kinetics of PMN entry into plaques was determined by fluorescence microscopy and confirmed by FLIM. Proteolytic activity of plaques after PMN delivery was assessed using *in situ* zymography.

Results: PMNs entered plaques after peri-adventitial delivery; the maximal entry was observed at one hour (mean= 1342±162 labelled cells per plaque). Once inside the plaque, PMNs significantly increased proteolytic activity for collagen type I and type IV (respectively 2.8- and 2.5-fold increase, $p<0.001$). Both activities were inhibited by the broad MMP inhibitor, 1-10 phenanthroline. Elastinolytic activity also increased after neutrophils had entered plaques (1.9-fold increase, $p<0.001$).

Conclusions: Neutrophils can penetrate the plaque by a peri-adventitial route. Once there, they release the content of their granules, leading to an increase in collagenolytic and elastinolytic activities. This model of peri-adventitial delivery is a suitable model to explore the effect of various factors on the plaque. This study suggests that PMNs might contribute to plaque rupture.

EAS-0647.
(−)-EPICATECHIN ATTENUATES HIGH GLUCOSE-INDUCED INFLAMMATION BY EPIGENETIC MODULATION IN HUMAN MONOCYTES

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Diabetes is a proinflammatory state that is associated with increased complications. We have previously shown increased monocyte activity in diabetics with complications than without. NFκB is crucial regulating the inflammatory process and different studies have shown that chromatin remodeling by epigenetic changes could regulate monocyte NFκB activation and cytokine expression under diabetic conditions. Cocoa and its main flavanol, (-)-epicatechin (EC), display anti-inflammatory and antidiabetic effects. The aim of the study was to test the potential anti-inflammatory role of EC via epigenetic changes in the diabetic milieu.

THP-1 cells were pre-treated with EC (5μM) and 4h later exposed to 25mM glucose for a total of 24h. Acetyl CBP-p300, HDAC4 and phosphorylated and total levels of p65-NFκB were analysed by Western blot in nuclear lysates. Total histone 3 (HH3) levels, K9H3 acetylation, K4H3me2 and K9H3me2 were analysed after histone purification. Histone acetyltransferase (HAT) activity was measured in nuclear lysates and TNF-α production was evaluated in the cell culture media with a Milliplex MAP Assay. ChIP for TNF-α was also assessed.

EC restored the changes induced by high glucose (HG) in p-p65/p65 ratio, acetyl CBP-p300 and HAT activity to control levels. Pre-treatment with EC counteracted the increased acetylation of H3K9 and H3K4 dimethylation and attenuated H3K9 dimethylation triggered by HG. EC also decreased HG-induced HDAC4 levels and significantly decreased TNF-α release.

EC induces epigenetic changes in human monocytes resulting in decreased NF-κB activation and TNFα release in diabetes.

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EAS-0664.
INFLAMMATORY MEDIATORS, OBESITY AND TYPE 2 DIABETES STIMULATE ADHESION OF HUMAN DENDRITIC CELLS TO CORONARY SMOOTH MUSCLE CELLS

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Inflammation is involved in atherosclerosis and vascular remodeling. Dendritic cells (DCs) can drive immune and non-immune inflammation, participate to blood vessel wall infiltrates and can adhere to vascular smooth muscle cells (SMCs), but it is still unclear how the interactions between these cell types are regulated. We have addressed this issue in vitro for human cells, with regard to the influence of inflammation mediators. The adhesion of monocyte-derived DCs (Mo-DCs) isolated from healthy subjects to coronary SMCs increased when the latter were stimulated by inflammatory cytokines. This effect was prevented by pretreatment of SMCs with a statin or a PPAR-gamma agonist. Mo-DCs obtained from obese (BMI>30) and type 2 diabetic patients displayed a morphology comparable to DCs obtained from healthy subjects, but showed higher ability to adhere to coronary SMCs accompanied by increase in cell adhesion molecules including CD18, CD11c and DC-SIGN. These findings suggest that: (1) an inflammatory microenvironment in the vascular wall may stimulate recruitment and retention of DCs by VSMCs, with perpetuation and worsening of local inflammation and atherosclerosis; (2) stimulation of DC adhesion to VASMCs may be one mechanism by which obesity and type 2 diabetes promote vascular inflammation and atherosclerosis.

EAS-0766.
ROLE OF 5-LIPOXYGENASE PATHWAY IN INTERMITTENT HYPOXIA-INDUCED ATHEROGENESIS IN APOE$^{-/-}$ MICE

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5-lipoxygenase (LOX)-pathway is activated in obstructive sleep apnea syndrome (OSA) and seems to be involved in the OSA-associated vascular remodeling[1]. Exposure of apolipoprotein-E deficient (ApoE$^{-/-}$) mice to intermittent hypoxia (IH) accelerated atherogenesis[2] but the link between IH, atherogenesis and the LOX-pathway remains te be explored.

ApoE$^{-/-}$ mice were exposed to IH or normoxia (N) for 8 weeks, and treated or not with the five-lipoxygenase activating protein (FLAP) inhibitor MK-886 (40mg/kg/d) or with the type 1 cysteinyl-leukotriene (CysLT1) receptor blocker montelukast (1mg/kg/d). The genomic expression of the LOX-pathway (RT-PCR) and the atherosclerotic lesion size (Oil-Red-O) were evaluated in aortas.

IH significantly aggravated atherosclerosis without changing cholesterol levels and activated the transcription of genes of the LOX-pathway (5-LOX, FLAP, Cys-LT1 receptor and leukotriene A4 hydrolase (LTA4H)) ($p< 0.05$). Atherosclerosis lesion sizes correlated to these mRNA levels (5-LOX: $r=0.552$, $p=0.05$; FLAP: $r=0.714$, $p=0.01$; LTA4H: $r=0.582$, $p=0.04$; Cys-LT1 receptor: $r=0.543$, $p=0.05$) in IH mice; whereas, the lesion sizes of N mice only correlated to cholesterol levels ($r=0.571$, $p=0.02$). FLAP inhibition had no impact on IH-related atherogenesis, while blockage of CysLT1 receptor reduced the lesion progression in IH mice only, notably on abdominal aorta ($p=0.02$).

Exposure of ApoE$^{-/-}$ mice to IH accelerated atherogenesis and activated the transcription of the LOX-pathway, both being highly correlated. HI-related atherosclerosis was reduced by blockage of CysLT1 receptor, suggesting that blockage of Cys-LT receptors could be an interesting pharmacological approach to treat the OSA-associated vascular remodeling.

References:
1 Stanke-Labesque et al, Cardiovasc Res 2013
2 Gautier-Veyret et al, Eur Respir J 2013