ex vivo mammalian prions are formed of paired double helical prion protein fibrils

Terry, C., Wenborn, A., Gros, N., Sells, J., Joiner, S., Hosszu, L.P., Tattum, M.H., Panico, S., Clare, D.K., Collinge, J., Saibil, H.R. and Wadsworth, J.D.F.
Open Biology, **6**, 160035 (2016)

Mammalian prions are hypothesized to be fibrillar or amyloid forms of prion protein (PrP), but structures observed to date have not been definitively correlated with infectivity and the three-dimensional structure of infectious prions has remained obscure. Recently, we developed novel methods to obtain exceptionally pure preparations of prions from mouse brain and showed that pathogenic PrP in these high-titre preparations is assembled into rod-like assemblies. Here, we have used precise cell culture-based prion infectivity assays to define the physical relationship between the PrP rods and prion infectivity and have used electron tomography to define their architecture. We show that infectious PrP rods isolated from multiple prion strains have a common hierarchical assembly comprising twisted pairs of short fibres with repeating substructure. The architecture of the PrP rods provides a new structural basis for understanding prion infectivity and can explain the inability to systematically generate high-titre synthetic prions from recombinant PrP.

2.238 Isolation of high density lipoproteins in ovine follicular and oviductal fluid

Bernećić, N.C., Gadella, B.M., de Graaf, S.P. and Leahy, T.
Animal Reproduction Science, **169**, 121.122 (2016)

Capacitation is a key maturation process in spermatozoa that is vital for successful fertilisation. Following the initiation of this process, cholesterol is lost from the sperm plasma membrane via a series of regulated mechanisms which then permits appropriate sperm binding to the zona pellucida and the induction of the acrosome reaction. High density lipoproteins (HDL) have been shown to play a key role in cholesterol efflux in somatic cells but their physiological role in sperm cholesterol efflux is uncertain. The objective of this study was to isolate and identify HDL in ovine follicular and oviductal fluid for potential use in media that supports ram sperm capacitation *in vitro*.

Density gradients were generated by diluting Iodixanol (OptiPrep; Sigma Aldrich, Australia) to 20% (v/v) with follicular or oviductal fluid and then layering this mixture underneath equal volumes of 6% and 12.5% iodixanol in PBS (v/v). Samples were centrifuged ($27,7320 \times g$ at 16°C for 5 h) and the resulting gradients were harvested into 10 fractions by aspiration. Proteins from each fraction were separated based on molecular weight using 1D SDS-PAGE. Liquid Chromatography Mass Spectrometry (LC-MS) was used to detect the presence of the major HDL apolipoprotein, apoA-I, in the relevant molecular weight band on the gels.

This proteomic approach confirmed the presence of apoA-I in fractions 7–10 of follicular fluid and fractions 8–10 of oviductal fluid. This indicates that HDLs in ovine follicular and oviductal fluid can be isolated using iodixanol and density ultracentrifugation. The establishment of this protocol in sheep provides an opportunity to investigate the function of HDLs as potential cholesterol acceptors from ram spermatozoa during *in vitro* capacitation, which will be the focus of future studies.

2.239 Self-assembly of size-controlled liposomes on DNA nanotemplates

Yang, Y., Wang, J., Shigematsu, H., Xu, W., Shih, W.M., Rothman, J.E. and Lin, C.

Artificial lipid-bilayer membranes are valuable tools for the study of membrane structure and dynamics. For applications such as the study of vesicular transport and drug delivery, there is a pressing need for artificial vesicles with controlled size. However, controlling vesicle size and shape with nanometre precision is challenging, and approaches to achieve this can be heavily affected by lipid composition. Here, we present a bio-inspired templating method to generate highly monodispersed sub-100-nm unilamellar vesicles, where liposome self-assembly was nucleated and confined inside rigid DNA nanotemplates. Using this method, we produce homogeneous liposomes with four distinct predefined sizes. We also show that the method can be used with a variety of lipid compositions and probe the mechanism of templated liposome formation by capturing key intermediates during membrane self-assembly. The DNA nanotemplating strategy represents a conceptually novel way to guide lipid bilayer formation and could be generalized to engineer complex membrane/protein structures with nanoscale precision.

2.240 PCSK9 Association With Lipoprotein(a)

Tavori, H., Christian, D., Minnier, J., Plubell, D., Shapiro, M.D., Yeang, C., Giunzioni, I., Croyal, M., Duell, P.B., Lambert, G., Tsimikas, S. and Fazio, S.

Circ. Res., **119**, 29-35 (2016)

Rationale: Lipoprotein(a) [Lp(a)] is a highly atherogenic low-density lipoprotein-like particle characterized by the presence of apoprotein(a) [apo(a)] bound to apolipoprotein B. Proprotein convertase subtilisin/kexin type 9 (PCSK9) selectively binds low-density lipoprotein; we hypothesized that it can also be associated with Lp(a) in plasma.

Objective: Characterize the association of PCSK9 and Lp(a) in 39 subjects with high Lp(a) levels (range 39–320 mg/dL) and in transgenic mice expressing either human apo(a) only or human Lp(a) (via coexpression of human apo(a) and human apolipoprotein B).

Methods and Results: We show that PCSK9 is physically associated with Lp(a) in vivo using 3 different approaches: (1) analysis of Lp(a) fractions isolated by ultracentrifugation; (2) immunoprecipitation of plasma using antibodies to PCSK9 and immunodetection of apo(a); (3) ELISA quantification of Lp(a)-associated PCSK9. Plasma PCSK9 levels correlated with Lp(a) levels, but not with the number of kringle IV-2 repeats. PCSK9 did not bind to apo(a) only, and the association of PCSK9 with Lp(a) was not affected by the loss of the apo(a) region responsible for binding oxidized phospholipids. Preferential association of PCSK9 with Lp(a) versus low-density lipoprotein (1.7-fold increase) was seen in subjects with high Lp(a) and normal low-density lipoprotein. Finally, Lp(a)-associated PCSK9 levels directly correlated with plasma Lp(a) levels but not with total plasma PCSK9 levels.

Conclusions: Our results show, for the first time, that plasma PCSK9 is found in association with Lp(a) particles in humans with high Lp(a) levels and in mice carrying human Lp(a). Lp(a)-bound PCSK9 may be pursued as a biomarker for cardiovascular risk.

2.241 TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia

Yeh, F.L., Wang, Y., Tom, I., Gonzales, L.C. and Sheng, M.

Neuron, **91**, 328-340 (2016)

Genetic variants of TREM2, a protein expressed selectively by microglia in the brain, are associated with Alzheimer's disease (AD). Starting from an unbiased protein microarray screen, we identified a set of lipoprotein particles (including LDL) and apolipoproteins (including CLU/APOJ and APOE) as ligands of TREM2. Binding of these ligands by TREM2 was abolished or reduced by disease-associated mutations. Overexpression of wild-type TREM2 was sufficient to enhance uptake of LDL, CLU, and APOE in heterologous cells, whereas TREM2 disease variants were impaired in this activity. *Trem2* knockout microglia showed reduced internalization of LDL and CLU. β -amyloid ($A\beta$) binds to lipoproteins and this complex is efficiently taken up by microglia in a TREM2-dependent fashion. Uptake of $A\beta$ -lipoprotein complexes was reduced in macrophages from human subjects carrying a *TREM2* AD variant. These data link three genetic risk factors for AD and reveal a possible mechanism by which mutant TREM2 increases risk of AD.

2.242 Cell-free expression, purification, and membrane reconstitution for NMR studies of the nonstructural protein 4B from hepatitis C virus

Fogeron, M-L. et al

J. Biomol. NMR, **65**(2), 87-98 (2016)

We describe the expression of the hepatitis C virus nonstructural protein 4B (NS4B), which is an integral membrane protein, in a wheat germ cell-free system, the subsequent purification and characterization of NS4B and its insertion into proteoliposomes in amounts sufficient for multidimensional solid-state NMR spectroscopy. First spectra of the isotopically [^2H , ^{13}C , ^{15}N]-labeled protein are shown to yield narrow ^{13}C resonance lines and a proper, predominantly α -helical fold. Clean residue-selective leucine, isoleucine and threonine-labeling is demonstrated. These results evidence the suitability of the wheat germ-produced integral membrane protein NS4B for solid-state NMR. Still, the proton linewidth under fast magic angle spinning is broader than expected for a perfect sample and possible causes are discussed.

2.243 Eicosapentaenoic Acid Inhibits Oxidation of ApoB-containing Lipoprotein Particles of Different Size In Vitro When Administered Alone or in Combination With Atorvastatin Active Metabolite Compared With Other Triglyceride-lowering Agents

Mason, R.P., Sherratt, S.C.R. and Jacob, R.F.
J. Cardiovasc. Pharmacol., **68**(1), 33-40 (2016)

Eicosapentaenoic acid (EPA) is a triglyceride-lowering agent that reduces circulating levels of the apolipoprotein B (apoB)-containing lipoprotein particles small dense low-density lipoprotein (sdLDL), very-low-density lipoprotein (VLDL), and oxidized low-density lipoprotein (LDL). These benefits may result from the direct antioxidant effects of EPA. To investigate this potential mechanism, these particles were isolated from human plasma, preincubated with EPA in the absence or presence of atorvastatin (active) metabolite, and subjected to copper-initiated oxidation. Lipid oxidation was measured as a function of thiobarbituric acid reactive substances formation. EPA inhibited sdLDL (IC₅₀ ~2.0 μM) and LDL oxidation (IC₅₀ ~2.5 μM) in a dose-dependent manner. Greater antioxidant potency was observed for EPA in VLDL. EPA inhibition was enhanced when combined with atorvastatin metabolite at low equimolar concentrations. Other triglyceride-lowering agents (fenofibrate, niacin, and gemfibrozil) and vitamin E did not significantly affect sdLDL, LDL, or VLDL oxidation compared with vehicle-treated controls. Docosahexaenoic acid was also found to inhibit oxidation in these particles but over a shorter time period than EPA. These data support recent clinical findings and suggest that EPA has direct antioxidant benefits in various apoB-containing subfractions that are more pronounced than those of other triglyceride-lowering agents and docosahexaenoic acid.

2.244 Increase of Positive Net Charge and Conformational Rigidity Enhances the Efficacy of d-Enantiomeric Peptides Designed to Eliminate Cytotoxic A β Species

Ziehm, T., Brener, O., van Groen, T., Kadish, I., Frenzel, D., Tusche, M., Kutzsche, J., Reiss, K., Gremer, L., Nagel-Steger, L. and Willbold, D.
ACS Chem. Neurosci., **7**(8), 1088-1096 (2016)

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common type of dementia. Until now, there is no curative therapy available. Previously, we selected the amyloid-beta (A β) targeting peptide D3 consisting of 12 d-enantiomeric amino acid residues by mirror image phage display as a potential drug candidate for the treatment of AD. In the current approach, we investigated the optimization potential of linear D3 with free C-terminus (D3_{COOH}) by chemical modifications. First, the impact of the net charge was investigated and second, cyclization was introduced which is a well-known tool for the optimization of peptides for enhanced target affinity. Following this strategy, three D3 derivatives in addition to D3_{COOH} were designed: C-terminally amidated linear D3 (D3_{CONH₂}), cyclic D3 (cD3), and cyclic D3 with an additional arginine residue (cD3r) to maintain the net charge of linear D3_{CONH₂}. These four compounds were compared to each other according to their binding affinities to A β (1–42), their efficacy to eliminate cytotoxic oligomers, and consequently their potency to neutralize A β (1–42) oligomer induced neurotoxicity. D3_{CONH₂} and cD3r versions with equally increased net charge showed superior properties over D3_{COOH} and cD3, respectively. The cyclic versions showed superior properties compared to their linear version with equal net charge, suggesting cD3r to be the most efficient compound among these four. Indeed, treatment of the transgenic AD mouse model Tg-SwDI with cD3r significantly enhanced spatial memory and cognition of these animals as revealed by water maze performance. Therefore, charge increase and cyclization imply suitable modification steps for an optimization approach of the A β targeting compound D3.

2.245 14-3-3 ζ Mediates Tau Aggregation in Human Neuroblastoma M17 Cells

Li, T. and Paudel, H.K.

PloS One, 11(8), e0160635 (2016)

Microtubule-associated protein tau is the major component of paired helical filaments (PHFs) associated with the neuropathology of Alzheimer's disease (AD). Tau in the normal brain binds and stabilizes microtubules. Tau isolated from PHFs is hyperphosphorylated, which prevents it from binding to microtubules. Tau phosphorylation has been suggested to be involved in the development of NFT pathology in the AD brain. Recently, we showed that 14-3-3 ζ is bound to tau in the PHFs and when incubated in vitro with 14-3-3 ζ , tau formed amorphous aggregates, single-stranded straight filaments, double stranded ribbon-like filaments and PHF-like filaments that displayed close resemblance with corresponding ultrastructures of AD brain. Surprisingly however, phosphorylated and non-phosphorylated tau aggregated in a similar manner, indicating that tau phosphorylation does not affect in vitro tau aggregation (Qureshi et al (2013) *Biochemistry* 52, 6445–6455). In this study, we have examined the role of tau phosphorylation in tau aggregation in cellular level. We have found that in human M17 neuroblastoma cells, tau phosphorylation by GSK3 β or PKA does not cause tau aggregation, but promotes 14-3-3 ζ -induced tau aggregation by destabilizing microtubules. Microtubule disrupting drugs also promoted 14-3-3 ζ -induced tau aggregation without changing tau phosphorylation in M17 cell. In vitro, when incubated with 14-3-3 ζ and microtubules, nonphosphorylated tau bound to microtubules and did not aggregate. Phosphorylated tau on the other hand did not bind to microtubules and aggregated. Our data indicate that microtubule-bound tau is resistant to 14-3-3 ζ -induced tau aggregation and suggest that tau

2.246 Hepatitis C virus (HCV) and atherosclerosis risk: A role for low-density immune complexes?

Bassendine, M., Nielsen, S. and Neely, D.

Atherosclerosis, 252, e206 (2016)

Objectives: HCV is associated with an increased risk of atherosclerosis, despite inducing a favourable lipid profile with accompanying low classical risk score. HCV lipo-viral particles and sub-viral particles are found in the low-density fraction (LDF) of blood (<1.08 g/ml) associated with apoB-containing lipoproteins. The aim of this study was to examine the proteome of LDF in chronic HCV, compared to non-HCV control.

Methods: Proteins in iodixanol fractions of blood were separated on SDS PAGE; 14 bands excised from the gel were analysed by MALDI TOF. Peptides generated by trypsin digestion were analysed by mass spectrometry. Protein bands were identified using the peptide mass fingerprint data and the Mascot search engine program, searched against NCBI protein.

Results: Several protein bands were detected in the LDF only in HCV patients. After identification of protein bands by mass spectrometry, lanes were scanned to produce an intensity profile. Peaks corresponding to proteins identified by mass spectrometry were labelled indicating that four proteins, IgM heavy chain, IgG3 heavy chain, IgG1 heavy chain and Kappa light chain were present only in LDF purified from HCV patients.

2.247 Mechanisms of selective delivery of xanthophylls to retinal pigment epithelial cells by human lipoproteins

Thomas, S.E. and Harrison, E.H.

J. Lipid Res., 57, 1865-1878 (2016)

The xanthophylls, lutein and zeaxanthin, are dietary carotenoids that selectively accumulate in the macula of the eye providing protection against age-related macular degeneration. To reach the macula, carotenoids cross the retinal pigment epithelium (RPE). Xanthophylls and β -carotene mostly associate with HDL and LDL, respectively. HDL binds to cells via a scavenger receptor class B1 (SR-B1)-dependent mechanism, while LDL binds via the LDL receptor. Using an in-vitro, human RPE cell model (ARPE-19), we studied the mechanisms of carotenoid uptake into the RPE by evaluating kinetics of cell uptake when delivered in serum or isolated LDL or HDL. For lutein and β -carotene, LDL delivery resulted in the highest rates and extents of uptake. In contrast, HDL was more effective in delivering zeaxanthin and *meso*-zeaxanthin leading to the highest rates and extents of uptake of all four carotenoids. Inhibitors of SR-B1 suppressed zeaxanthin delivery via HDL. Results show a selective HDL-mediated uptake of zeaxanthin and *meso*-zeaxanthin via SR-B1 and a LDL-mediated uptake of lutein. This demonstrates a plausible mechanism for the selective accumulation of zeaxanthin greater than lutein and xanthophylls over β -carotene in the retina. We found no evidence of xanthophyll metabolism to apocarotenoids or lutein conversion to *meso*-zeaxanthin.

2.248 Characterizing the Effect of Multivalent Conjugates Composed of A β -Specific Ligands and Metal Nanoparticles on Neurotoxic Fibrillar Aggregation

Streich, C., Akkari, L., Decker, C., Bormann, J., Rehbock, C., Müller-Sciffmann, A., Carlsson Niemayer, F., Nagel-Steger, L., Willbold, d., Sacca, B., Korth, C., Schrader, T. and Barcikowski, S.
ACS Nano, **10**(8), 7582-7597 (2016)

Therapeutically active small molecules represent promising nonimmunogenic alternatives to antibodies for specifically targeting disease-relevant receptors. However, a potential drawback compared to antibody–antigen interactions may be the lower affinity of small molecules toward receptors. Here, we overcome this low-affinity problem by coating the surface of nanoparticles (NPs) with multiple ligands. Specifically, we explored the use of gold and platinum nanoparticles to increase the binding affinity of A β -specific small molecules to inhibit A β peptide aggregation into fibrils *in vitro*. The interactions of bare NPs, free ligands, and NP-bound ligands with A β are comprehensively studied *via* physicochemical methods (spectroscopy, microscopy, immunologic tests) and cell assays. Reduction of thioflavin T fluorescence, as an indicator for β -sheet content, and inhibition of cellular A β excretion are even more effective with NP-bound ligands than with the free ligands. The results from this study may have implications in the development of therapeutics for treating Alzheimer’s disease.

2.249 Size Determination of a Liposomal Drug by Small-Angle X-ray Scattering Using Continuous Contrast Variation

Garcia-Diez, R., Gollwitzer, C., Krumrey, M. and Varga, Z.
Langmuir, **32**(3), 772-778 (2016)

The continuously growing complexity of nanodrugs urges for complementary characterization techniques which can elude the current limitations. In this paper, the applicability of continuous contrast variation in small-angle X-ray scattering (SAXS) for the accurate size determination of a complex nanocarrier is demonstrated on the example of PEGylated liposomal doxorubicin (Caelyx). The mean size and average electron density of Caelyx was determined by SAXS using a gradient of aqueous iodixanol (Optiprep), an iso-osmolar suspending medium. The study is focused on the isoscattering point position and the analysis of the Guinier region of the scattering curves recorded at different solvent densities. An average diameter of (69 ± 5) nm and electron density of (346.2 ± 1.2) nm⁻³ were determined for the liposomal formulation of doxorubicin. The response of the liposomal nanocarrier to increasing solvent osmolality and the structure of the liposome-encapsulated doxorubicin after the osmotic shrinkage of the liposome are evaluated with sucrose contrast variation in SAXS and wide-angle X-ray scattering (WAXS). In the case of using sucrose as contrast agent, a clear osmolality threshold at 670 mOsm kg⁻¹ was observed, above which the liposomal drug carriers start to shrink, though preserving the intraliposomal doxorubicin structure. The average size obtained by this technique is smaller than the value measured by dynamic light scattering (DLS), though this difference is expected due to the hydrodynamic size of the PEG moieties attached to the liposomal surface, which are not probed with solvent contrast variation in SAXS. The advantages and drawbacks of the proposed technique are discussed in comparison to DLS, the most frequently used sizing method in nanomedicine.

2.250 High-Affinity Binding of Monomeric but Not Oligomeric Amyloid- β to Ganglioside GM1 Containing Nanodiscs

Thomaier, M., Gremer, L., Dammers, C., Fabig, J., Neudecker, P. and Willbold, D.
Biochemistry, **55**(48), 6662-6672 (2016)

The interaction of the amyloid- β protein (A β) with neuronal cell membranes plays a crucial role in Alzheimer’s disease. A β undergoes structural changes upon binding to ganglioside GM1 containing membranes leading to altered molecular characteristics of the protein. The physiological role of the A β interaction with the ganglioside GM1 is still unclear. In order to further elucidate the molecular requirements of A β membrane binding, we tested different nanodiscs varying in their lipid composition, regarding the charge of the headgroups as well as ganglioside GM1 concentration. Nanodiscs are excellent model membrane systems for studying protein membrane interactions, and we show here their suitability to investigate the membrane interaction of A β . In particular, we set out to investigate whether the binding activity of GM1 to A β is specific for the assembly state of A β and compared the binding affinities of monomeric with oligomeric A β . Using fluorescence titration experiments, we demonstrate high-affinity binding of A β (1–40) to GM1 containing nanodiscs, with dissociation constants, K_D , in the range from 25 to 41 nM, in a GM1 concentration-dependent manner. Biolayer interferometry experiments confirmed the

high-affinity binding of monomeric A β (1–40) (K_D of 24 nM to 49 nM) as well as of A β (1–42) (K_D of 30 nM) to GM1 containing nanodiscs, and no binding to phospholipid containing nanodiscs. Interestingly, and in contrast to monomeric A β , neither oligomeric A β (1–40) nor oligomeric A β (1–42) binds to GM1 nanodiscs. To the best of our knowledge, this is the first report of a loss of function for monomeric A β upon aggregation.

2.251 Plasma transport of ergocalciferol and cholecalciferol and their 25-hydroxylated metabolites in dairy cows

Hymøller, L. and Jensen, S.K.

Domestic Animal Endocrinology, **59**, 44-52 (2017)

In cattle, there are 2 significant forms of vitamin D: ergocalciferol (ERG) from fungi on roughage and cholecalciferol (CHO) from vitamin supplements or endogenous synthesis in the skin. The hypothesis of the present study is that vitamin D from the 3 sources is transported in different plasma fractions in the body. This is hypothesized to explain the lower efficiency of ERG compared to CHO in securing a sufficient plasma status of 25-hydroxyvitamin D and explain the inefficient excretion of dietary CHO into milk compared to endogenous CHO. Twenty vitamin D–depleted cows were assigned to 5 treatments: D2, housed indoor and fed 625- μ g/d (25,000 IU) ERG; D3, housed indoor and fed 625- μ g/d CHO; D2+D3, housed indoor and fed 625- μ g/d ERG and 625- μ g/d CHO; SUN, let out for daily pasture to facilitate CHO synthesis from sunlight; and D2+SUN, fed 625- μ g/d ERG and let out for daily pasture. Blood samples were taken twice weekly and plasma fractionated by ultracentrifugation into 3 fractions: light lipoprotein (LLP), heavy lipoprotein (HLP), and protein and analyzed for content of ERG and CHO and their liver derived metabolites 25-hydroxyergocalciferol (25ERG) and 25-hydroxycholecalciferol (25CHO), respectively. Liver biopsies were taken on the last day of the study to assess gene expression related to vitamin D metabolism. During 4 wk of study, the vitamin D status in plasma increased to 19.3 to 22.8 ng/mL 25ERG in ERG-treated cows with the highest concentration in D2 ($P \leq 0.05$) and to 25.0 to 33.4 ng/mL 25CHO in pasture or CHO-treated cows with the highest concentration in SUN ($P \leq 0.01$). In plasma fractions, CHO was mainly found in the HLP fraction, whereas 25CHO was almost exclusively found in the protein fraction, probably due to its reported high binding affinity to vitamin D–binding protein. About 70% to 90% of 25ERG was found in the protein fraction and the remaining 25ERG was found in HLP, whereas ERG was found in both HLP and LLP fractions. In liver tissue, the expression of vitamin D-25-hydroxylase was lower in D2+D3 ($P \leq 0.05$) and SUN ($P \leq 0.05$) than that in the remaining groups, and the vitamin D receptor was expressed in the liver to a larger extent in D2+SUN than that in D2+D3 ($P \leq 0.05$) and SUN ($P \leq 0.05$). In conclusion, different plasma transport mechanisms may explain the lower physiological efficiency of ERG compared to CHO in securing the vitamin D status in plasma but do not explain the lower efficiency of synthetic CHO compared to endogenous CHO from sunlight or UV light in securing a high CHO content in milk.

2.252 Omega-3 fatty acid fish oil dietary supplements contain saturated fats and oxidized lipids that may interfere with their intended biological benefits

Preston Mason, R. and Sherratt, A.C.R.

Biochem. Biophys. Res. Comm., **483**, 425-429 (2017)

Widely available fish oil dietary supplements (DS) may contain fats and oxidized lipids in addition to the beneficial omega-3 fatty acids (OM3FAs) for which they are purchased. Little is known about the potential biological effects of these oxidized lipids. The objective of this study was to assess the fatty acid content, oxidation products, and biological effects of leading fish oil DS available in the United States. Three top-selling fish oil DS in the US were included in this analysis. Fatty acid composition was measured using gas chromatography. Lipid oxidation (primary and secondary products) was measured by spectroscopy in both DS and a prescription OM3FA product. OM3FAs were also isolated and concentrated from DS and were tested for the ability to inhibit copper-induced oxidation of human small dense low-density lipoprotein particles (sdLDL) *in vitro*. Fish oil DS were found to contain more than 30 different fatty acids, including 10 to 14 different saturated species comprising up to 36% of the total fatty acid content. Levels of OM3FAs also varied widely among DS (33%–79%). Primary (peroxide), secondary (anisidine), and total oxidation products exceeded maximum levels established by international standards of quality in the DS but not the prescription OM3FA product. Oxidation of sdLDL was inhibited by >95% ($P < 0.001$) with non-oxidized forms of OM3FA but not with OM3FAs isolated from DS, which were a mixture of oxidized and non-oxidized OM3FAs. These data indicate that levels of saturated fat and oxidized OM3FAs found in common DS may interfere with their intended/potential biological benefits.

2.253 Controlling the gastrointestinal fate of nutraceutical and pharmaceutical-enriched lipid nanoparticles: From mixed micelles to chylomicrons

Yao, M., McClements, D.J., Zhao, F., Craig, R.W. and Xiao, H.
NanoImpact, **5(1)**, 13-21 (2017)

The oral bioavailability of lipophilic bioactive compounds such as many pharmaceuticals and nutraceuticals can be enhanced using triacylglycerol-based lipid nanoparticle delivery systems. These digestible lipid nanoparticles are disassembled in the gastrointestinal tract to form mixed micelles that solubilize and transport the lipophilic bioactives to the intestinal epithelium cells where they are absorbed. In these cells, the lipid digestion products and bioactive agents contained within the mixed micelles are then packaged into biological lipid protein nanoparticles (e.g., chylomicrons) that are secreted into the lymph. In this study, we examined the influence of fatty acid type (i.e., oleic acid, linoleic acid, and linolenic acid) on the properties of mixed micelles, cellular lipid droplets, and lipoprotein nanoparticles, and on the bioavailability of a highly lipophilic nutraceutical: 5-demethylnobiletin (5DN). There were distinct differences in the structural properties of lipoprotein nanoparticles formed depending on fatty acid unsaturation. Oleic acid (C_{18:1}) was most effective in enhancing intestinal uptake of 5DN and led to the formation of the largest chylomicrons. Linoleic acid (C_{18:2}) and linolenic acid (C_{18:3}) also promoted intestinal uptake of 5DN and formation of chylomicrons, but they were less efficient than oleic acid. The metabolism of 5DN within the intestinal epithelium cells was greatly reduced when 5DN was incorporated into chylomicrons, presumably because they were isolated from metabolic enzymes in the cytoplasm. These results have important implications for the rational design of lipid nanoparticle-based delivery systems for lipophilic nutraceuticals and pharmaceuticals by targeting them to the lymphatic circulation.

2.254 Anionic Phospholipids and the Alb3 Translocase Activate Signal Recognition Particle-Receptor Interaction during Light-harvesting Chlorophyll a/b-binding Protein Targeting

Chandrasekar, S. and Shan, S-o.
J. Biol. Chem., **292(1)**, 397-406 (2017)

The universally conserved signal recognition particle (SRP) co-translationally delivers newly synthesized membrane and secretory proteins to the target cellular membrane. The only exception is found in the chloroplast of green plants, where the chloroplast SRP (cpSRP) post-translationally targets light-harvesting chlorophyll a/b-binding proteins (LHCP) to the thylakoid membrane. The mechanism and regulation of this post-translational mode of targeting by cpSRP remain unclear. Using biochemical and biophysical methods, here we show that anionic phospholipids activate the cpSRP receptor cpFtsY to promote rapid and stable cpSRP54-cpFtsY complex assembly. Furthermore, the stromal domain of the Alb3 translocase binds with high affinity to and regulates GTP hydrolysis in the cpSRP54-cpFtsY complex, suggesting that cpFtsY is primarily responsible for initial recruitment of the targeting complex to Alb3. These results suggest a new model for the sequential recruitment, remodeling, and unloading of the targeting complex at membrane translocase sites in the post-translational cpSRP pathway.

2.255 Identification of Novel Functions for Hepatitis C Virus Envelope Glycoprotein E1 in Virus Entry and Assembly

Haddad, J.G., Rouille, Y., Hanouille, X., Descamps, V., Hamze, M., Dabbousi, F., Baumert, T.F., Duverlie, G., Lavie, M. and Dubuisson, J.
J. Virol., **91(8)**, e-00048-17 (2017)

Hepatitis C virus (HCV) envelope glycoprotein complex is composed of E1 and E2 subunits. E2 is the receptor-binding protein as well as the major target of neutralizing antibodies, whereas the functions of E1 remain poorly defined. Here, we took advantage of the recently published structure of the N-terminal region of the E1 ectodomain to interrogate the functions of this glycoprotein by mutating residues within this 79-amino-acid region in the context of an infectious clone. The phenotypes of the mutants were characterized to determine the effects of the mutations on virus entry, replication, and assembly. Furthermore, biochemical approaches were also used to characterize the folding and assembly of E1E2 heterodimers. Thirteen out of 19 mutations led to viral attenuation or inactivation. Interestingly, two attenuated mutants, T213A and I262A, were less dependent on claudin-1 for cellular entry in Huh-7 cells. Instead, these viruses relied on claudin-6, indicating a shift in receptor dependence for these two mutants in the target cell line. An unexpected phenotype was also observed for mutant D263A which was no longer infectious but still showed a good level of core protein secretion. Furthermore, genomic RNA was absent from these noninfectious viral particles, indicating that the D263A mutation leads to the assembly and release of viral particles devoid of genomic RNA. Finally, a change in subcellular colocalization between

HCV RNA and E1 was observed for the D263A mutant. This unique observation highlights for the first time cross talk between HCV glycoprotein E1 and the genomic RNA during HCV morphogenesis.

2.256 **Lipid Membrane Encapsulation of a 3D DNA Nano Octahedron**

Perrault, S.D. and Shih, W.M.

Methods in Mol. Biol., **1500**, 165-184 (2017)

Structural DNA nanotechnology methods such as DNA origami allow for the synthesis of highly precise nanometer-scale materials (Rothenmund, *Nature* 440:297–302, 2006; Douglas et al., *Nature* 459:414–418, 2009). These offer compelling advantages for biomedical applications. Such materials can suffer from structural instability in biological environments due to denaturation and nuclease digestion (Hahn et al., *ACS Nano* 2014; Perrault and Shih, *ACS Nano* 8:5132–5140, 2014). Encapsulation of DNA nanostructures in a lipid membrane compartmentalizes them from their environment and prevents denaturation and nuclease digestion (Perrault and Shih, *ACS Nano* 8:5132–5140, 2014). Here, we describe the encapsulation of a 50 nm DNA nanostructure having the geometry of a wireframe octahedron in a phospholipid membrane containing poly-(ethylene glycol), resulting in biocompatible DNA nanostructures.

2.257 **The Survival of Motor Neuron Protein Acts as a Molecular Chaperone for mRNP Assembly**

Donlin-Asp, P.G., Fallini, C., Campos, J., Chou, C.C., Merritt, C.C., Bassell, G.J. and Rossoll, W.

Cell Reports, **18**, 1660-1673 (2017)

Spinal muscular atrophy (SMA) is a motor neuron disease caused by reduced levels of the survival of motor neuron (SMN) protein. SMN is part of a multiprotein complex that facilitates the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). SMN has also been found to associate with mRNA-binding proteins, but the nature of this association was unknown. Here, we have employed a combination of biochemical and advanced imaging methods to demonstrate that SMN promotes the molecular interaction between IMP1 protein and the 3' UTR zipcode region of β -actin mRNA, leading to assembly of messenger ribonucleoprotein (mRNP) complexes that associate with the cytoskeleton to facilitate trafficking. We have identified defects in mRNP assembly in cells and tissues from SMA disease models and patients that depend on the SMN Tudor domain and explain the observed deficiency in mRNA localization and local translation, providing insight into SMA pathogenesis as a ribonucleoprotein (RNP)-assembly disorder.

2.258 **Protease resistance of infectious prions is suppressed by removal of a single atom in the cellular prion protein**

Leske, H., Hornemann, U., Zhu, C. et al

PloS One, **12(2)**, e0170503 (2017)

Resistance to proteolytic digestion has long been considered a defining trait of prions in tissues of organisms suffering from transmissible spongiform encephalopathies. Detection of proteinase K-resistant prion protein (PrP^{Sc}) still represents the diagnostic gold standard for prion diseases in humans, sheep and cattle. However, it has become increasingly apparent that the accumulation of PrP^{Sc} does not always accompany prion infections: high titers of prion infectivity can be reached also in the absence of protease resistant PrP^{Sc}. Here, we describe a structural basis for the phenomenon of protease-sensitive prion infectivity. We studied the effect on proteinase K (PK) resistance of the amino acid substitution Y169F, which removes a single oxygen atom from the β 2– α 2 loop of the cellular prion protein (PrP^C). When infected with RML or the 263K strain of prions, transgenic mice lacking wild-type (wt) PrP^C but expressing MoPrP^{169F} generated prion infectivity at levels comparable to wt mice. The newly generated MoPrP^{169F} prions were biologically indistinguishable from those recovered from prion-infected wt mice, and elicited similar pathologies *in vivo*. Surprisingly, MoPrP^{169F} prions showed greatly reduced PK resistance and density gradient analyses showed a significant reduction in high-density aggregates. Passage of MoPrP^{169F} prions into mice expressing wt MoPrP led to full recovery of protease resistance, indicating that no strain shift had taken place. We conclude that a subtle structural variation in the β 2– α 2 loop of PrP^C affects the sensitivity of PrP^{Sc} to protease but does not impact prion replication and infectivity. With these findings a specific structural feature of PrP^C can be linked to a physicochemical property of the corresponding PrP^{Sc}.

2.259 **CD63 Regulates Epstein-Barr Virus LMP1 Exosomal Packaging, Enhancement of Vesicle Production, and Noncanonical NF- κ B Signaling**

Hurwitz, S.N., Nkosi, D., Conlon, M.M., York, S.B., Liu, X., Tremblay, D.C. and Meckes, D.G.

Latent membrane protein 1 (LMP1) is an Epstein-Barr virus (EBV)-encoded oncoprotein that is packaged into small extracellular vesicles (EVs) called exosomes. Trafficking of LMP1 into multivesicular bodies (MVBs) alters the content and function of exosomes. LMP1-modified exosomes enhance the growth, migration, and invasion of malignant cells, demonstrating the capacity to manipulate the tumor microenvironment and enhance the progression of EBV-associated cancers. Despite the growing evidence surrounding the significance of LMP1-modified exosomes in cancer, very little is understood about the mechanisms that orchestrate LMP1 incorporation into these vesicles. Recently, LMP1 was shown to be copurified with CD63, a conserved tetraspanin protein enriched in late endosomal and lysosomal compartments. Here, we demonstrate the importance of CD63 presence for exosomal packaging of LMP1. Nanoparticle tracking analysis and gradient purification revealed an increase in extracellular vesicle secretion and exosomal proteins following LMP1 expression. Immunoprecipitation of CD63-positive exosomes exhibited accumulation of LMP1 in this vesicle population. Functionally, CRISPR/Cas9 knockout of CD63 resulted in a reduction of LMP1-induced particle secretion. Furthermore, LMP1 packaging was severely impaired in CD63 knockout cells, concomitant with a disruption in the perinuclear localization of LMP1. Importantly, LMP1 trafficking to lipid rafts and activation of NF- κ B and PI3K/Akt pathways remained intact following CD63 knockout, while mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and noncanonical NF- κ B activation were observed to be increased. These results suggest that CD63 is a critical player in LMP1 exosomal trafficking and LMP1-mediated enhancement of exosome production and may play further roles in limiting downstream LMP1 signaling.

2.260 Post-translational modifications in PrP expand the conformational diversity of prions in vivo

Aguilar-Calvo, P., Xiao, X., Bett, C., Erana, H., Soldau, K., Castilla, J., Nilsson, K.P.R., Surewicz, W.K. and Sigurdson, C.J.

Scientific Reports, **7**:43295 (2017)

Misfolded prion protein aggregates (PrP^{Sc}) show remarkable structural diversity and are associated with highly variable disease phenotypes. Similarly, other proteins, including amyloid- β , tau, α -synuclein, and serum amyloid A, misfold into distinct conformers linked to different clinical diseases through poorly understood mechanisms. Here we use mice expressing glycosylphosphatidylinositol (GPI)-anchorless prion protein, PrP^C, together with hydrogen-deuterium exchange coupled with mass spectrometry (HXMS) and a battery of biochemical and biophysical tools to investigate how post-translational modifications impact the aggregated prion protein properties and disease phenotype. Four GPI-anchorless prion strains caused a nearly identical clinical and pathological disease phenotype, yet maintained their structural diversity in the anchorless state. HXMS studies revealed that GPI-anchorless PrP^{Sc} is characterized by substantially higher protection against hydrogen/deuterium exchange in the C-terminal region near the N-glycan sites, suggesting this region had become more ordered in the anchorless state. For one strain, passage of GPI-anchorless prions into wild type mice led to the emergence of a novel strain with a unique biochemical and phenotypic signature. For the new strain, histidine hydrogen-deuterium mass spectrometry revealed altered packing arrangements of β -sheets that encompass residues 139 and 186 of PrP^{Sc}. These findings show how variation in post-translational modifications may explain the emergence of new protein conformations in vivo and also provide a basis for understanding how the misfolded protein structure impacts the disease.

2.261 Actin-Interacting Protein 1 Contributes to Intranuclear Rod Assembly in Dictyostelium discoideum

Ishikawa-Ankerhold, H.C., Daszkiewicz, W., Schleicher, M. and Müller-Taubenberger, A.

Scientific Reports, **7**:40310 (2017)

Intranuclear rods are aggregates consisting of actin and cofilin that are formed in the nucleus in consequence of chemical or mechanical stress conditions. The formation of rods is implicated in a variety of pathological conditions, such as certain myopathies and some neurological disorders. It is still not well understood what exactly triggers the formation of intranuclear rods, whether other proteins are involved, and what the underlying mechanisms of rod assembly or disassembly are. In this study, *Dictyostelium discoideum* was used to examine appearance, stages of assembly, composition, stability, and dismantling of rods. Our data show that intranuclear rods, in addition to actin and cofilin, are composed of a distinct set of other proteins comprising actin-interacting protein 1 (Aip1), coronin (CorA), filactin (Fia), and the 34 kDa actin-bundling protein B (AbpB). A finely tuned spatio-temporal pattern of protein recruitment was found during formation of rods. Aip1 is important for the final state of rod compaction indicating that Aip1 plays a major role in shaping the intranuclear rods. In the absence of both Aip1 and CorA, rods are not formed in the nucleus, suggesting that a sufficient supply of monomeric actin is a prerequisite for rod

formation.

2.262 Enhanced neuroinvasion by smaller, soluble prions

Bett, C., Lawrence, J., Kurt, T.D., Orru, C., Aguilar-Calvo, P., Kincaid, A.E., Surewicz, W.K., Caughey, B., Wu, C. and Sigurdson, C.J.

Acta Neuropathol. Commun., 5:32 (2017)

Infectious prion aggregates can propagate from extraneural sites into the brain with remarkable efficiency, likely transported via peripheral nerves. Yet not all prions spread into the brain, and the physical properties of a prion that is capable of transit within neurons remain unclear. We hypothesized that small, diffusible aggregates spread into the CNS via peripheral nerves. Here we used a structurally diverse panel of prion strains to analyze how the prion conformation impacts transit into the brain. Two prion strains form fibrils visible ultrastructurally in the brain in situ, whereas three strains form diffuse, subfibrillar prion deposits and no visible fibrils. The subfibrillar strains had significantly higher levels of soluble prion aggregates than the fibrillar strains. Primary neurons internalized both the subfibrillar and fibril-forming prion strains by macropinocytosis, and both strain types were transported from the axon terminal to the cell body in vitro. However in mice, only the predominantly soluble, subfibrillar prions, and not the fibrillar prions, were efficiently transported from the tongue to the brain. Sonication of a fibrillar prion strain increased the solubility and enabled prions to spread into the brain in mice, as evident by a 40% increase in the attack rate, indicating that an increase in smaller particles enhances prion neuroinvasion. Our data suggest that the small, highly soluble prion particles have a higher capacity for transport via nerves. These findings help explain how prions that predominantly assemble into subfibrillar states can more effectively traverse into and out of the CNS, and suggest that promoting fibril assembly may slow the neuron-to-neuron spread of protein aggregates.

2.263 Multiple pathogen biomarker detection using an encoded bead array in droplet PCR

Rajeswari, P.K.P., Soderberg, L.M., Yacoub, A., Leijon, M., Andersson Svahn, H. and Joensson, H.N.

J. Microbiol. Methods, 139, 22-28 (2017)

We present a droplet PCR workflow for detection of multiple pathogen DNA biomarkers using fluorescent color-coded Luminex® beads. This strategy enables encoding of multiple singleplex droplet PCRs using a commercially available bead set of several hundred distinguishable fluorescence codes. This workflow provides scalability beyond the limited number offered by fluorescent detection probes such as TaqMan probes, commonly used in current multiplex droplet PCRs. The workflow was validated for three different Luminex bead sets coupled to target specific capture oligos to detect hybridization of three microorganisms infecting poultry: avian influenza, infectious laryngotracheitis virus and *Campylobacter jejuni*. In this assay, the target DNA was amplified with fluorescently labeled primers by PCR in parallel in monodisperse picoliter droplets, to avoid amplification bias. The color codes of the Luminex detection beads allowed concurrent and accurate classification of the different bead sets used in this assay. The hybridization assay detected target DNA of all three microorganisms with high specificity, from samples with average target concentration of a single DNA template molecule per droplet. This workflow demonstrates the possibility of increasing the droplet PCR assay detection panel to detect large numbers of targets in parallel, utilizing the scalability offered by the color-coded Luminex detection beads.

2.264 Covalently linked dengue virus envelope glycoprotein dimers reduce exposure of the immunodominant fusion loop epitope

Rouvinski, A. et al

Nature Communications, 8:15411 (2017)

A problem in the search for an efficient vaccine against dengue virus is the immunodominance of the fusion loop epitope (FLE), a segment of the envelope protein E that is buried at the interface of the E dimers coating mature viral particles. Anti-FLE antibodies are broadly cross-reactive but poorly neutralizing, displaying a strong infection enhancing potential. FLE exposure takes place via dynamic 'breathing' of E dimers at the virion surface. In contrast, antibodies targeting the E dimer epitope (EDE), readily exposed at the E dimer interface over the region of the conserved fusion loop, are very potent and broadly neutralizing. We here engineer E dimers locked by inter-subunit disulfide bonds, and show by X-ray crystallography and by binding to a panel of human antibodies that these engineered dimers do not expose the FLE, while retaining the EDE exposure. These locked dimers are strong immunogen candidates

for a next-generation vaccine.

2.265 **Phosphorylation of TXNIP by AKT Mediates Acute Influx of Glucose in Response to Insulin**

Waldhart, A.N., Dykstra, H., Peck, A.S., Cantley, L.C., Graw, T.E. and Wu, N.
Cell Reports, **19**, 2005-2013 (2017)

Growth factors, such as **insulin**, can induce both acute and long-term **glucose** uptake into cells. Apart from the rapid, insulin-induced fusion of **glucose transporter (GLUT)4** storage **vesicles** with the **cell surface** that occurs in **muscle** and **adipose tissues**, the mechanism behind acute induction has been unclear in other systems. **Thioredoxin** interacting protein (TXNIP) has been shown to be a negative regulator of cellular glucose uptake. TXNIP is transcriptionally induced by glucose and reduces glucose influx by promoting GLUT1 **endocytosis**. Here, we report that TXNIP is a direct substrate of **protein kinase B (AKT)** and is responsible for mediating AKT-dependent acute glucose influx after **growth factor** stimulation. Furthermore, TXNIP functions as an adaptor for the basal endocytosis of GLUT4 **in vivo**, its absence allows excess glucose uptake in muscle and **adipose tissues**, causing **hypoglycemia** during fasting. Altogether, TXNIP serves as a key node of signal regulation and response for modulating glucose influx through GLUT1 and GLUT4.

2.266 **Apolipoprotein(a) inhibits hepatitis C virus entry through interaction with infectious particles**

Oliveira, C. et al
Hepatology, **65**(6), 1851-1864 (2017)

The development of different cell culture models has greatly contributed to increased understanding of the hepatitis C virus (HCV) life cycle. However, it is still challenging to grow HCV clinical isolates in cell culture. If overcome, this would open new perspectives to study HCV biology, including drug-resistant variants emerging with new antiviral therapies. In this study we hypothesized that this hurdle could be due to the presence of inhibitory factors in patient serum. Combining polyethylene glycol precipitation, iodixanol gradient, and size-exclusion chromatography, we obtained from HCV-seronegative sera a purified fraction enriched in inhibitory factors. Mass spectrometric analysis identified apolipoprotein(a) (apo[a]) as a potential inhibitor of HCV entry. Apo(a) consists of 10 kringle IV domains (KIVs), one kringle V domain, and an inactive protease domain. The 10 KIVs are present in a single copy with the exception of KIV type 2 (KIV2), which is encoded in a variable number of tandemly repeated copies, giving rise to numerous apo(a) size isoforms. In addition, apo(a) covalently links to the apolipoprotein B component of a low-density lipoprotein through a disulfide bridge to form lipoprotein(a). Using a recombinant virus derived from the JFH1 strain, we confirmed that plasma-derived and recombinant lipoprotein(a) as well as purified recombinant apo(a) variants were able to specifically inhibit HCV by interacting with infectious particles. Our results also suggest that small isoforms are less inhibitory than the large ones. Finally, we observed that the lipoprotein moiety of HCV lipovirions was essential for inhibition, whereas functional lysine-binding sites in KIV7, KIV8, and KIV10 were not required. Conclusions: Our results identify apo(a) as an additional component of the lipid metabolism modulating HCV infection.

2.267 **Heterogeneous Defect Domains in Single-Crystalline Hexagonal WS₂**

Jeong, H.Y., Jin, Y., Yun, S.J., Zhao, J., Baik, J., Keum, D.H., Lee, H.S. and Lee, Y.H.
Advanced Materials, **29**:160543 (2017)

Single-crystalline monolayer hexagonal WS₂ is segmented into alternating triangular domains: sulfur-vacancy (SV)-rich and tungsten-vacancy (WV)-rich domains. The WV-rich domain with deep-trap states reveals an electron-dedoping effect, and the electron mobility and photoluminescence are lower than those of the SV-rich domain with shallow-donor states by one order of magnitude. The vacancy-induced strain and doping effects are investigated via Raman and scanning photoelectron microscopy.

2.268 **Placing and shaping liposomes with reconfigurable DNA nanocages**

Zhang, Z., Yang, Y., Pincet, F., Llaguno, M.C. and Lin, C.
Nature Chem., **9**(7), 653-659 (2017)

The diverse structure and regulated deformation of lipid bilayer membranes are among a cell's most fascinating features. Artificial membrane-bound vesicles, known as liposomes, are versatile tools for modelling biological membranes and delivering foreign objects to cells. To fully mimic the complexity of cell membranes and optimize the efficiency of delivery vesicles, controlling liposome shape (both

statically and dynamically) is of utmost importance. Here we report the assembly, arrangement and remodelling of liposomes with designer geometry: all of which are exquisitely controlled by a set of modular, reconfigurable DNA nanocages. Tubular and toroid shapes, among others, are transcribed from DNA cages to liposomes with high fidelity, giving rise to membrane curvatures present in cells yet previously difficult to construct *in vitro*. Moreover, the conformational changes of DNA cages drive membrane fusion and bending with predictable outcomes, opening up opportunities for the systematic study of membrane mechanics.

- 2.269 Discrete cytosolic macromolecular BRAF complexes exhibit distinct activities and composition**
Diedrich, B., Rigbolt, K.T.G., Röring, M., Herr, R., Kaeser-Pebernard, S., Gretzmeier, C., Murphy, R.F., Brummer, T. and Dengjel, J.
EMBO J., **36**(5), 646-663 (2017)

As a central element within the RAS/ERK pathway, the serine/threonine kinase BRAF plays a key role in development and homeostasis and represents the most frequently mutated kinase in tumors. Consequently, it has emerged as an important therapeutic target in various malignancies. Nevertheless, the BRAF activation cycle still raises many mechanistic questions as illustrated by the paradoxical action and side effects of RAF inhibitors. By applying SEC-PCP-SILAC, we analyzed protein-protein interactions of hyperactive BRAF^{V600E} and wild-type BRAF (BRAF^{WT}). We identified two macromolecular, cytosolic BRAF complexes of distinct molecular composition and phosphorylation status. Hyperactive BRAF^{V600E} resides in large complexes of higher molecular mass and activity, while BRAF^{WT} is confined to smaller, slightly less active complexes. However, expression of oncogenic K-Ras^{G12V}, either by itself or in combination with RAF dimer promoting inhibitors, induces the incorporation of BRAF^{WT} into large, active complexes, whereas pharmacological inhibition of BRAF^{V600E} has the opposite effect. Thus, the quaternary structure of BRAF complexes is shaped by its activation status, the conformation of its kinase domain, and clinically relevant inhibitors.

- 2.270 Impact of Lipid Phase on the Bioavailability of Vitamin E in Emulsion-Based Delivery Systems: Relative Importance of Bioaccessibility, Absorption, and Transformation**
Yang, Y., Xiao, H. and McClements, D.J.
Agric. Food Chem., **65**, 3946-3955 (2017)

A simulated gastrointestinal tract/Caco-2 cell culture model was used to investigate the effects of lipid phase type on vitamin E (VE) bioavailability. Oil-in-water emulsions fortified with α -tocopherol acetate were fabricated using a natural emulsifier (quillaja saponin) and long or medium chain triglycerides (LCTs or MCTs) as lipids. The impact of lipid type on VE bioaccessibility, absorption, and transformation was determined. VE bioaccessibility was greater for LCT (46%) than MCT (19%) due to greater solubilization in mixed micelles assembled from longer fatty acids. VE absorption by Caco-2 cells was similar for LCT (28%) and MCT (30%). The transformation of α -tocopherol acetate to α -tocopherol was higher for LCT (90%) than MCT (75%) due to differences in esterase accessibility to VE. Emulsion-based delivery systems formulated using LCT are therefore more suitable for encapsulating and delivering vitamin E than those formulated using MCT.

- 2.271 Microfluidic platform for efficient Nanodisc assembly, membrane protein incorporation, and purification**
Wade, J.H., Jones, J.D., Ienov, I., Riordan, C.M., Sligar, S.G. and Bailey, R.C.
Lab on a Chip, **17**, 2951-2959 (2017)

The characterization of integral membrane proteins presents numerous analytical challenges on account of their poor activity under non-native conditions, limited solubility in aqueous solutions, and low expression in most cell culture systems. Nanodiscs are synthetic model membrane constructs that offer many advantages for studying membrane protein function by offering a native-like phospholipid bilayer environment. The successful incorporation of membrane proteins within Nanodiscs requires experimental optimization of conditions. Standard protocols for Nanodisc formation can require large amounts of time and input material, limiting the facile screening of formation conditions. Capitalizing on the miniaturization and efficient mass transport inherent to microfluidics, we have developed a microfluidic platform for efficient Nanodisc assembly and purification, and demonstrated the ability to incorporate functional membrane proteins into the resulting Nanodiscs. In addition to working with reduced sample volumes, this platform simplifies membrane protein incorporation from a multi-stage protocol requiring several hours or days into a single platform that outputs purified Nanodiscs in less than one hour. To

demonstrate the utility of this platform, we incorporated Cytochrome P450 into Nanodiscs of variable size and lipid composition, and present spectroscopic evidence for the functional active site of the membrane protein. This platform is a promising new tool for membrane protein biology and biochemistry that enables tremendous versatility for optimizing the incorporation of membrane proteins using microfluidic gradients to screen across diverse formation conditions.

2.272 Reversible unfolding of infectious prion assemblies reveals the existence of an oligomeric elementary brick

Igel-Egalon, A., Moudjou, M., Martin, D., Busley, A., Knäpple, T., Herzog, L., Reine, F., Lepajova, N., Richard, C-A., Beringue, V. and Rezaei, H.
PLoS Pathogens, **13**(9), e1006557 (2017)

Mammalian prions, the pathogens that cause transmissible spongiform encephalopathies, propagate by self-perpetuating the structural information stored in the abnormally folded, aggregated conformer (PrP^{Sc}) of the host-encoded prion protein (PrP^C). To date, no structural model related to prion assembly organization satisfactorily describes how strain-specified structural information is encoded and by which mechanism this information is transferred to PrP^C. To achieve progress on this issue, we correlated the PrP^{Sc} quaternary structural transition from three distinct prion strains during unfolding and refolding with their templating activity. We reveal the existence of a mesoscopic organization in PrP^{Sc} through the packing of a highly stable oligomeric elementary subunit (suPrP), in which the strain structural determinant (SSD) is encoded. Once kinetically trapped, this elementary subunit reversibly loses all replicative information. We demonstrate that acquisition of the templating interface and infectivity requires structural rearrangement of suPrP, in concert with its condensation. The existence of such an elementary brick scales down the SSD support to a small oligomer and provide a basis of reflexion for prion templating process and propagation.

2.273 A human APOC3 missense variant and monoclonal antibody accelerate apoC-III clearance and lower triglyceride-rich lipoprotein levels

Khetarpal, S.A. et al
Nature Med., **23**(9), 1086-1094 (2017)

Recent large-scale genetic sequencing efforts have identified rare coding variants in genes in the triglyceride-rich lipoprotein (TRL) clearance pathway that are protective against coronary heart disease (CHD), independently of LDL cholesterol (LDL-C) levels¹. Insight into the mechanisms of protection of these variants may facilitate the development of new therapies for lowering TRL levels. The gene APOC3 encodes apoC-III, a critical inhibitor of triglyceride (TG) lipolysis and remnant TRL clearance². Here we report a detailed interrogation of the mechanism of TRL lowering by the APOC3 Ala43Thr (A43T) variant, the only missense (rather than protein-truncating) variant in APOC3 reported to be TG lowering and protective against CHD^{3, 4, 5}. We found that both human APOC3 A43T heterozygotes and mice expressing human APOC3 A43T display markedly reduced circulating apoC-III levels. In mice, this reduction is due to impaired binding of A43T apoC-III to lipoproteins and accelerated renal catabolism of free apoC-III. Moreover, the reduced content of apoC-III in TRLs resulted in accelerated clearance of circulating TRLs. On the basis of this protective mechanism, we developed a monoclonal antibody targeting lipoprotein-bound human apoC-III that promotes circulating apoC-III clearance in mice expressing human APOC3 and enhances TRL catabolism in vivo. These data reveal the molecular mechanism by which a missense variant in APOC3 causes reduced circulating TG levels and, hence, protects from CHD. This protective mechanism has the potential to be exploited as a new therapeutic approach to reduce apoC-III levels and circulating TRL burden.

2.274 Metalloprotease-mediated cleavage of PlexinD1 and its sequestration to actin rods in the motoneuron disease spinal muscular atrophy (SMA)

Rademacher, S., Verheijen, B.M., Hensel, N., Peters, M., Bora, G., Brandes, G., de Sa, R.V., Heidrich, N., Fischer, S., Brinkmann, H., van der Pol, W.L., Wirth, B., Pasterkamp, R.J. and Claus, P.
Hum. Mol. Genet., **26**(20), 3946-3959 (2017)

Cytoskeletal rearrangement during axon growth is mediated by guidance receptors and their ligands which act either as repellent, attractant or both. Regulation of the actin cytoskeleton is disturbed in Spinal Muscular Atrophy (SMA), a devastating neurodegenerative disease affecting mainly motoneurons, but receptor-ligand interactions leading to the dysregulation causing SMA are poorly understood. In this study,

we analysed the role of the guidance receptor PlexinD1 in SMA pathogenesis. We showed that PlexinD1 is cleaved by metalloproteases in SMA and that this cleavage switches its function from an attractant to repellent. Moreover, we found that the PlexinD1 cleavage product binds to actin rods, pathological aggregate-like structures which had so far been described for age-related neurodegenerative diseases. Our data suggest a novel disease mechanism for SMA involving formation of actin rods as a molecular sink for a cleaved PlexinD1 fragment leading to dysregulation of receptor signaling.

Conclusions Our findings highlight a new mechanism in lipid metabolism regulation and interaction of the lipid metabolism with the HCV life cycle, which may be important for viral pathogenesis and might also be explored for antiviral therapy.

2.275 **Human Herpesvirus 8 Interleukin-6 Interacts with Calnexin Cycle Components and Promotes Protein Folding**

Chen, D., Xiang, Q. and Nicholas, J.
J. Virol., **91**(22), e00965-17 (2017)

Viral interleukin-6 (vIL-6) encoded by human herpesvirus 8 (HHV-8) is believed to contribute via mitogenic, survival, and angiogenic activities to HHV-8-associated Kaposi's sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman's disease through autocrine or paracrine mechanisms during latency or productive replication. There is direct evidence that vIL-6 promotes latently infected PEL cell viability and proliferation and also viral productive replication in PEL and endothelial cells. These activities are mediated largely through endoplasmic reticulum (ER)-localized vIL-6, which can induce signal transduction via the gp130 signaling receptor, activating mitogen-activated protein kinase and signal transducer and activator of transcription signaling, and interactions of vIL-6 with the ER membrane protein vitamin K epoxide reductase complex subunit 1 variant 2 (VKORC1v2). The latter functional axis involves suppression of proapoptotic lysosomal protein cathepsin D by promotion of the ER-associated degradation of ER-transiting, preproteolytically processed procathepsin D. Other interactions of VKORC1v2 and activities of vIL-6 via the receptor have not been reported. We show here that both vIL-6 and VKORC1v2 interact with calnexin cycle proteins UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1), which catalyzes monoglucosylation of N-glycans, and oppositely acting glucosidase II (GlucII), and that vIL-6 can promote protein folding. This activity was found to require VKORC1v2 and UGGT1, to involve vIL-6 associations with VKORC1v2, UGGT1, and GlucII, and to operate in the context of productively infected cells. These findings document new VKORC1v2-associated interactions and activities of vIL-6, revealing novel mechanisms of vIL-6 function within the ER compartment.

2.276 **Optimization of d-Peptides for A β Monomer Binding Specificity Enhances Their Potential to Eliminate Toxic A β Oligomers**

Klein, A.N., Ziehm, T., van Groen, T., Kadish, I., Elfgen, A., Tusche, M., Thomaier, M., Reiss, K., Brener, O., Gremer, I., Kutzsche, J. and Wilbold, D.
ACS Chem. Neurosci., **8**, 1889-1900 (2017)

Amyloid-beta (A β) oligomers are thought to be causative for the development and progression of Alzheimer's disease (AD). Starting from the A β oligomer eliminating d-enantiomeric peptide D3, we developed and applied a two-step procedure based on peptide microarrays to identify D3 derivatives with increased binding affinity and specificity for monomeric A β (1-42) to further enhance the A β oligomer elimination efficacy. Out of more than 1000 D3 derivatives, we selected seven novel d-peptides, named ANK1 to ANK7, and characterized them in more detail in vitro. All ANK peptides bound to monomeric A β (1-42), eliminated A β (1-42) oligomers, inhibited A β (1-42) fibril formation, and reduced A β (1-42)-induced cytotoxicity more efficiently than D3. Additionally, ANK6 completely inhibited the prion-like propagation of preformed A β (1-42) seeds and showed a nonsignificant tendency for improving memory performance of tg-APP^{SwDI} mice after i.p. application for 4 weeks. This supports the hypothesis that stabilization of A β monomers and thereby induced elimination of A β oligomers is a suitable therapeutic strategy.

2.277 **How We Make DNA Origami**

Wagenbauer, K.F., Engelhardt, F.A.S., Stahl, E., Hechtel, V.K., Stömmer, P., Seebacher, F., Meregalli, L., Ketterer, P., Gerling, T. and Dietz, H.
ChemBioChem., **18**(19), 1873-1885 (2017)

DNA origami has attracted substantial attention since its invention ten years ago, due to the seemingly

infinite possibilities that it affords for creating customized nanoscale objects. Although the basic concept of DNA origami is easy to understand, using custom DNA origami in practical applications requires detailed know-how for designing and producing the particles with sufficient quality and for preparing them at appropriate concentrations with the necessary degree of purity in custom environments. Such know-how is not readily available for newcomers to the field, thus slowing down the rate at which new applications outside the field of DNA nanotechnology may emerge. To foster faster progress, we share in this article the experience in making and preparing DNA origami that we have accumulated over recent years. We discuss design solutions for creating advanced structural motifs including corners and various types of hinges that expand the design space for the more rigid multilayer DNA origami and provide guidelines for preventing undesired aggregation and on how to induce specific oligomerization of multiple DNA origami building blocks. In addition, we provide detailed protocols and discuss the expected results for five key methods that allow efficient and damage-free preparation of DNA origami. These methods are agarose-gel purification, filtration through molecular cut-off membranes, PEG precipitation, size-exclusion chromatography, and ultracentrifugation-based sedimentation. The guide for creating advanced design motifs and the detailed protocols with their experimental characterization that we describe here should lower the barrier for researchers to accomplish the full DNA origami production workflow.

2.278 The A β oligomer eliminating D-enantiomeric peptide RD2 improves cognition without changing plaque pathology

Van Groen, T., Schemmert, S., Brener, O., Gremer, I., Ziehm, T., Tusche, M., Nagel-Steger, L., Kadish, I., Schartmann, E., Elfgen, A., Jürgens, D., Willuweit, A., Kutzsche, J. and Willbold, D.
Scientific Reports, 7.16275 (2017)

While amyloid- β protein (A β) aggregation into insoluble plaques is one of the pathological hallmarks of Alzheimer's disease (AD), soluble oligomeric A β has been hypothesized to be responsible for synapse damage, neurodegeneration, learning, and memory deficits in AD. Here, we investigate the *in vitro* and *in vivo* efficacy of the d-enantiomeric peptide RD2, a rationally designed derivative of the previously described lead compound D3, which has been developed to efficiently eliminate toxic A β 42 oligomers as a promising treatment strategy for AD. Besides the detailed *in vitro* characterization of RD2, we also report the results of a treatment study of APP/PS1 mice with RD2. After 28 days of treatment we observed enhancement of cognition and learning behaviour. Analysis on brain plaque load did not reveal significant changes, but a significant reduction of insoluble A β 42. Our findings demonstrate that RD2 was significantly more efficient in A β oligomer elimination *in vitro* compared to D3. Enhanced cognition without reduction of plaque pathology in parallel suggests that synaptic malfunction due to A β oligomers rather than plaque pathology is decisive for disease development and progression. Thus, A β oligomer elimination by RD2 treatment may be also beneficial for AD patients.

2.279 UNC-45a promotes myosin folding and stress fiber assembly

Lehtimäki, J.I., Fenix, A.M., Kotila, T.M., Balistreri, G., Paavolainen, L., varjosalo, M., Burnette, D.T. and Lappalainen, P.
J. Cell Biol., 216(12), 4053-4072 (2017)

Contractile actomyosin bundles, stress fibers, are crucial for adhesion, morphogenesis, and mechanosensing in nonmuscle cells. However, the mechanisms by which nonmuscle myosin II (NM-II) is recruited to those structures and assembled into functional bipolar filaments have remained elusive. We report that UNC-45a is a dynamic component of actin stress fibers and functions as a myosin chaperone *in vivo*. UNC-45a knockout cells display severe defects in stress fiber assembly and consequent abnormalities in cell morphogenesis, polarity, and migration. Experiments combining structured-illumination microscopy, gradient centrifugation, and proteasome inhibition approaches revealed that a large fraction of NM-II and myosin-1c molecules fail to fold in the absence of UNC-45a. The remaining properly folded NM-II molecules display defects in forming functional bipolar filaments. The C-terminal UNC-45/Cro1/She4p domain of UNC-45a is critical for NM-II folding, whereas the N-terminal tetratricopeptide repeat domain contributes to the assembly of functional stress fibers. Thus, UNC-45a promotes generation of contractile actomyosin bundles through synchronized NM-II folding and filament-assembly activities.

2.280 A stretch of residues within the protease-resistant core is not necessary for prion structure and infectivity

Munoz-Montesino, C., Sizun, C., Moudjou, M., Herzog, L., Reine, F., Igel-Egalon, A., Barbereau, C., Chapuis, J., Ciric, D., Laude, H., Beringue, V., Rezaei, H. and Dron, M.

Mapping out regions of PrP influencing prion conversion remains a challenging issue complicated by the lack of prion structure. The portion of PrP associated with infectivity contains the α -helical domain of the correctly folded protein and turns into a β -sheet-rich insoluble core in prions. Deletions performed so far inside this segment essentially prevented the conversion. Recently we found that deletion of the last C-terminal residues of the helix H2 was fully compatible with prion conversion in the RK13-ovPrP cell culture model, using 3 different infecting strains. This was in agreement with preservation of the overall PrP^C structure even after removal of up to one-third of this helix. Prions with internal deletion were infectious for cells and mice expressing the wild-type PrP and they retained prion strain-specific characteristics. We thus identified a piece of the prion domain that is neither necessary for the conformational transition of PrP^C nor for the formation of a stable prion structure.

2.281 **Circulating ApoJ is closely associated with insulin resistance in human subjects**

Seo, J.A., Kang, M-C., Ciaraldi, T.P., Kim, S.S., Park, K.S., Choe, C., Hwang, W.H., Lim, D.M., Farr, O., Mantzoros, C., Henry, R. and Kim, Y-B.
Metabolism, **78**, 155-166 (2018)

Objective

[Insulin](#) resistance is a major risk factor for [type 2 diabetes](#). ApolipoproteinJ (ApoJ) has been implicated in altered [pathophysiologic](#) states including cardiovascular and [Alzheimer's disease](#). However, the function of ApoJ in regulation of [glucose](#) homeostasis remains unclear. This study sought to determine whether serum ApoJ levels are associated with insulin resistance in human subjects and if they change after interventions that improve insulin sensitivity.

Methods

Serum ApoJ levels and insulin resistance status were assessed in nondiabetic (ND) and type 2 diabetic (T2D) subjects. The impacts of [rosiglitazone](#) or [metformin](#) therapy on serum ApoJ levels and glucose disposal rate (GDR) during a [hyperinsulinemic/euglycemic clamp](#) were evaluated in a separate cohort of T2D subjects. Total ApoJ protein or that associated with the HDL and LDL fractions was measured by [immunoblotting](#) or ELISA.

Results

Fasting serum ApoJ levels were greatly elevated in T2D subjects (ND vs T2D; 100 ± 8.3 vs. 150.6 ± 8.5 AU, $P < 0.0001$). Circulating ApoJ levels strongly correlated with [fasting glucose](#), fasting insulin, [HOMA-IR](#), and BMI. ApoJ levels were significantly and independently associated with HOMA-IR, even after adjustment for age, sex, and BMI. Rosiglitazone treatment in T2D subjects resulted in a reduction in serum ApoJ levels (before vs. after treatment; 100 ± 13.9 vs. 77 ± 15.2 AU, $P = 0.015$), whereas metformin had no effect on ApoJ levels. The change in ApoJ levels during treatment was inversely associated with the change in GDR. Interestingly, ApoJ content in the LDL fraction was inversely associated with HOMA-IR.

Conclusion

Serum ApoJ levels are closely correlated with the magnitude of insulin resistance regardless of obesity, and decrease along with improvement of insulin resistance in response only to rosiglitazone in type 2 diabetes.

2.282 **Eicosapentaenoic acid inhibits oxidation of high density lipoprotein particles in a manner distinct from docosahexaenoic acid**

Sherratt, S.C.R. and mason, R.P.

Biochem. Biophys. Res. Comm., **496**, 315-338 (2018)

The [omega-3 fatty acid eicosapentaenoic acid](#) (EPA) reduces oxidation of ApoB-containing particles *in vitro* and in patients with [hypertriglyceridemia](#). EPA may produce these effects through a potent antioxidant mechanism, which may facilitate LDL clearance and slow plaque progression. We hypothesize that EPA antioxidant effects may extend to ApoA-containing particles like HDL, potentially preserving certain atheroprotective functions. HDL was isolated from [human plasma](#) and incubated at 37 °C in the absence (vehicle) or presence of EPA and/or DHA; 5.0 or 10.0 μ M each. Samples were then subjected to copper-induced oxidation (10 μ M). HDL oxidation was inhibited similarly by EPA and DHA up to 1 h. EPA (10 μ M) maintained significant HDL oxidation inhibition of 89% (0.622 ± 0.066 μ M MDA; $p < .001$) at 4 h, with continued inhibition of 64% at 14 h, vs. vehicle (5.65 ± 0.06 to 2.01 ± 0.10 μ M MDA; $p < .001$). Conversely, DHA (10 μ M) antioxidant benefit was lost by 4 h. At a lower concentration (5 μ M), EPA antioxidant activity remained at 81% (5.53 ± 0.15 to 1.03 ± 0.10 μ M MDA; $p < .001$) at 6 h, while DHA

lost all antioxidant activity by 4 h. The antioxidant activity of EPA was preserved when combined with an equimolar concentration of DHA (5 μ M each). EPA pretreatment prevented HDL oxidation in a dose-dependent manner that was preserved over time. These results suggest unique lipophilic and electron stabilization properties for EPA as compared to DHA with respect to inhibition of HDL oxidation. These antioxidant effects of EPA may enhance certain atheroprotective functions for HDL.

2.283 Update on the laboratory investigation of dyslipidemias

Ramasamy, I.

Clin. Chim. Acta, **479**, 103-125 (2018)

The role of the clinical laboratory is evolving to provide more information to clinicians to assess [cardiovascular disease](#) (CVD) risk and target therapy more effectively. Current routine methods to measure [LDL-cholesterol](#) (LDL-C), the Friedewald calculation, ultracentrifugation, [electrophoresis](#) and homogeneous direct methods have established limitations. Studies suggest that LDL and HDL size or particle concentration are alternative methods to predict future CVD risk. At this time there is no consensus role for [lipoprotein](#) particle or subclasses in CVD risk assessment. LDL and HDL particle concentration are measured by several methods, namely gradient gel electrophoresis, ultracentrifugation-vertical auto profile, nuclear magnetic resonance and [ion mobility](#). It has been suggested that HDL functional assays may be better predictors of CVD risk. To assess the issue of lipoprotein subclasses/particles and HDL function as potential CVD risk markers robust, simple, validated [analytical methods](#) are required. In patients with small dense LDL particles, even a perfect measure of LDL-C will not reflect LDL particle concentration. [Non-HDL-C](#) is an alternative measurement and includes [VLDL](#) and CM remnant cholesterol and LDL-C. However, [apolipoprotein B](#) measurement may more accurately reflect LDL particle numbers. Non-fasting lipid measurements have many practical advantages. Defining thresholds for treatment with new measurements of CVD risk remain a challenge. In families with genetic variants, [ApoCIII](#) and lipoprotein (a) may be additional risk factors. Recognition of familial causes of [dyslipidemias](#) and diagnosis in childhood will result in early treatment. This review discusses the limitations in current [laboratory technologies](#) to predict CVD risk and reviews the evidence for emergent approaches using newer biomarkers in clinical practice.

2.284 Isolation and Characterization of Endogenous RNPs from Brain Tissues

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Methods in Mol. Biol., **1649**, 419-426 (2018)

Identification of physiological target RNAs and protein interactors bound to RNA-binding proteins is a key prerequisite to understand the underlying mechanisms of posttranscriptional expression control and RNA granule assembly. Here, we describe a multistep biochemical approach to isolate endogenous ribonucleoprotein particles from brain tissues by exploiting differential centrifugation and gradient fractionation followed by immunoprecipitation with monospecific, affinity-purified antibodies directed against selected RNA-binding proteins. This protocol results in highly enriched endogenous ribonucleoprotein particles that then can be analyzed by mass spectrometry (for proteins composition) and microarray or RNA sequencing technologies (for target mRNAs).

2.285 Functional innate immunity restricts Hepatitis C Virus infection in induced pluripotent stem cell-derived hepatocytes

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Knowledge of activation and interplay between the hepatitis C virus (HCV) and the hosts' innate immunity is essential to understanding the establishment of chronic HCV infection. Human hepatoma cell lines, widely used as HCV cell culture system, display numerous metabolic alterations and a defective innate immunity, hindering the detailed study of virus-host interactions. Here, we analysed the suitability of induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (iHLCs) as a physiologically relevant model to study HCV replication *in vitro*. Density gradients and triglyceride analysis revealed that iHLCs secreted very-low density lipoprotein (VLDL)-like lipoproteins, providing a putative platform for *bona fide* lipoviroparticles. iHLCs supported the full HCV life cycle, but in contrast to Huh7 and Huh7.5 cells, replication and viral RNA levels decreased continuously. Following HCV infection, interferon-stimulated gene (ISG)-expression significantly increased in iHLCs, whereas induction was almost absent in Huh7/7.5 cells. However, IFN α -stimulation equally induced ISGs in iHLCs and hepatoma cells. JAK-STAT pathway inhibition increased HCV replication in mature iHLCs, but not in Huh7 cells. Additionally, HCV

replication levels were higher in STAT2-, but not STAT1-knockdown iHLCs. Our findings support iHLCs as a suitable model for HCV-host interaction regarding functional innate immunity and lipoprotein synthesis.