**Workshop track 2**
**Workshop: Hematopoietic cells and CVD**

**EAS19-0667.**
**MITOCHONDRIAL DYSFUNCTION IN M2 MACROPHAGES DIFFERENTIATED FROM HUMAN NON-CLASSICAL MONOCYTES IS LINKED TO FOAM CELL FORMATION**

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**Background and Aims:** Macrophages scavenge lipids during atherogenesis. It remains unclear whether M1 (inflammatory) or M2 (pro-resolving) macrophages have differing lipid-handling capacities. Intermediate ($CD14^{+}CD16^{+}$) and non-classical ($CD14^{dim}CD16^{+}$), but not classical ($CD14^{+}CD16^{-}$) monocytes, are elevated in patients with cardiovascular disease. It is unknown if and how these specific subsets contribute to foam cell formation. Therefore we aimed to determine the lipid-handling and metabolic phenotype of macrophages derived from different human monocyte subsets.

**Methods:** Classical, intermediate and non-classical monocytes were isolated from healthy donors by FACS, differentiated into macrophages using M-CSF and then stimulated with LPS+IFNg(M1) or IL-4 (M2) followed by incubation with oxLDL. Cells were analysed using flow cytometry, RT-PCR and XFe-96 seahorse bioanalyzer.
**Results:** M2 macrophages from all human monocyte subsets took up oxLDL, whereas M1 macrophages did not. Following oxLDL loading, M2 macrophages from classical and intermediate monocytes increased cholesterol efflux to apoA-I, but this was not observed in M2 macrophages derived from non-classical monocytes, suggesting impaired cholesterol efflux capacity. Moreover, glycolytic capacity significantly increased while mitochondrial activity was decreased in these cells after oxLDL loading due to a robust increase in mitochondrial reactive oxygen species (mtROS) production in M2 macrophages from human non-classical monocytes. Importantly, when mitochondrial activity of oxLDL-loaded M2 macrophages from non-classical monocytes was restored by reducing mtROS, cholesterol efflux capacity was restored.
**Conclusions:** We suggest that non-classical monocyte derived M2 macrophages are involved in foam cell formation, which is caused by mitochondrial dysfunction. Restoring mitochondrial function restores cholesterol efflux and could potentially aid in atherosclerotic lesion regression.

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**EAS19-0382.**
**ADIPOSE TISSUE MACROPHAGES INDUCE HEPATIC NEUTROPHIL RECRUITMENT AND MACROPHAGE ACCUMULATION WITHOUT AFFECTING ATHEROSCLEROSIS DEVELOPMENT IN MICE.**

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**Background and Aims:** Obesity is a risk factor for both non-alcoholic steatohepatitis (NASH) and atherosclerosis. This risk has been attributed to visceral adipose tissue (vAT) expansion associated with increased proinflammatory mediators. Accumulation of $CD11c^{+}$ proinflammatory adipose tissue macrophages (ATM) is an important driver of vAT inflammation. We investigated the role of ATMs in hepatic inflammation and atherosclerotic plaque development.

**Methods:** vAT isolated from lean, obese or ATM-depleted (using clodronate liposomes) obese mice was transplanted to lean $ldlr^{-/-}$ acceptor mice. Systemic and hepatic inflammation and atherosclerotic plaque formation were investigated. Microarray data were collected from vAT and liver from obese individuals.
**Results:** Transplanting donor vAT from obese mice increased diet-induced hepatic macrophage content compared with lean-transplanted mice, worsening liver damage. ATM depletion prior to vAT transplantation reduced this increased hepatic macrophage accumulation. On chow, vAT transplantation induced a more pronounced increase in circulating and hepatic neutrophil numbers in obese-transplanted than lean-transplanted mice, while ATM depletion prior to vAT transplantation reversed this effect. Microarray analysis of fluorescence-activated cell sorted CD11c+ and CD11c- macrophages isolated from donor adipose tissue showed that obesity resulted in enhanced expression of neutrophil chemotaxis genes specifically in CD11c+ ATMs. Surprisingly, AT transplantation did not influence atherosclerotic plaque size, phenotype, or stability. In humans, CD11c expression in vAT of obese individuals correlated with vAT expression of neutrophil chemotactic genes and with hepatic expression of neutrophil and macrophage marker genes.
**Conclusions:** ATMs from obese vAT induce hepatic macrophage accumulation during NASH development without affecting atherosclerosis, possibly by enhancing neutrophil recruitment.

**Late breaking abstracts session**
**Late Breaking session on Experimental Atherosclerosis and Genetics**

**EAS19-1090.**
**FUNCTION AND MUTATION OF NETRIN-1 IN PREMATURE ATHEROSCLEROSIS**

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**Background and Aims:** Cardiovascular disease risk is largely genetically determined. However, the molecular pathology is not elucidated in many families suffering from atherosclerosis.

**Methods:** .
**Results:** We found that plasma Netrin-1 levels, measured by a bioactive ELISA assay, were significantly lower in individuals with calcified plaques (11.25 ng/ml, N=120), on coronary calcium CT-scans, compared to individuals without (28.01 ng/ml, N=60) (p=<0.001). Furthermore, we observed a significant negative correlation between arterial wall inflammation and Netrin-1 plasma levels (P=<0.001, Slope -10.1±2.0, R square: 0.65). Upon whole exome sequencing in a pedigree comprising 2 generations of 7 family members who suffered from premature atherosclerosis, we found a rare possible deleterious variant (MAF<0.05, CADD=34) located in the Netrin-1 gene, cG1769T (pR590L). Purified mutated Netrin-1 protein (mutNetrin-1) showed significant decreased binding capacity to receptors UNC5B, DCC, and β3-integrin, and significant increased binding capacity to neogenin, heparin, and heparan sulfate compared to wild type Netrin-1 (wtNetrin-1). Importantly, mutNetrin-1 did not have an anti-inflammatory effect on endothelial cells (monocyte binding and cytokine expression) as wtNetrin-1 had. Moreover, migration assays of macrophages and smooth muscle cells revealed inhibited migration in the presence of mutNetrin-1 compared to wtNetrin-1.
**Conclusions:** In conclusion, we show that high levels of Netrin-1 are associated with healthier arteries. Besides we found a rare functional variant of Netrin-1 that leads to altered Netrin-1 binding and cellular functions, which could translated to more unstable plaques and explain the premature atherosclerosis in this family. These studies provide novel insights into the importance and mechanisms of netrin-1 in human atherosclerosis.