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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Urolithins, Berry Ellagitannin Metabolites, Exert Anti- inflammatory Effects *In vivo*

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CONTENT

ELLAGITANNINS, ELLAGIC ACID

- ✓ Diet
- ✓ Metabolism (urolithins)
- ✓ Urolithins in Nature

BIOLOGICAL ACTIVITY

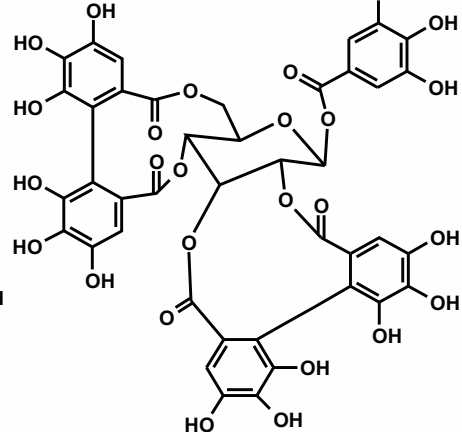
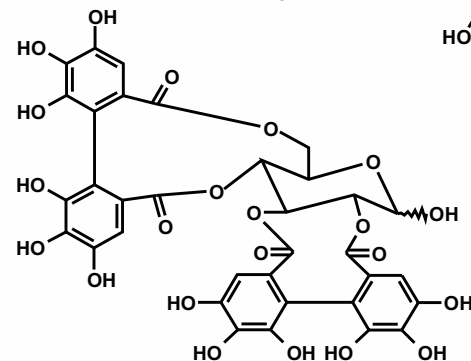
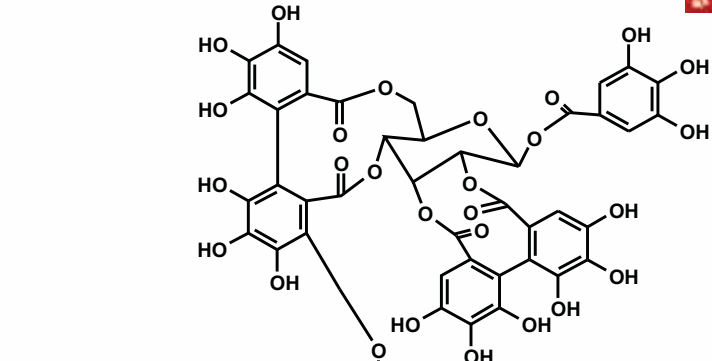
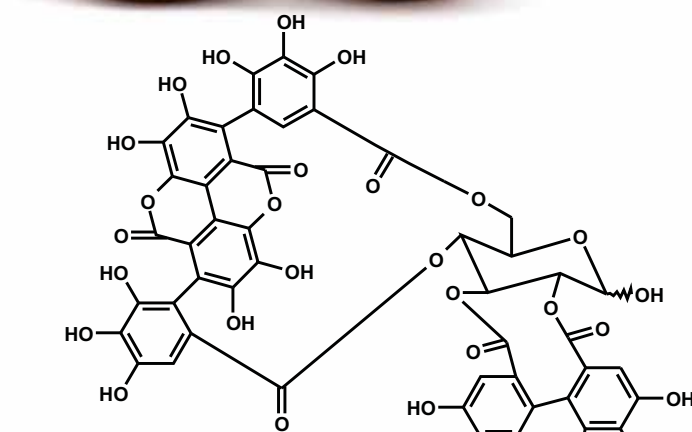
- ✓ Inflammatory bowel disease (IBD)

TARGET ORGANS

- ✓ Human prostate as target organ for urolithins

CONCLUSIONS





ELLAGITANNINS

ELLAGITANNINS & ELLAGIC ACID

CONTENT



25-85 mg/100 g fresh weight



51-330 mg/100 g fresh weight



56-360 mg/100 g fresh weight



Biological activity: Ellagitannin-containing foodstuffs (animal models and humans)

-Cancer

-Diabetes

-Cardiovascular

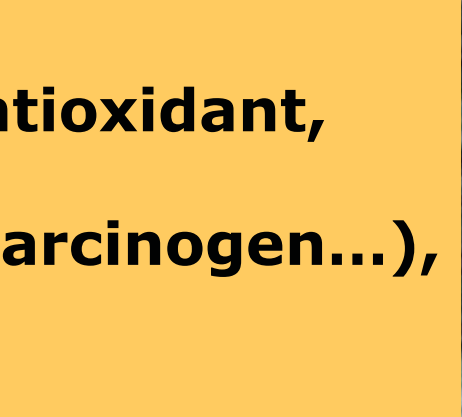
-Alzheimer's

-.....

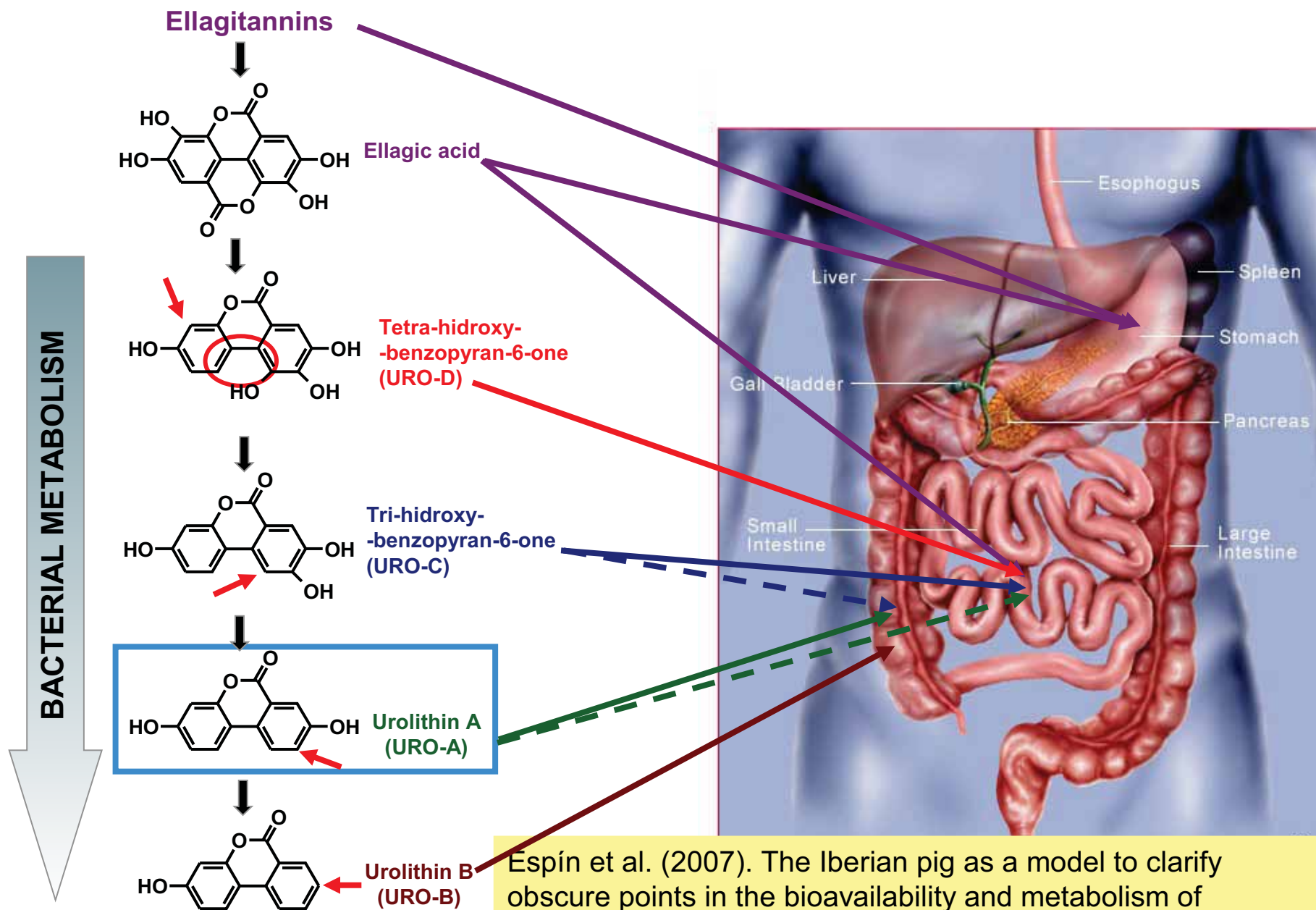
Multitarget action (antioxidant, anti-inflammatory, anticarcinogen...),

BUT...

Are ellagitannins or ellagic acid the real active molecules in vivo?

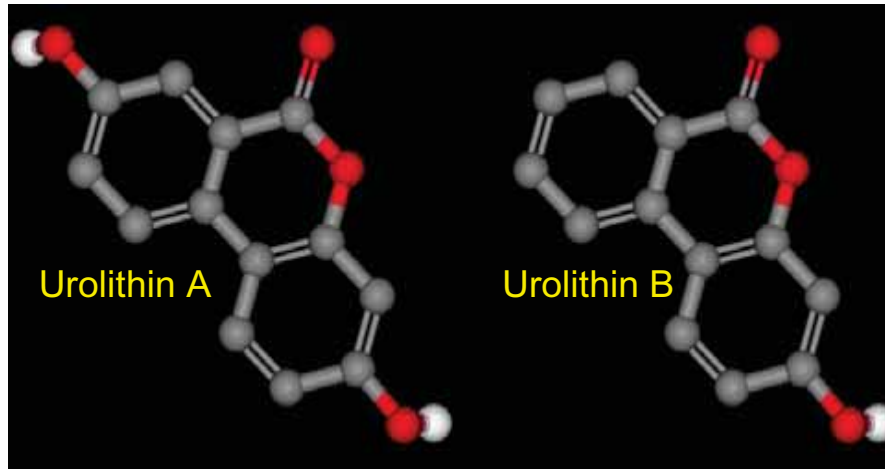


METABOLISM OF ELLAGITANNINS (What do we know?)



Espín et al. (2007). The Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. *J. Agric. Food Chem.* 55, 10476–10485

The Urolithins



Doyle B, Griffiths LA (1980). The metabolism of ellagic acid in the rat. *Xenobiotica*, 10, 247-256



Jeong et al. (2000). Hyaluronidase Inhibitory Active 6H-Dibenzo[b,d]pyran-6-ones from the Feces of *Trogopterus xanthipes*. *Planta Med.* 66, 76-77.



Urolithins in the phylogenetic scale



Urolithins are produced by mammals. Not found in birds and insects

RAT

- Cerdá et al., 2003, Eur. J. Nutr. 42, 18-28.
- Cerdá et al., 2003, J. Agric. Food Chem. 51, 3493-3501.
- González-Sarrías et al., 2009, J. Agric. Food Chem. 57, 5623-5632.
- Larrosa et al., 2009, J. Nutr. Biochem. 21, 717-725.

BEAVER, MICE, SHEEP, COW....

González-Barrio et al. 2010, J. Agric. Food Chem. 59, 1152-1162

PIG

- Espín et al., 2007, J. Agric. Food Chem. 55, 10476-10485.

HUMANS

- Cerdá et al., 2004, Eur. J. Nutr. 43, 205-220.
- Cerdá et al., 2005a, J. Agric. Food Chem. 53, 227-235.
- Cerdá et al., 2005b, J. Agric. Food Chem. 53, 5571-5576.
- Cerdá et al., 2006, Eur. J. Clin. Nutr. 63, 245-253.
- González-Sarrías et al., 2010, Mol. Nutr. Food Chem. 54, 311-3



ABSORPTION AND METABOLISM OF ELLAGITANNINS (Key points)

- ✓ Ellagitannins are not absorbed but hydrolyzed to yield ellagic acid.
- ✓ Ellagic acid is very poorly absorbed and mainly metabolized by gut microbiota to yield urolithins.
- ✓ Human subjects can be divided into high, and low-urolithin producers (due to their microbiota)
- ✓ Urolithins can reach high micromolar concentrations in the colon (aglycones) and in the bloodstream (glucuronides)



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- ✓ Metabolism
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BIOLOGICAL ACTIVITY

- ✓ Inflammatory bowel disease (IBD)

TARGET ORGANS

- ✓ Human prostate as target organ for urolithins

CONCLUSIONS



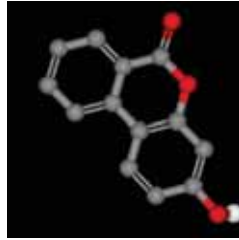
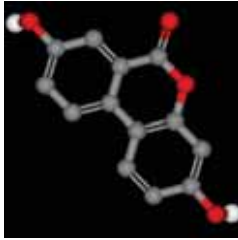
BIOLOGICAL ACTIVITY OF UROLITHINS



TRADITIONAL CHINESE MEDICINE

- Lower stomach and duodenum ulcers
- Abdominal pain
- Menstrual pain and postpartum infections

(Trogopterus xanthipes)

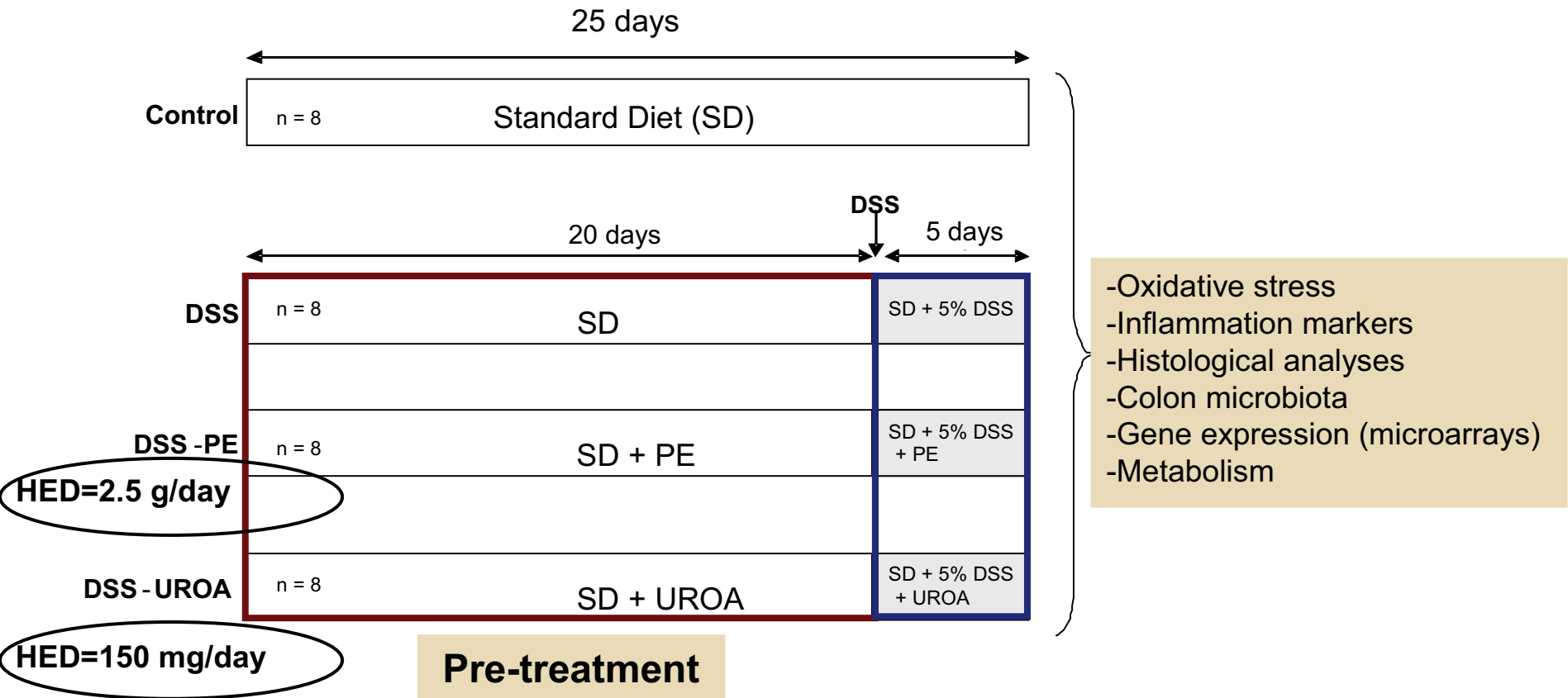


POMEGRANATE EXTRACTS IN INFLAMMATORY BOWEL DISEASE (IBD): The role of urolithins

Fisher 344 rats



Chronic inflammation increases CRC risk in IBD patients

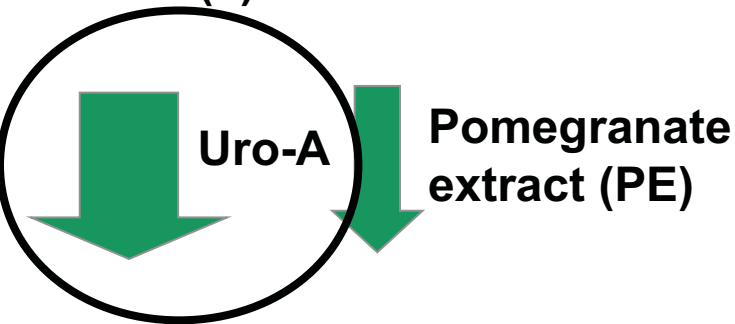


Larrosa et al. (2010). Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on the phenolic metabolism. *J. Nutr. Biochem.* 21, 717-725.

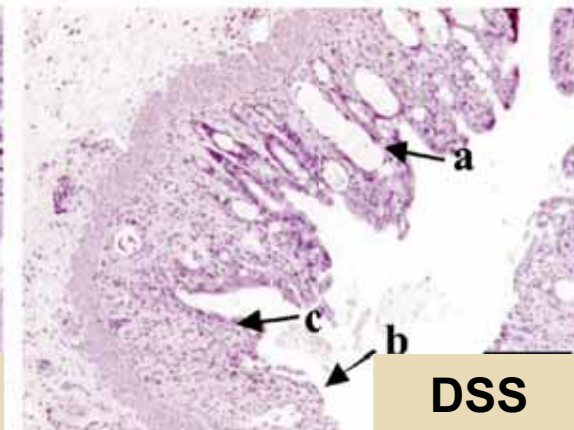
Histological analyses of colon samples



- Crypts damaging (a)
- Epithelium loss (b)
- Infiltration of inflammatory cells (c)



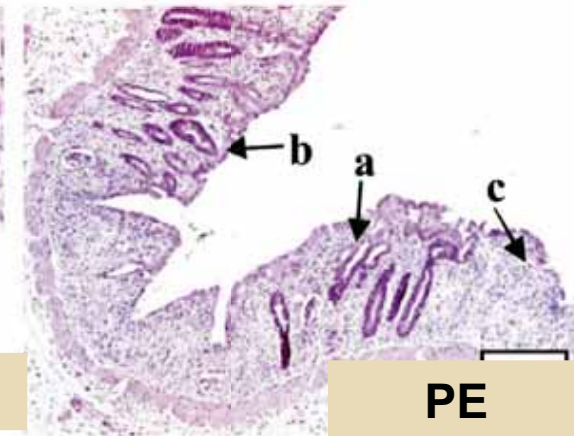
Control



DSS



Uro-A



PE

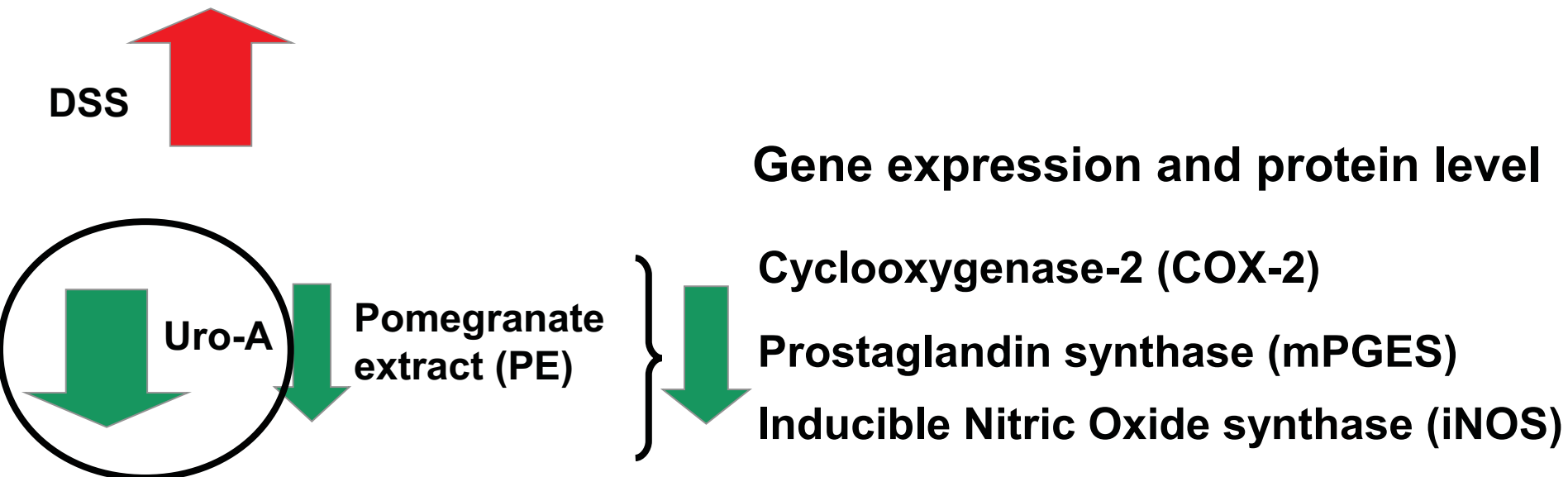
✓ PE and Uro-A protected colon from tissue damage

Larrosa et al. (2010). Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on the phenolic metabolism. *J. Nutr. Biochem.* 21, 717-725.

Inflammatory markers in colon mucosa



Prostaglandins (PGE₂), Nitric Oxide (NO)



✓ PE and Uro-A decreased NO and prostaglandins by downregulating the enzymes involved in their synthesis

Larrosa et al. (2010). Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on the phenolic metabolism. *J. Nutr. Biochem.* 21, 717-725.

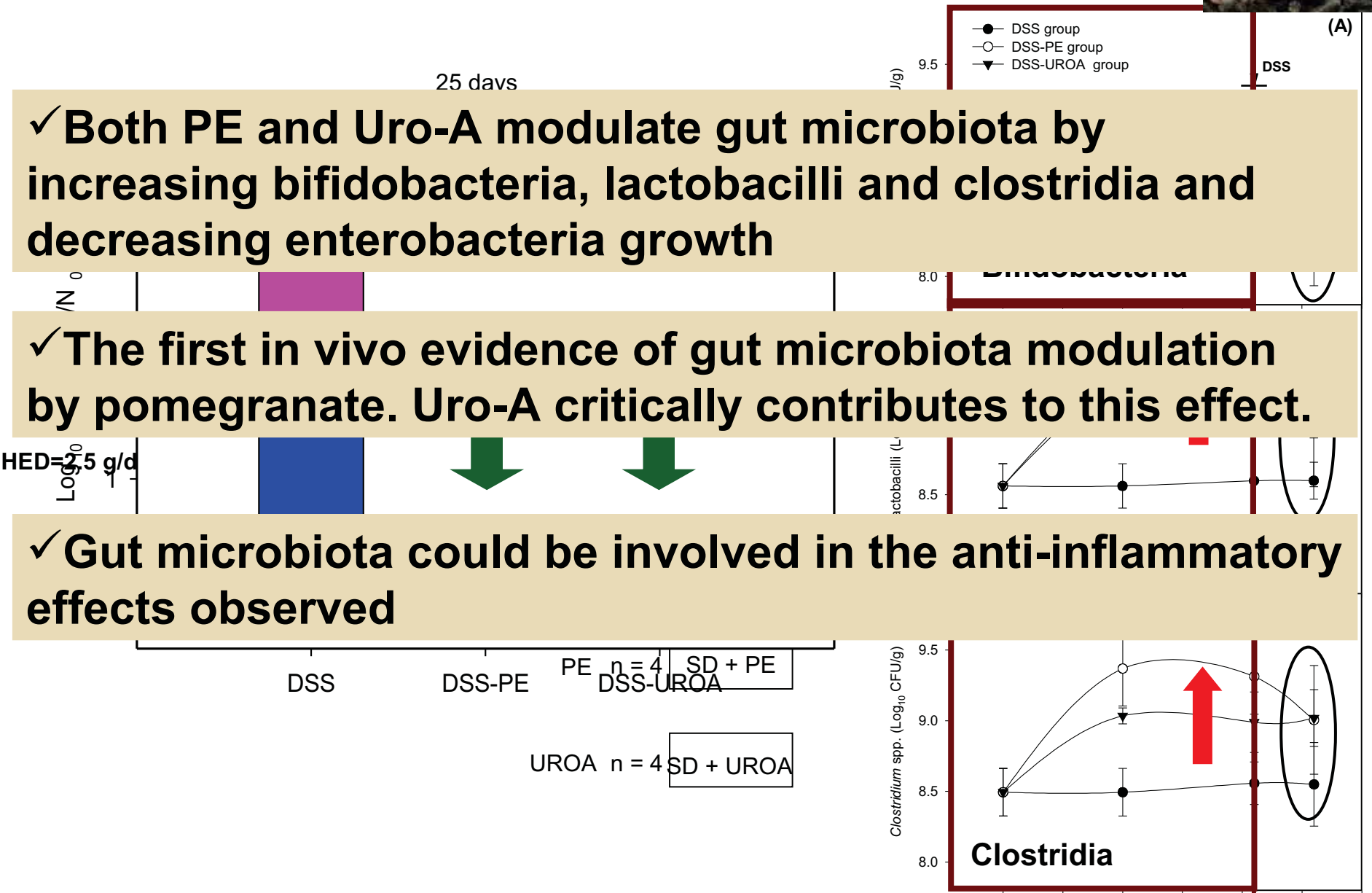
Effect of PE and Uro-A on gut microbiota



✓ Both PE and Uro-A modulate gut microbiota by increasing bifidobacteria, lactobacilli and clostridia and decreasing enterobacteria growth

✓ The first in vivo evidence of gut microbiota modulation by pomegranate. Uro-A critically contributes to this effect.

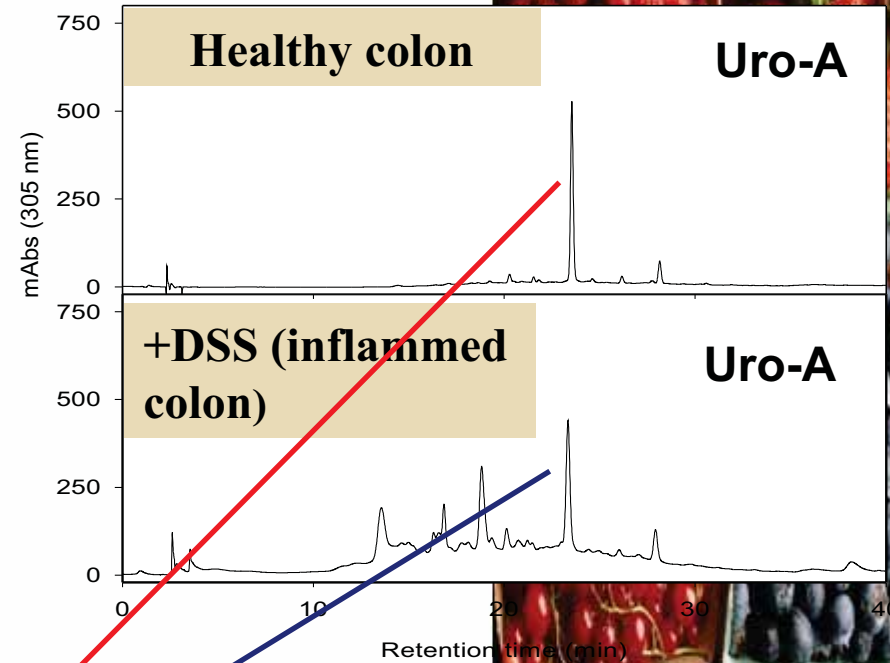
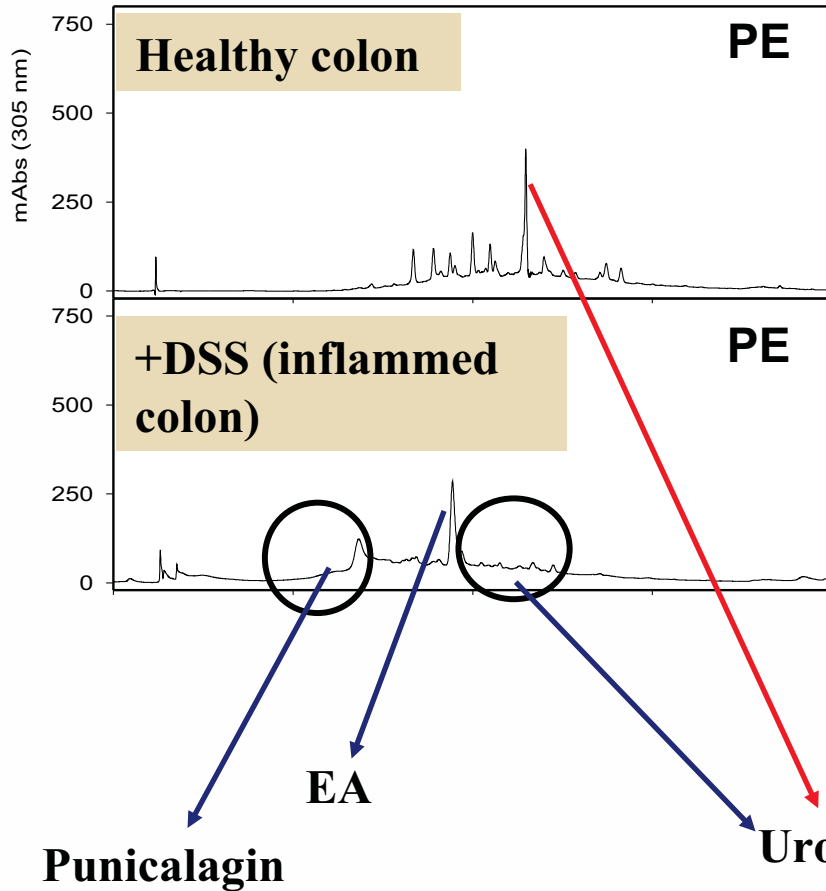
✓ Gut microbiota could be involved in the anti-inflammatory effects observed



Effect of DSS on pomegranate polyphenols metabolism

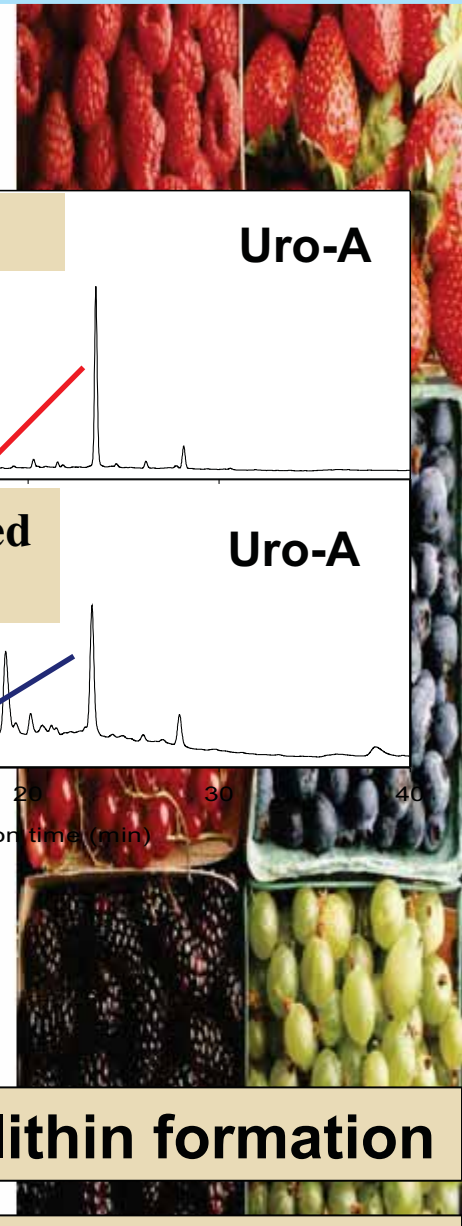
Larrosa et al. (2010). *J. Nutr. Biochem.* 21, 717-725.

Colon



Imbalance in gut microbiota prevents normal urolithin formation

Urolithin A: a promising targeting active molecule to the colon



Gene expression in colon mucosa (transcriptomic)



Affymetrix: Approx. 22,000 human genes

Differential expression at least 2-fold, $P < 0.001$ (colon mucosa)

	<i>DSS-PE vs DSS</i>	<i>DSS-UroA vs DSS</i>
<i>Down-regulated probes</i>	329	3,008
<i>Up-regulated probes</i>	1,728	3,987

2,057 genes

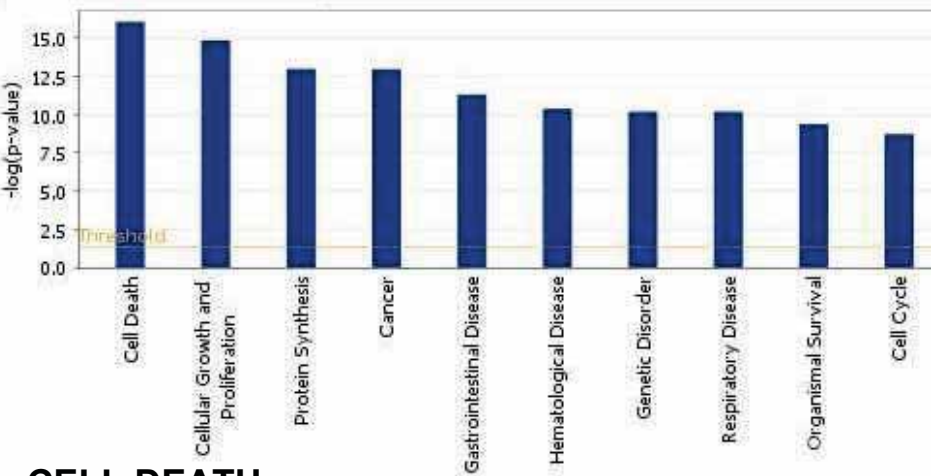
6,995 genes

667 common genes

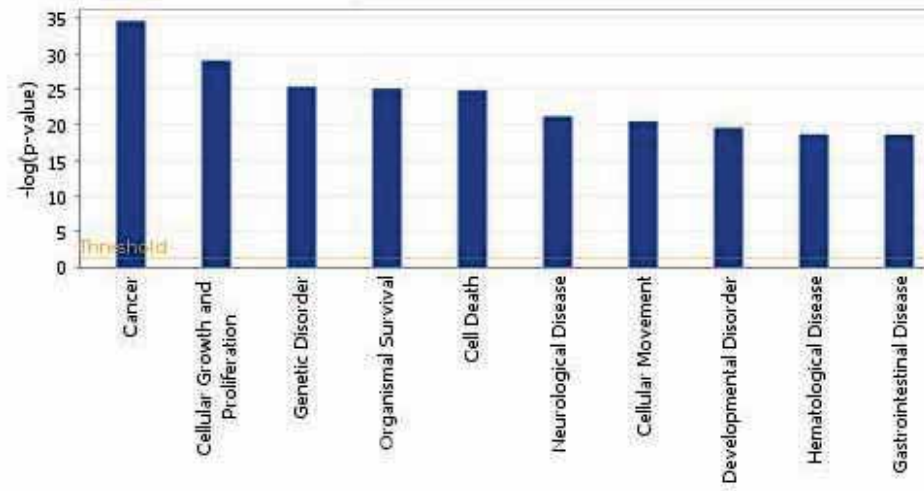
✓ Both PE and Uro-A modulate gene profile of colon mucosa

Functional analysis (top ten). (Ingenuity Software)

Pomegranate extract (PE)



Urolithin A



CELL DEATH
 CELLULAR GROWTH AND PROLIFERATION
 CANCER
 GASTROINTESTINAL DISEASE
 ORGANISMAL SURVIVAL
 CELL CYCLE

Common: PE-UroA (667 genes)



Gastrointestinal disease
Cellular growth and proliferation
Cancer
Organism
Cell cycle

URO-A
 PE

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BIOLOGICAL ACTIVITY

- ✓ Inflammatory bowel disease (IBD)
- ✓ Mechanisms of action
- ✓ Role of urolithins as anti-inflammatory compounds

TARGET ORGANS

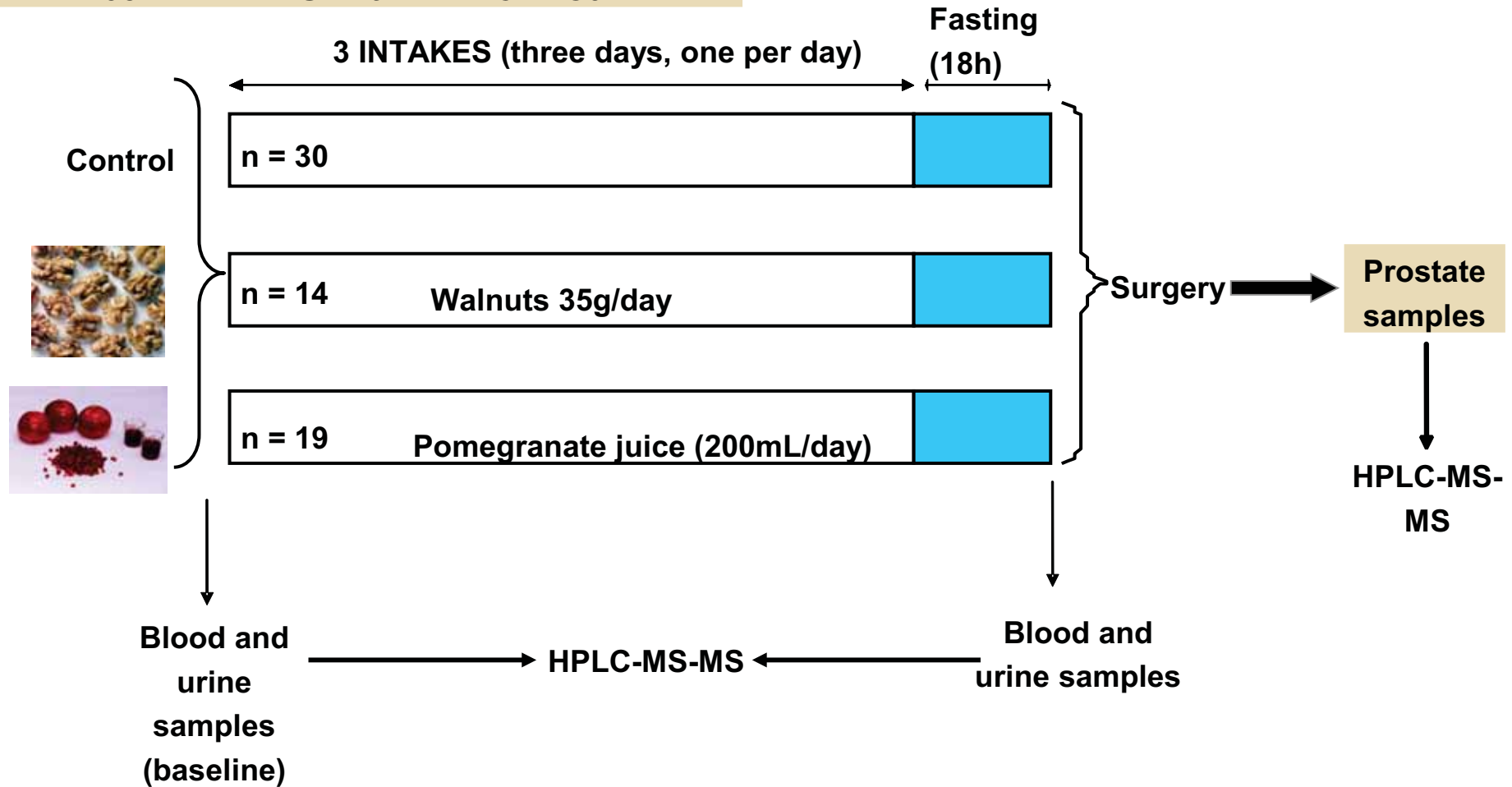
- ✓ Human prostate as target organ for urolithins

CONCLUSIONS



Pomegranate juice and prostate cancer: Could urolithins be behind these effects? The human prostate as target organ

63 PATIENTS with BPH or PCa



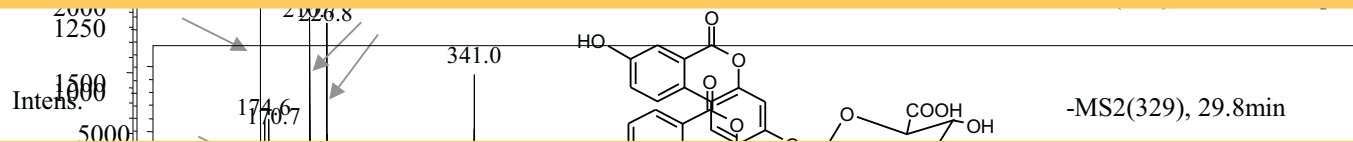
González-Sarrías et al. (2010). Occurrence of urolithins, gut microbiota ellagic acid-derived metabolites, in the human prostate gland upon consumption of walnuts and pomegranate juice. *Mol. Nutr. Food Res.* 54, 311-352.

Analyses of human prostates

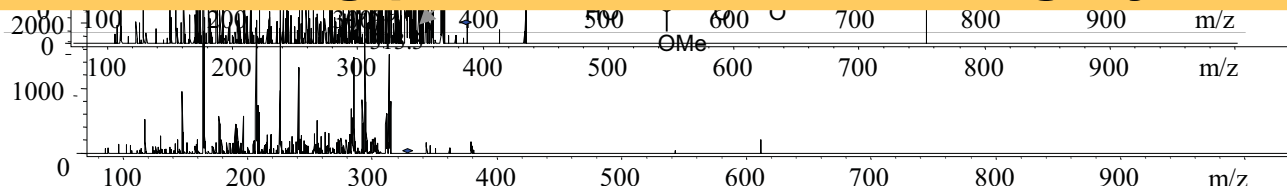
- High interindividual variability
- Metabolites in 8 prostate samples (high urolithin producers): 24% of patients
- Uro-A glc: 6 samples (0.5-2 ng/g tissue) → UV, MS, MS/MS
- Uro-B glc: 2 samples → MS and MS/MS
- Dimethyl ellagic acid (DMEA): 4 samples → MS and MS/MS



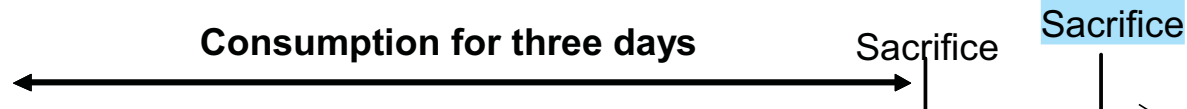
No correlation was observed between type of tissue (prostate cancer or benign hyperplasia) and metabolites detection



**Metabolites were detected at very low concentration:
Fasting period before the surgery?**

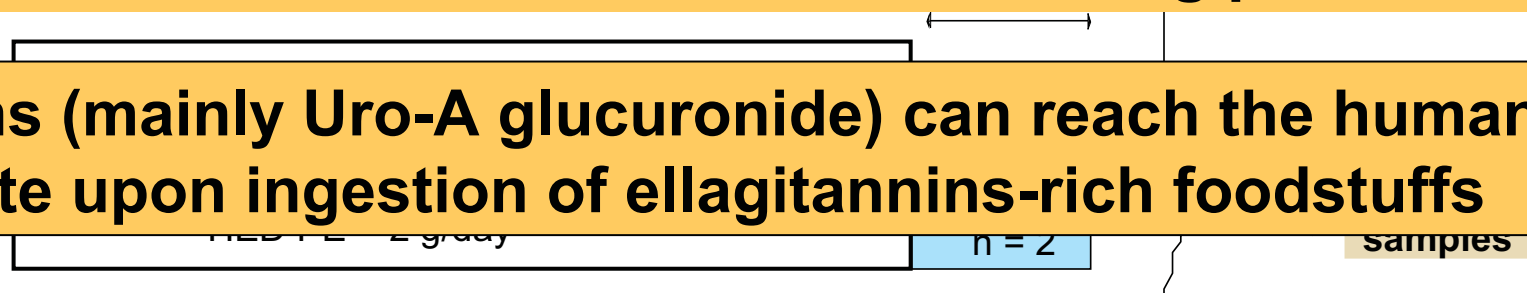


Analyses of rat prostates: Influence of the fasting period



In both groups, urolithin A glucuronide was only detected in rats with free access to feed, with no fasting period

Urolithins (mainly Uro-A glucuronide) can reach the human prostate upon ingestion of ellagitannins-rich foodstuffs



These metabolites could be involved in the protective effects of pomegranate juice intake against prostate cancer

Uro-A

(Without fasting) The presence of higher urolithins levels cannot be discarded in the human prostate

González-Sarrías et al. (2010). Occurrence of urolithins, gut microbiota ellagic acid-derived metabolites, in the human prostate gland upon consumption of walnuts and pomegranate juice. *Mol. Nutr. Food Res.* 54, 311-352.

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UROLITHINS: MULTITARGET MOLECULES PRODUCED BY THE GUT MICROBIOTA (anti-inflammatory, cancer cell regulation....)

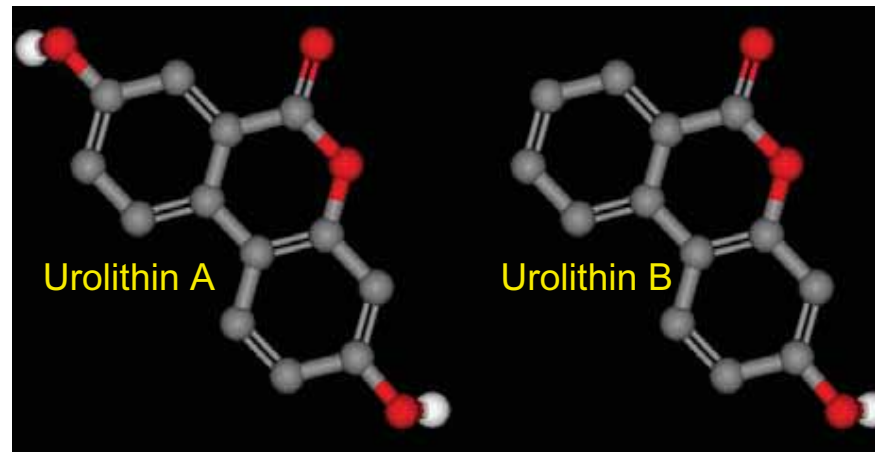
REGULATION OF GENE EXPRESSION:

- tumor suppressor genes,
- transcription factors,
- COX-2, mPGES-1, iNOS.....

HIGH CONCENTRATION
IN THE GUT

HIGH BIOAVAILABILITY

REGULATION OF GUT
MICROBIOTA



THE HUMAN PROSTATE
AS TARGET ORGAN

INHIBITION OF PROSTAGLANDIN E2
AND NO SYNTHESIS

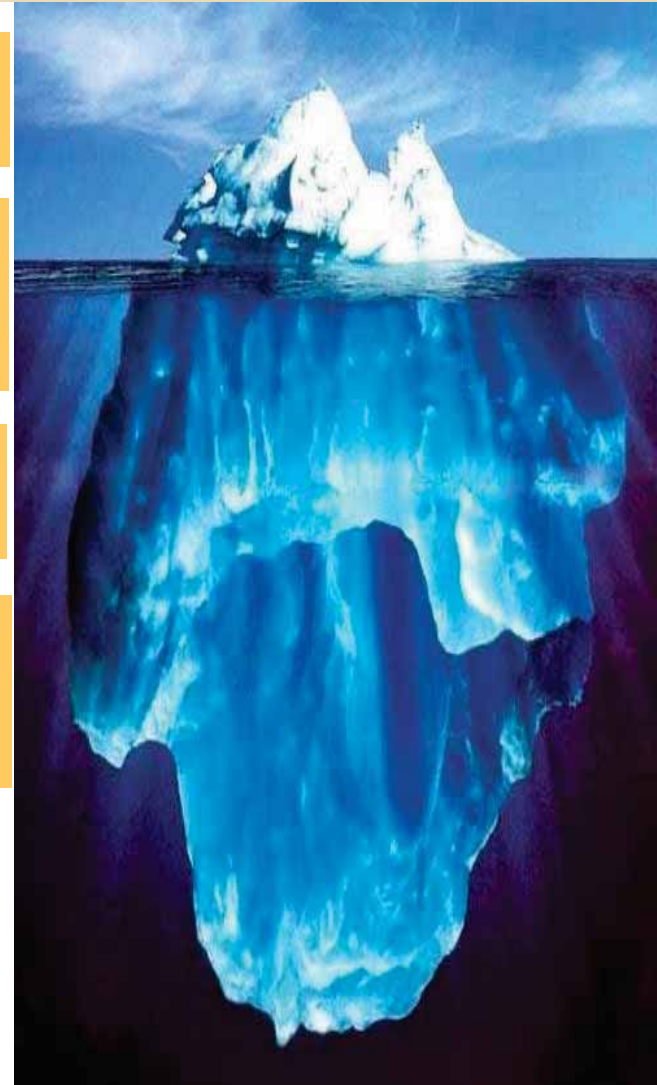
Urolithins as an iceberg: A long way to go for this emerging topic...

Identification of the microbiota involved in urolithins production

To study in depth the role of urolithins in colon inflammation and cancer: Many important markers

'Systemic' effect of urolithin conjugates: cardiovascular, other cancers....

Metabolism of ellagitannins in very low urolithin producers: What happens? Toxicity? Other effects?



Acknowledgements



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- *J.C. Espín de Gea*
- *F. Tomás-Barberán*
- *M. T. García-Conesa*
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- *M. Azorín*
- *F. Vallejo*
- *J.A. Giménez*
- *J. Tomé-Carneiro*
- *B. Cerdá*
- *R. González Barrio*

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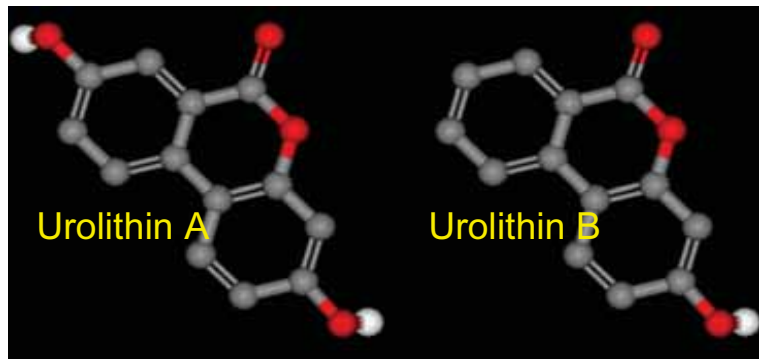
UROLITHINS: Multitarget molecules produced by the gut microbiota

REGULATION OF GENE EXPRESSION:

- tumor suppressor genes,
- transcription factors,
- COX-2, mPGES-1, iNOS.....

HIGH
CONCENTRATION IN
THE GUT

HIGH BIOAVAILABILITY



REGULATION OF GUT
MICROBIOTA

THE HUMAN PROSTATE
AS TARGET ORGAN

INHIBITION OF PROSTAGLANDIN E2
AND NO SYNTHESIS

**THANK YOU FOR
YOUR ATTENTION!**

