NBS' contact meeting, 19th – 22nd of January 2017, Storefjell, Norway

# Polyphenol metabolites display immune inhibiting effects on acute inflammation in human monocyte and macrophage cells

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**Background:** The dietary polyphenols ellagitannins, present in strawberry, raspberry and pomegranate, are catabolized to ellagic acid and further to urolithins by the colonic microbiota. Urolithin A is one of the main metabolites found in plasma. Ellagic acid is only found in minor amounts. Many studies support the positive effect on the immune system of polyphenols; however, usually the effects are of polyphenols only found in foods, and not of the metabolites present in circulation.

**Aim:** To examine and compare immune inhibiting effects on acute inflammation of ellagic acid and its catabolite urolithin A, which are both present in the circulation after consumption of certain fruits and berries.

**Conclusion:**
Both ellagic acid and urolithin A displayed immune inhibiting effects on acute inflammation, however, urolithin A had a stronger effect than ellagic acid. The reason for this might be different regulation of NF-κB.

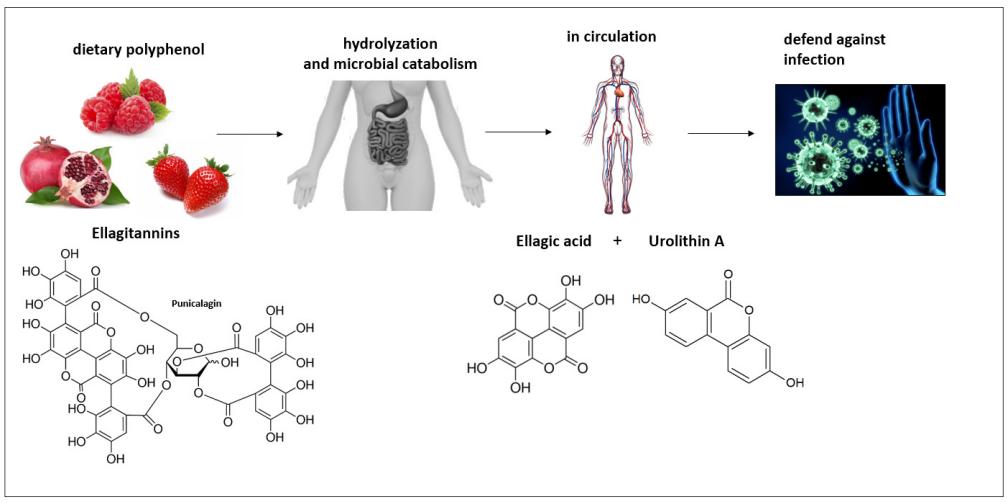


*Figure 1: Ellagitannins are converted to ellagic acid which is converted to among others urolithin A during digestion. Both urolithin A and ellagic acid are present in the blood and affect the immune system.*

## Results

### 1. Ellagic acid and urolithin A decreased NF-κB activity during acute inflammation

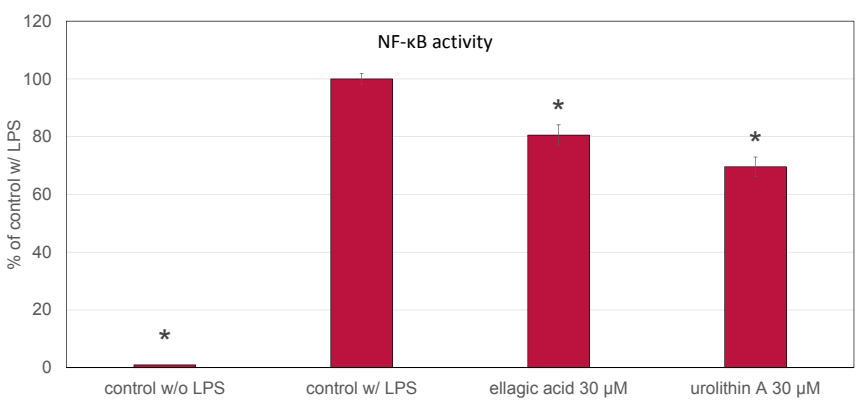


*Figure 2: Bars show NF-κB activity relative to control w/ LPS only during acute inflammation in U937 cells. Asterisk denote significant differences between metabolites compared with cells incubated with LPS only. Bars presented are mean of minimum three independent experiments ±SEM.*

### 2. Urolithin A reduced the mRNA expression and cytokine secretion of both IL-6 and TNF-α, while ellagic acid only reduced the TNF-α cytokine secretion.

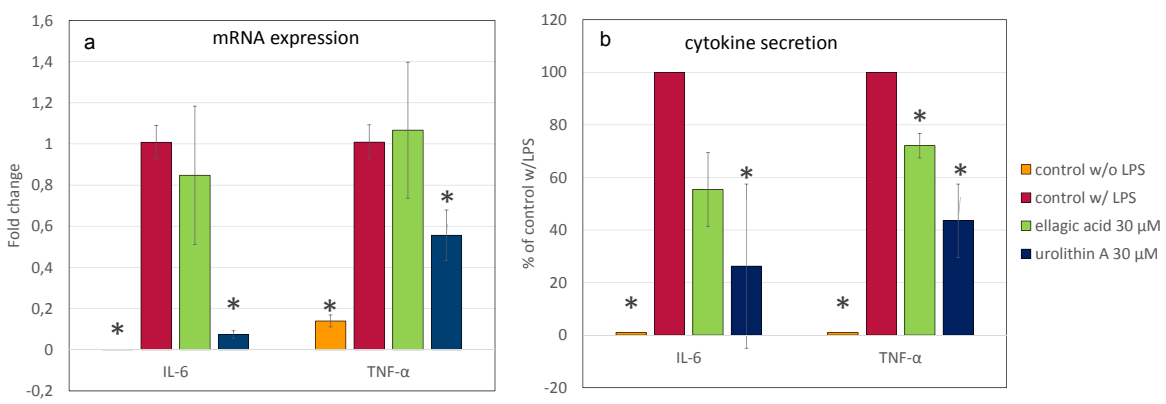


*Figure 3a) Bars show the relative gene expression of IL-6 and TNF-α in U937 cells during acute inflammation. b) Bars show cytokine secretion level of IL-6 and TNF-α relative to control w/ LPS only during acute inflammation in THP-1 cells. Asterisk denote significant differences between metabolites compared with cells incubated with LPS only. Bars presented are mean of minimum three independent experiments ±SEM.*

### 3. Ellagic acid and urolithin A did not affect the relative gene expression of the immune receptor TLR-4

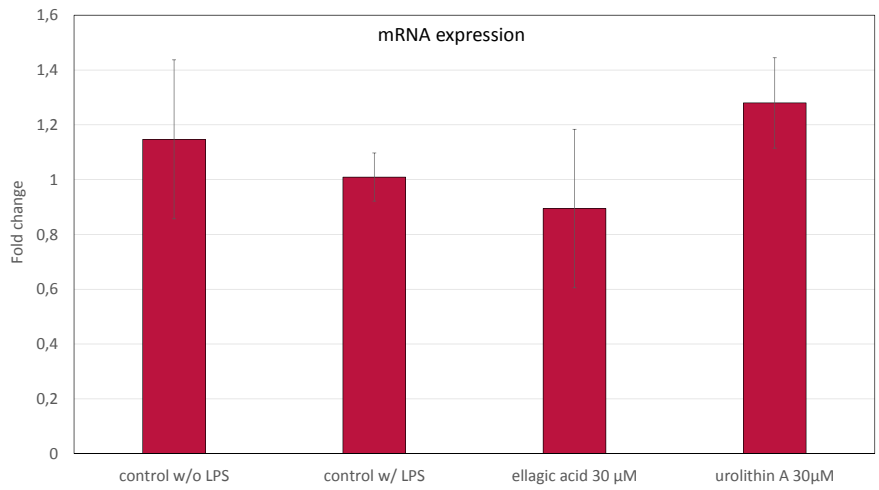


*Figure 4: Bars show the relative gene expression of the immunereceptor TLR-4 during acute inflammation in U937 cells. No significant differences between metabolites compared with cells incubated LPS only were found. Bars presented are mean of minimum three independent experiments ±SEM.*

### 4. Urolithin A reduced the nuclear localisation of phosphorylated NF-κB units p65 and p50, while ellagic acid only reduced the nuclear localisation of *p*65

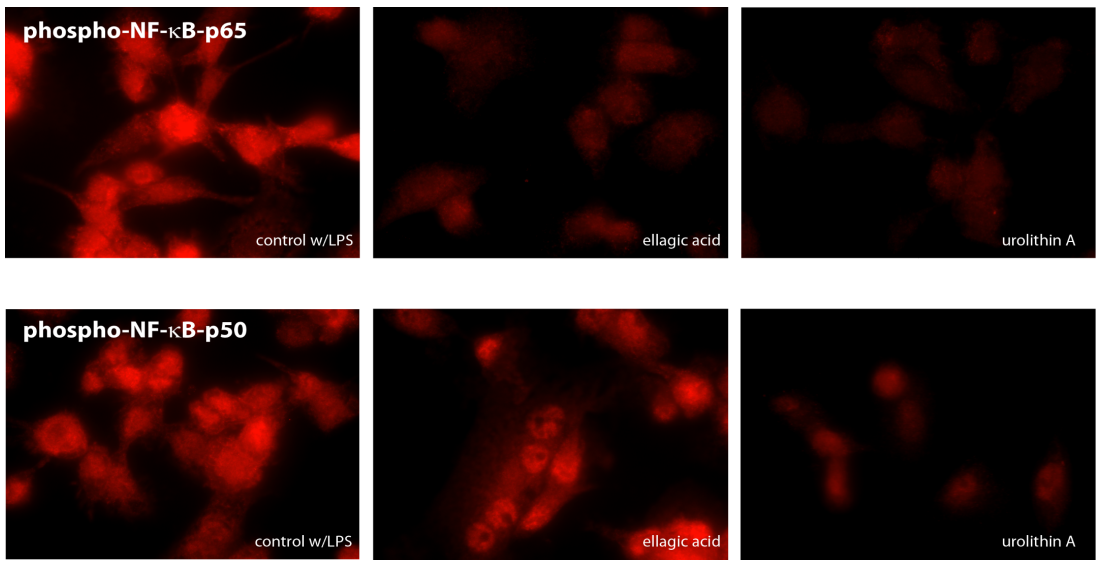


*Figure 5: Immunostaining of the phosphorylated NF-κB subunits p65 and p50 during actue inflammation in THP-1 cells demonstrate a nuclear localisation in control cells, and a reduced expression in cells treated with urolithin A. No reduction in nuclear localisation or expression of p50 was observed when treated with ellagic acid.*

## Methods

Urolithin A, that was kindly provided by partners in the EU-project 'BACCHUS' (FP7, no. 312090), and ellagic acid were examined for their effects on acute inflammation responses caused by the bacterial endotoxin LPS in human monocyte and macrophage cells. U937 3xκB-LUC cells and differentiated THP-1 cells were stimulated with 30 µM metabolites for 6 or up to 24 hours during acute inflammation induced by LPS. To measure the immune inhibiting effects, U937 was used to measure NF-κB activity with luminescence, and mRNA expression of the pro-inflammatory cytokines IL-6 and TNF-α, and the immune receptor TLR-4 using RT-PCR. In addition, THP-1 cells were measured for secretion of the pro-inflammatory cytokines IL-6 and TNF-α using sandwich ELISA, and for nuclear localisation of p50 and p65 using immunostaining. Dunnett's Multiple Comparisons with a control was performed using the Minitab 17 software for calculation of significant difference between samples.

**Acknowledgments**
The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement nº 312090, and Norwegian Levy on Agricultural Products under grant agreement nº 224892.

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