Concurrent Session 8A: PUFA/Heart Disease

**Influence of catechin and epicatechin on oleic acid fat loaded HepG2 cells**

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**Background** – Non-alcoholic steatohepatitis (NASH) is a condition in which hepatocytes become fat loaded and further damaged by oxidative stress.

**Objectives** – To induce oxidative stress by fat loading HepG2 cells and investigate the ability of catechin/epicatechin to counter this effect. To investigate the influence of these conditions on the expression of key NADPH oxidase (NOX) genes responsible for the production of reactive oxygen species (ROS).

**Design** – HepG2 cells were exposed to one of three conditions: control media, control media with 0.5mM oleic acid (48hrs), or control media with oleic acid (48hrs) followed by a catechin (0.16µmol/L) epicatechin (5.92µmol/L) combined treatment (2hrs). Triglyceride concentration was measured and ROS detected using a dihydroethidium stain (DHE). Relative gene expression was analysed by real time PCR for NOX1, NOX3, NOX4 and p47phox.

**Outcomes** – Fat loading HepG2 cells increased ROS by 90% compared to control and increased triglyceride concentration 1.5 fold compared to control (p=0.01). Treatment with catechin/epicatechin decreased ROS by 60% compared to fat loaded cells. Triglyceride concentration decreased with treatment by 2.7 fold compared to control (p<0.01) and a 4 fold decrease when compared to fat loaded cells (p<0.01). Interestingly only NOX1 relative gene expression increased in catechin/epicatechin treated groups compared to control and fat loaded cells (p<0.05).

**Conclusion** – Fat loading HepG2 cells increases intracellular triglycerides, a common finding in NASH, and also increases ROS. Despite lower levels of ROS in catechin/epicatechin treated cells, relative expression of NOX1 was increased, indicating that NOX1 may act as a signalling pathway to reduce ROS in liver disease.

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**Pure dietary flavonoids, quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men**

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**Background** – Dietary flavonoids may reduce cardiovascular disease risk by improving endothelial function.

**Objective** – To investigate whether pure dietary flavonoids may improve endothelial function by modulating nitric oxide (vasodilator), endothelin-1 (vasoconstrictor) and F2-isoprostane (marker of oxidative stress) production.

**Design** – A randomised, placebo controlled, cross-over trial in 12 healthy men was conducted to compare the acute effects on nitric oxide, endothelin-1 and F2-isoprostanes after oral administration of 200 mg of quercetin, (-)-epicatechin and epigallocatechin gallate. A blood and spot urine sample were taken at baseline, a blood sample was taken at 2 h post treatment, and a 5 h urine collection was performed. Measurements performed in plasma and urine included: S-nitrosothiols (plasma only), nitrite and nitrate concentrations (markers of nitric oxide production); endothelin-1; F2-isoprostanes, and quercetin, (-)-epicatechin and epigallocatechin gallate.

**Outcomes** – Relative to water (control), quercetin and (-)-epicatechin resulted in a significant increase in plasma S-nitrosothiols, plasma nitrite, and urinary nitrate concentrations (p < 0.05 for all), but not plasma nitrate or urinary nitrite. Epigallocatechin gallate did not alter any measures of nitric oxide production. Quercetin and (-)-epicatechin, but not epigallocatechin gallate, resulted in a significant reduction in plasma endothelin-1 concentration (p < 0.05), and only quercetin significantly decreased urinary endothelin-1 concentration (p < 0.05). None of the treatments significantly altered plasma or urinary F2-isoprostane concentrations. Significant increases in the circulating concentrations of the three flavonoids were observed after the corresponding treatment (p < 0.05).

**Conclusion** – Dietary flavonoids such as quercetin and (-)-epicatechin, but not epigallocatechin gallate, can augment nitric oxide status and reduce endothelin-1 concentrations. These may be important pathways to improved endothelial function. We have also found that the flavonoids tested had no acute effect on systemic oxidative stress assessed using F2-isoprostanes.