



# CSIC

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

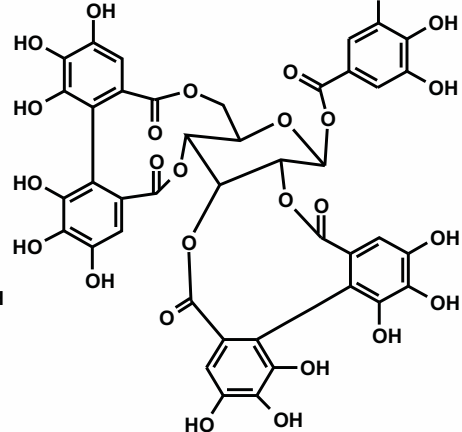
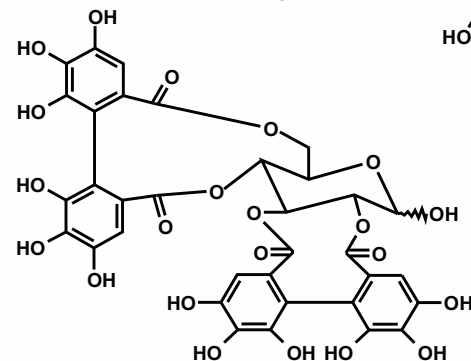
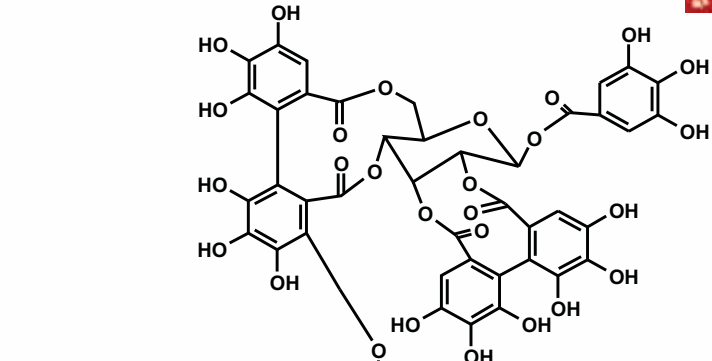
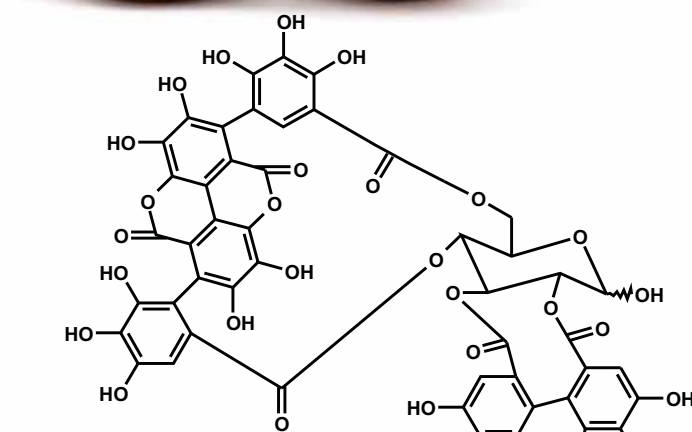
- ✓ Inflammatory bowel disease (IBD)

## 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

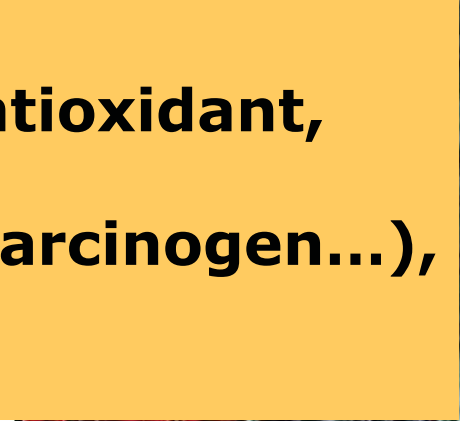
-.....

Multitarget action (antioxidant,

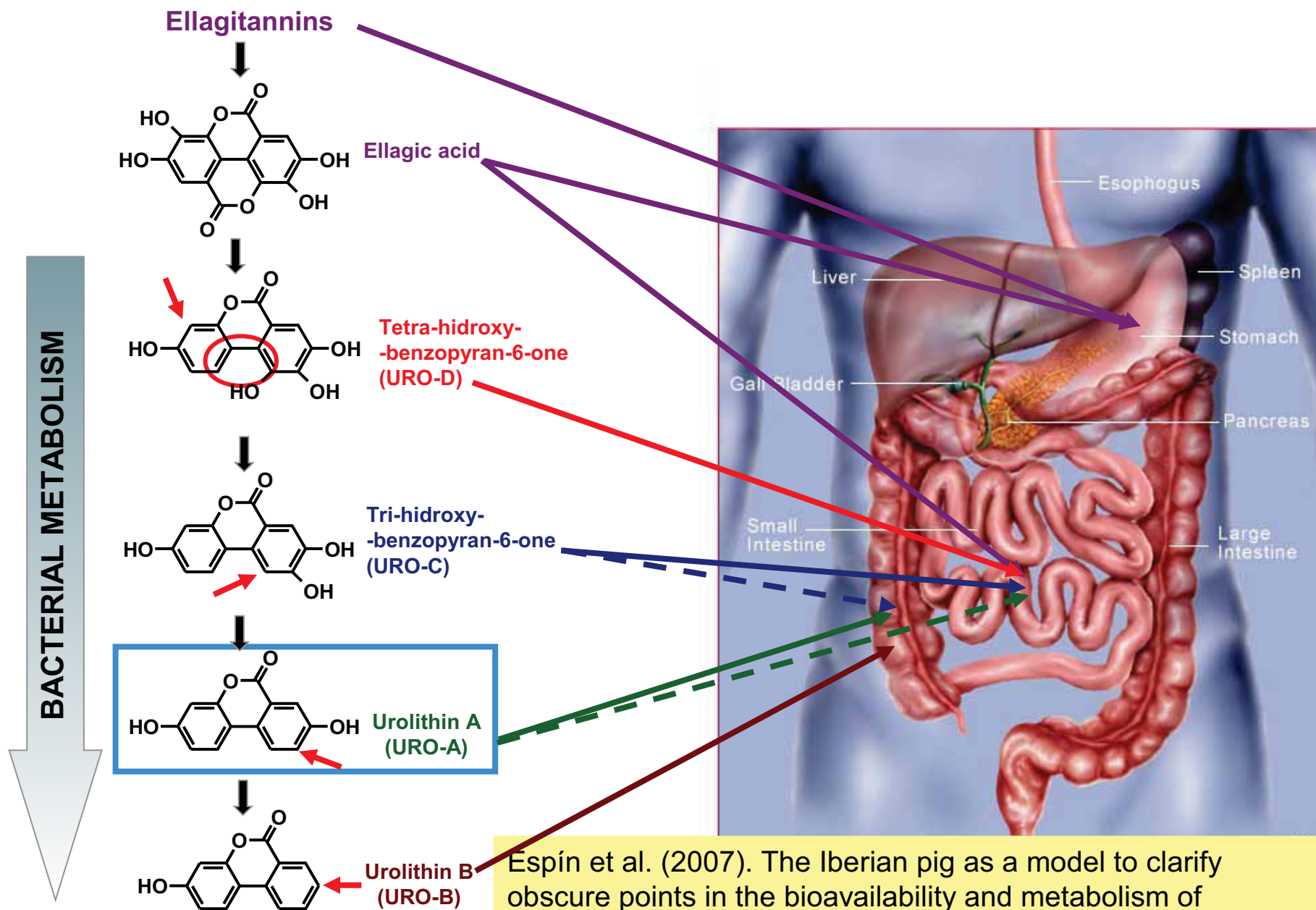
anti-inflammatory, anticarcinogen...),

**BUT...**

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

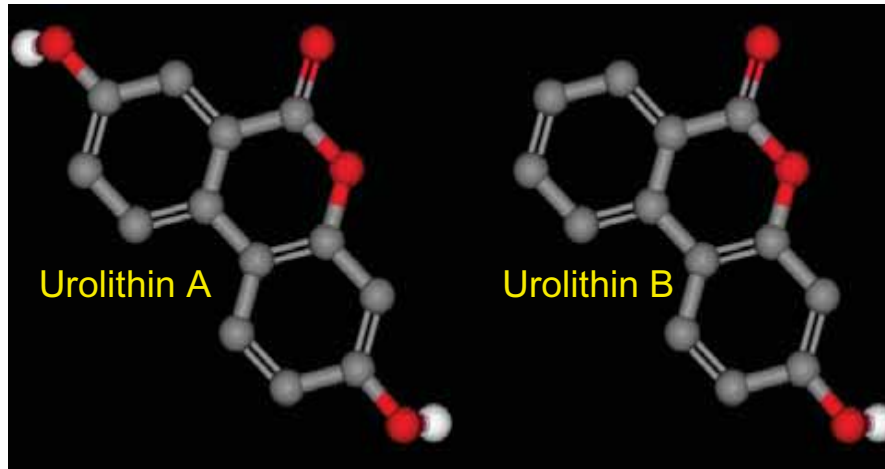


# METABOLISM OF ELLAGITANNINS (What do we know?)



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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## ELLAGITANNINS, ELLAGIC ACID AND UROLITHINS

- ✓ Metabolism
- ✓ Urolithins in Nature



## BIOLOGICAL ACTIVITY

- ✓ Inflammatory bowel disease (IBD)

## TARGET ORGANS

- ✓ Human prostate as target organ for urolithins

## CONCLUSIONS



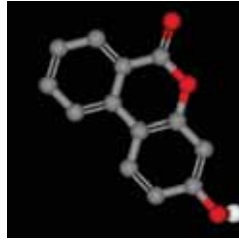
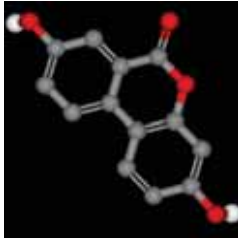
# BIOLOGICAL ACTIVITY OF UROLITHINS



## TRADITIONAL CHINESE MEDICINE

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

*(Trogopterus xanthipes)*

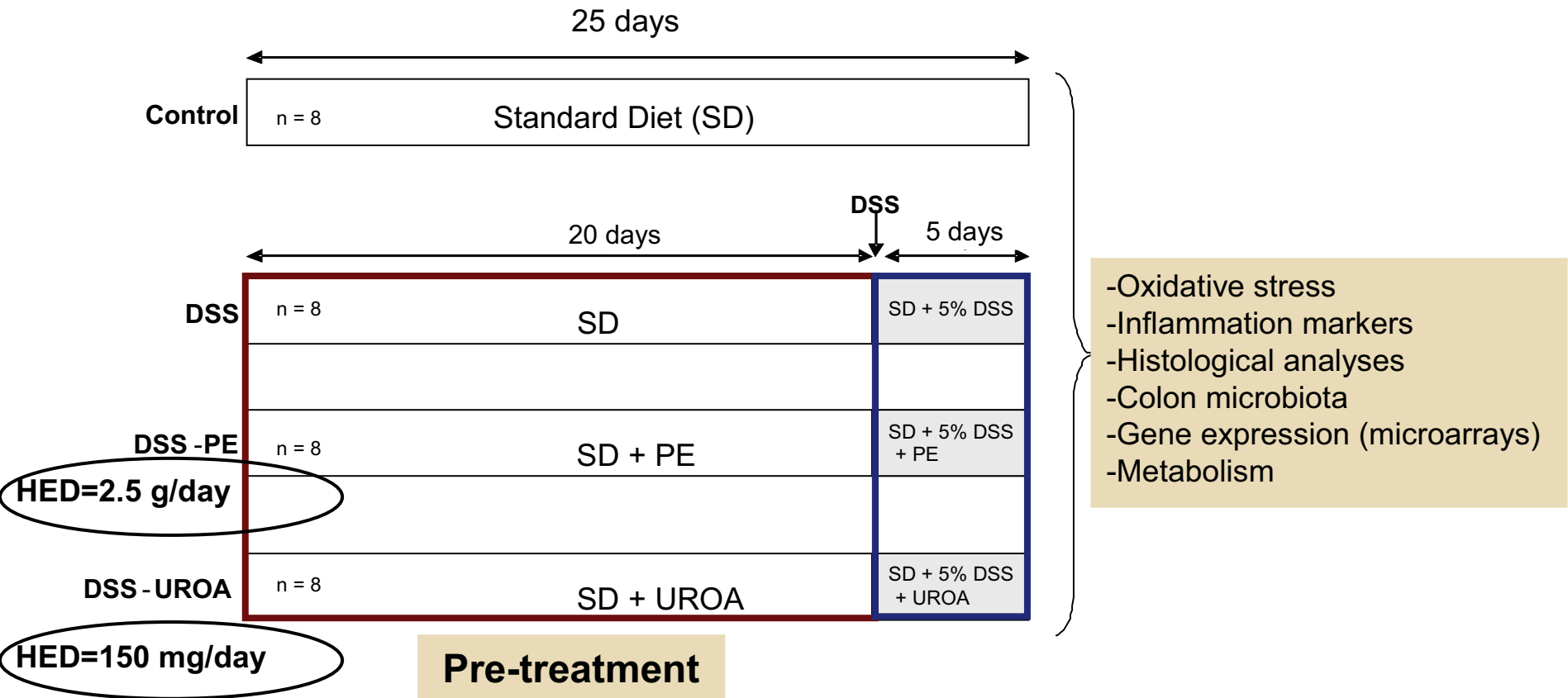


# POMEGRANATE EXTRACTS IN INFLAMMATORY BOWEL DISEASE (IBD): The role of urolithins

Fisher 344 rats



## Chronic inflammation increases CRC risk in IBD patients

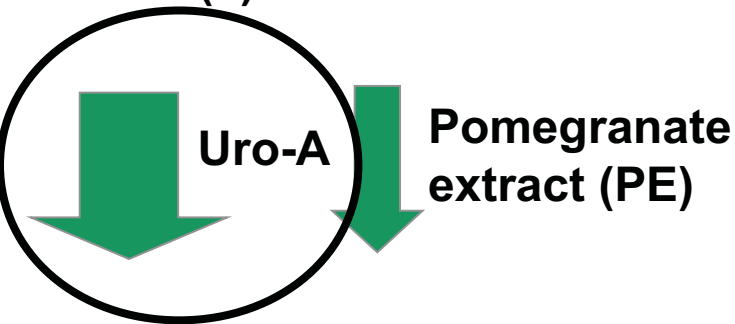


Larrosa et al. (2010). Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on the phenolic metabolism. *J. Nutr. Biochem.* 21, 717-725.

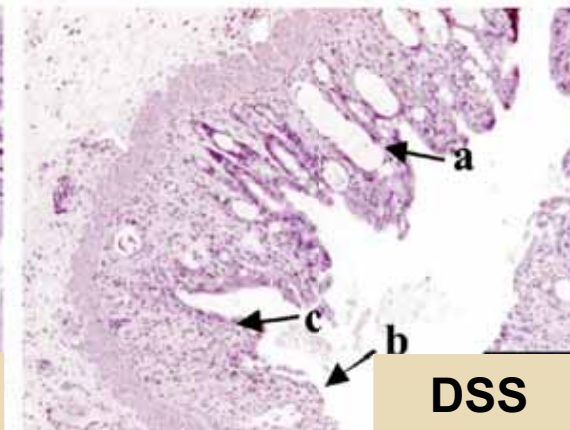
# Histological analyses of colon samples



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



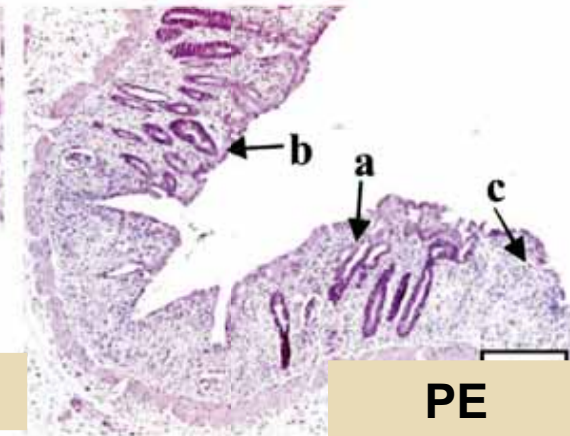
Control



DSS



Uro-A



PE

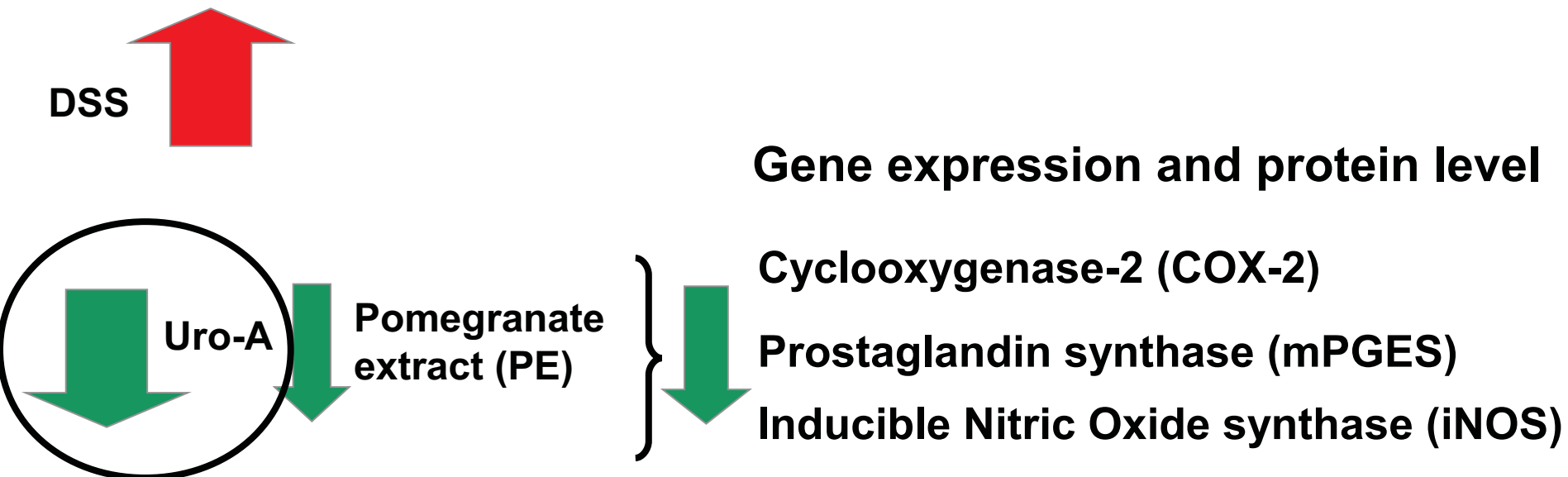
✓ PE and Uro-A protected colon from tissue damage

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 (PGE<sub>2</sub>), 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.

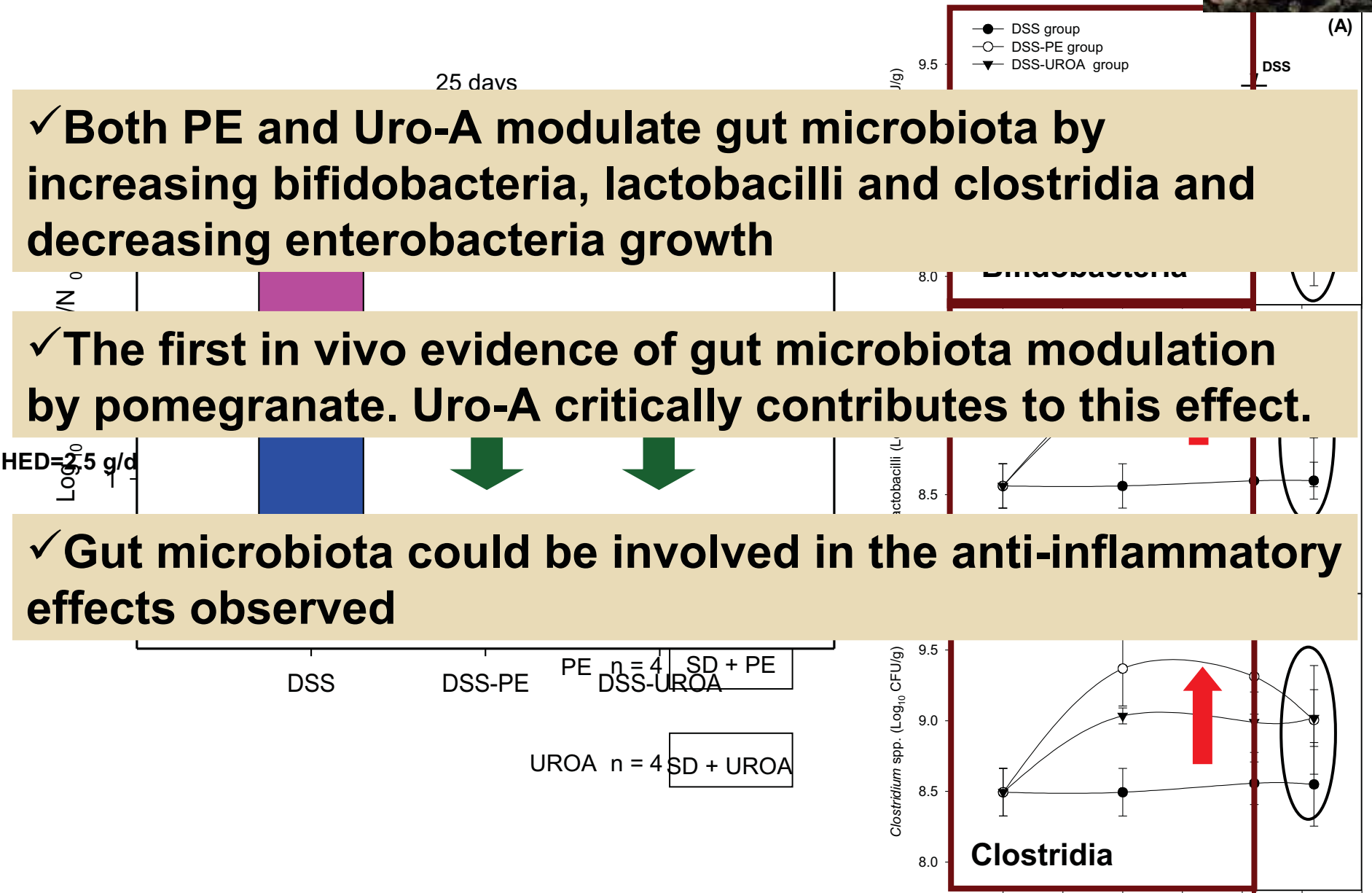
# Effect of PE and Uro-A on gut microbiota



✓ 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.

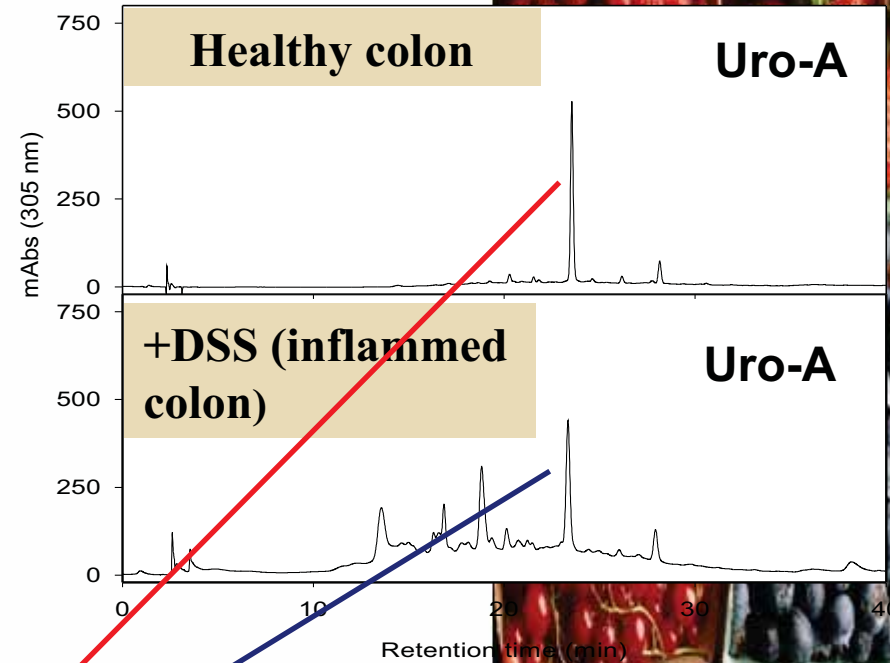
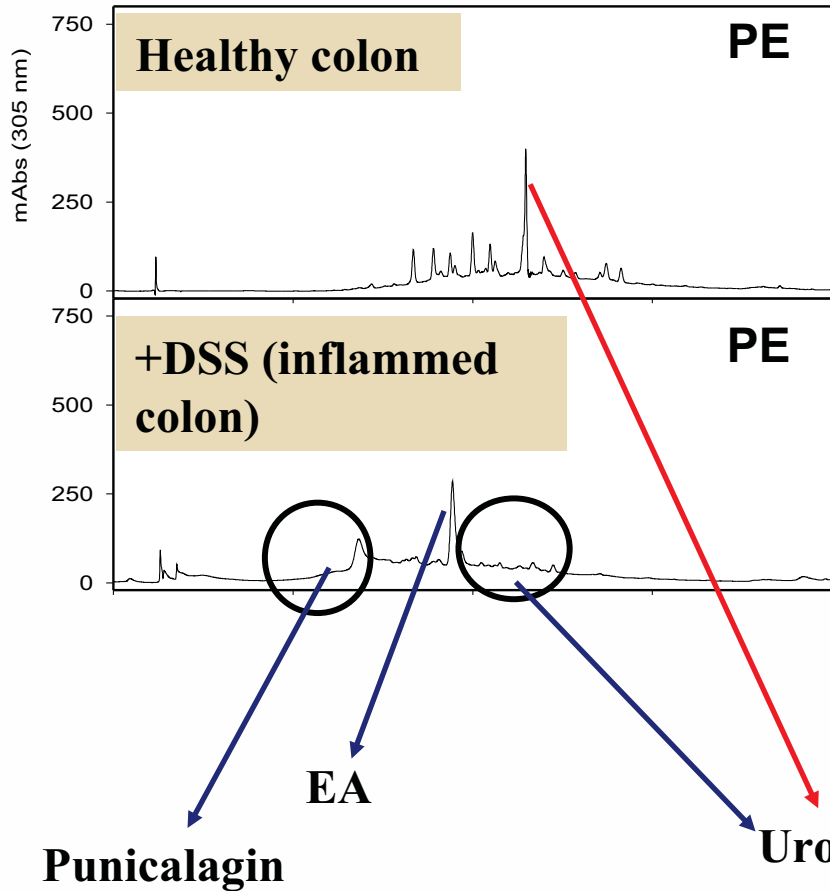
✓ Gut microbiota could be involved in the anti-inflammatory effects observed



# Effect of DSS on pomegranate polyphenols metabolism

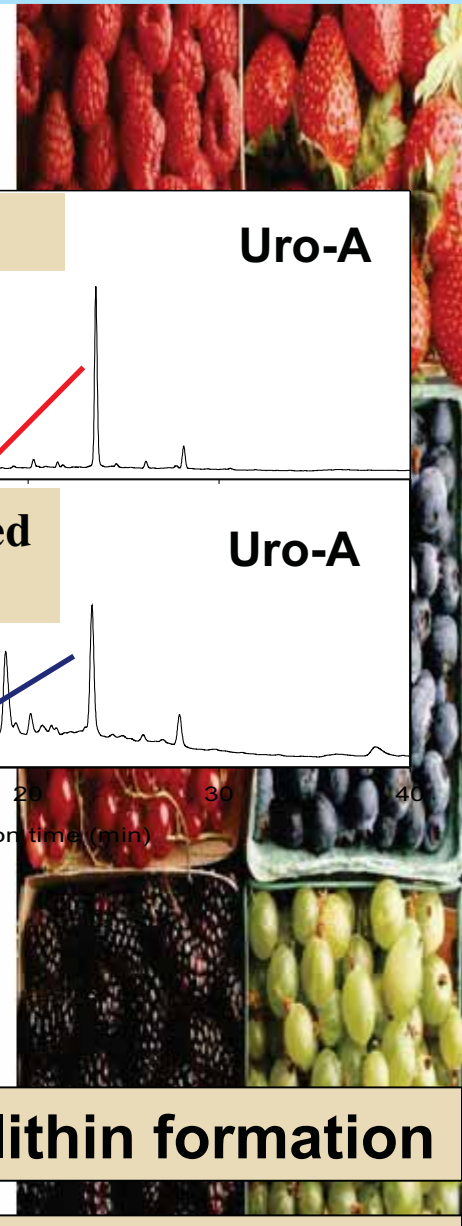
Larrosa et al. (2010). *J. Nutr. Biochem.* 21, 717-725.

## 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

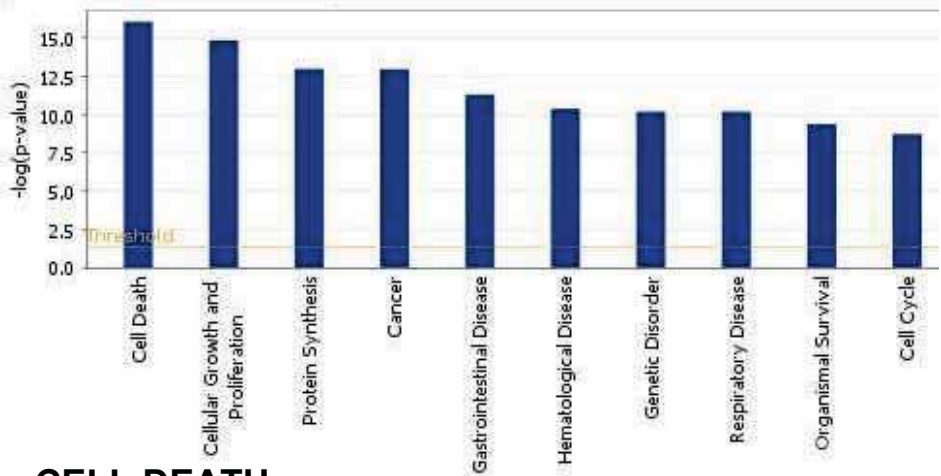
6,995 genes

667 common genes

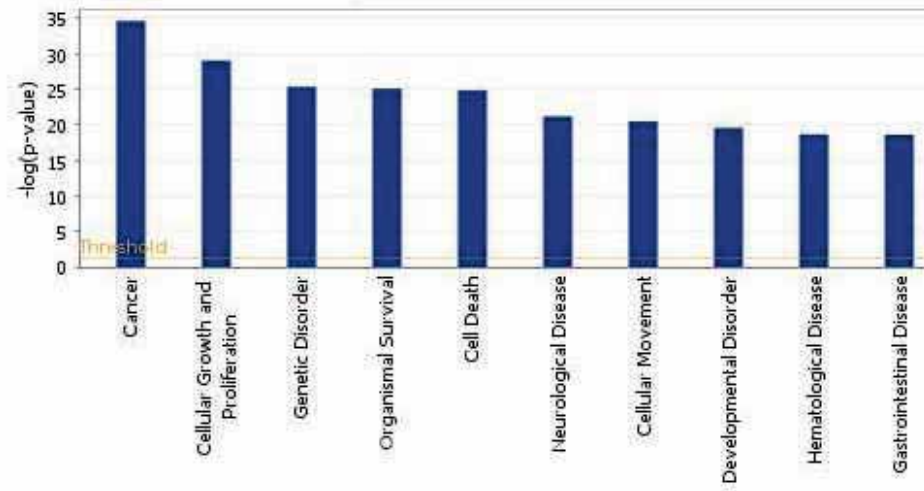
✓ Both PE and Uro-A modulate gene profile of colon mucosa

# 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**  
**Organismal survival**  
**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

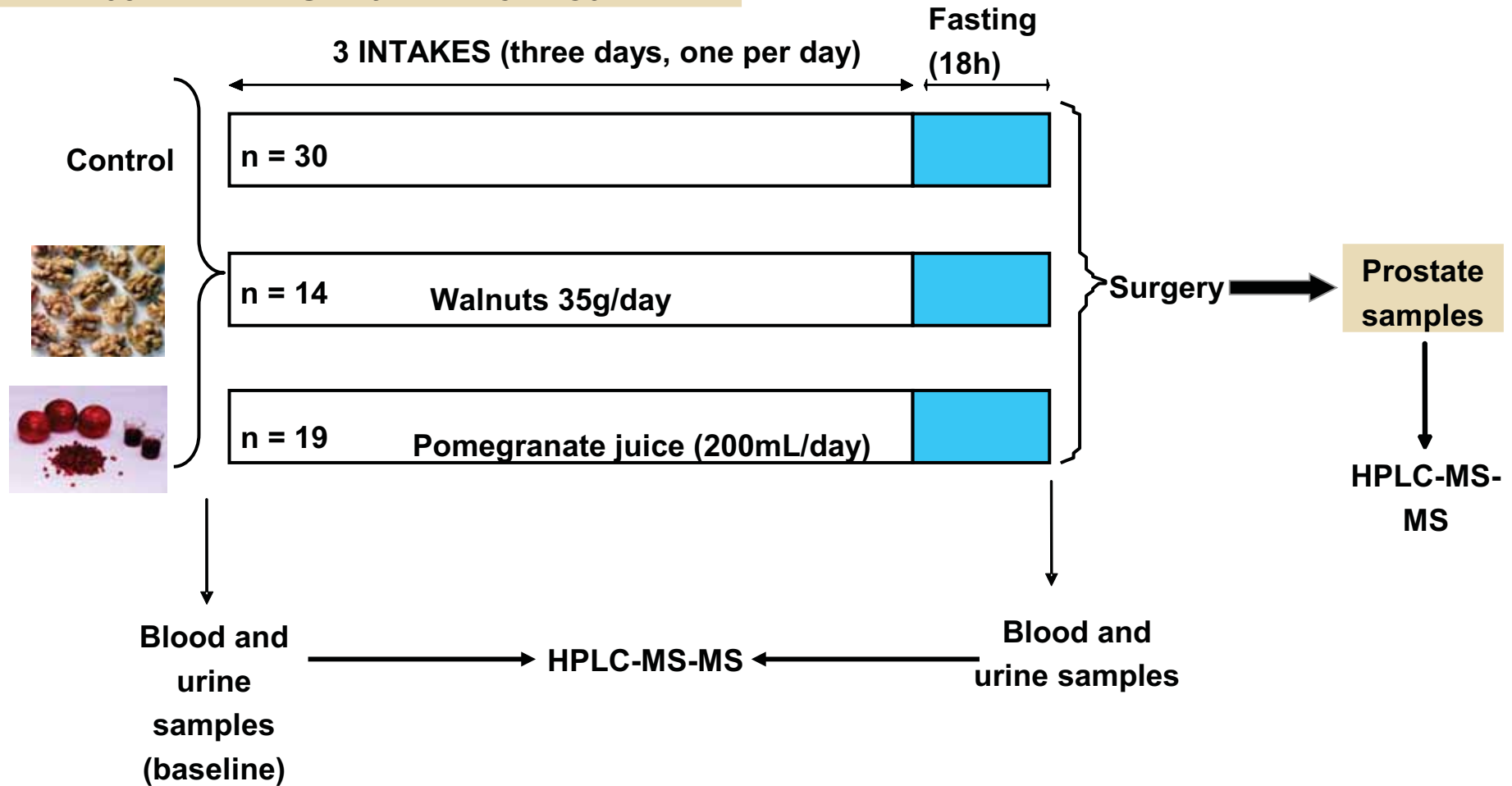
- ✓ Human prostate as target organ for urolithins

## CONCLUSIONS



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

63 PATIENTS with BPH or PCa



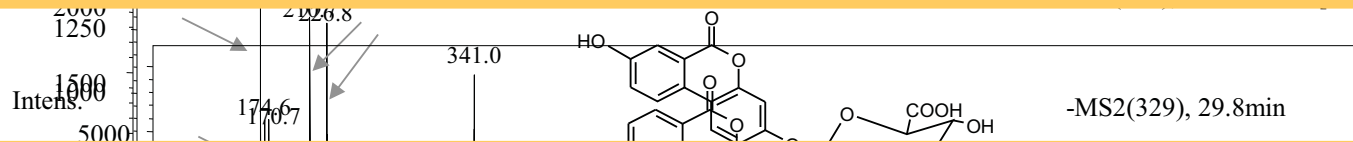
González-Sarrías et al. (2010). Occurrence of urolithins, gut microbiota ellagic acid-derived metabolites, in the human prostate gland upon consumption of walnuts and pomegranate juice. *Mol. Nutr. Food Res.* 54, 311-352.

# Analyses of human prostates

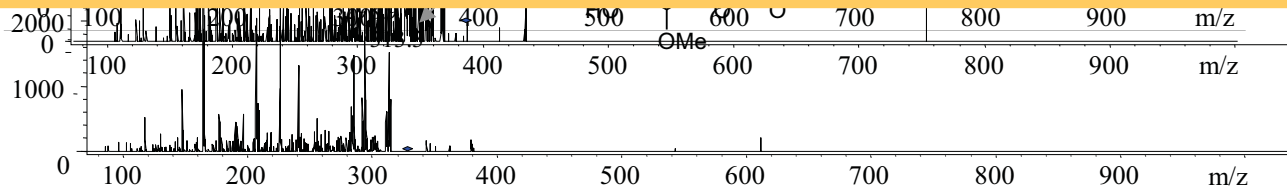
- High interindividual variability
- Metabolites in 8 prostate samples (high urolithin producers): 24% of patients
- Uro-A glc: 6 samples (0.5-2 ng/g tissue) → UV, MS, MS/MS
- Uro-B glc: 2 samples → MS and MS/MS
- Dimethyl ellagic acid (DMEA): 4 samples → MS and MS/MS



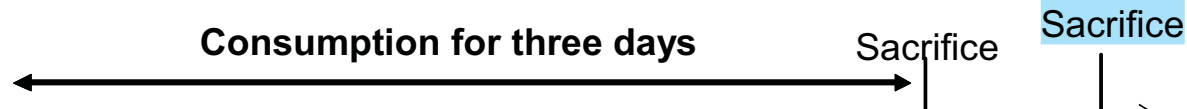
**No correlation was observed between type of tissue (prostate cancer or benign hyperplasia) and metabolites detection**



**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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## TARGET ORGANS

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

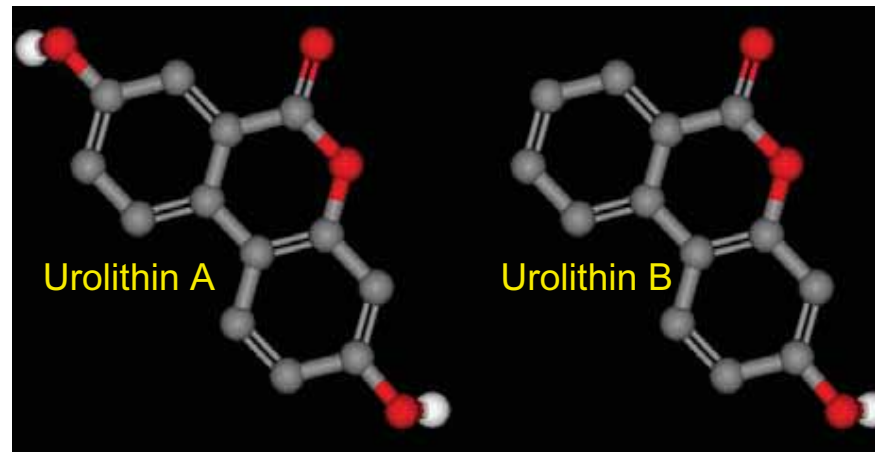
## REGULATION OF GENE EXPRESSION:

- 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

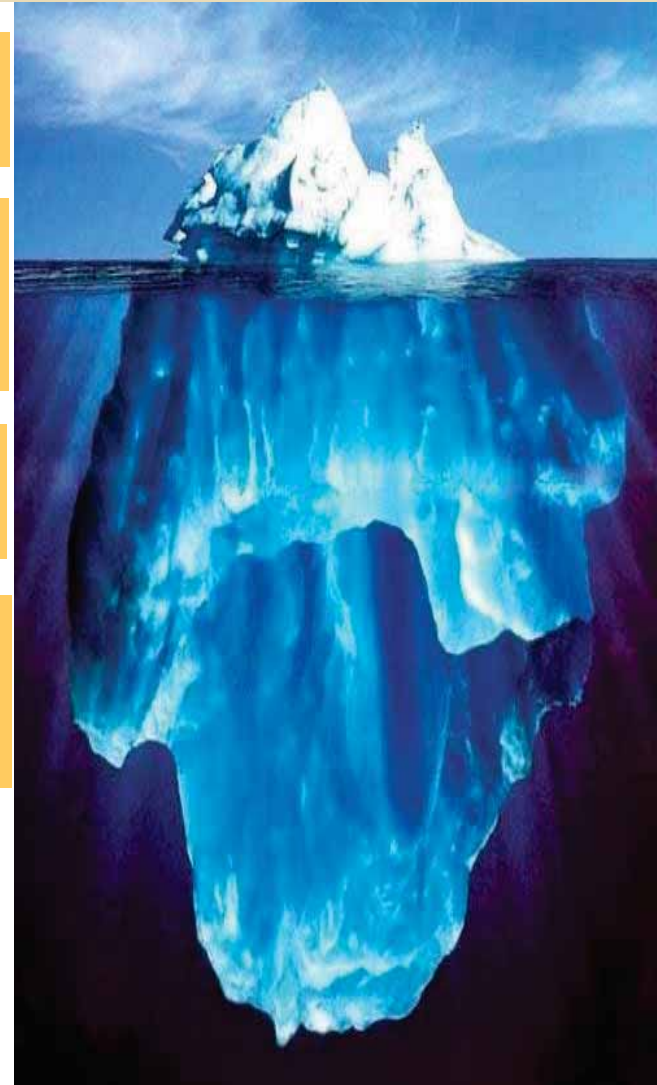
# Urolithins as an iceberg: A long way to go for this emerging topic...

**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



## **CEBAS-CSIC**

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- *F. Vallejo*
- *J.A. Giménez*
- *J. Tomé-Carneiro*
- *B. Cerdá*
- *R. González Barrio*

**Collaborations:** *Hospital V. Arrixaca, Univ. Complutense-Madrid, Univ. Murcia, Hospital Reina Sofia, Veterinary Hospital (Murcia)*

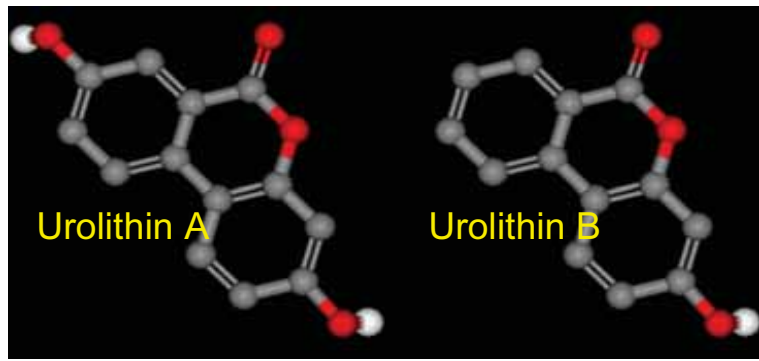
# UROLITHINS: Multitarget molecules produced by the gut microbiota

## REGULATION OF GENE EXPRESSION:

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

HIGH  
CONCENTRATION IN  
THE GUT

HIGH BIOAVAILABILITY



REGULATION OF GUT  
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THE HUMAN PROSTATE  
AS TARGET ORGAN

INHIBITION OF PROSTAGLANDIN E2  
AND NO SYNTHESIS

**THANK YOU FOR  
YOUR ATTENTION!**

