

## Effect of cocoa, cocoa polyphenols, theobromine and hesperidin on the gene expression of tight junction proteins in Caco-2 cells

Camps-Bossacoma M<sup>1,2\*</sup>, Pérez-Cano FJ<sup>1</sup>, Franch À<sup>1</sup>, Untermayr E<sup>2</sup>, Castell M<sup>1</sup>

<sup>1</sup>Secció de Fisiologia, Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Av Joan XXIII 27-31, 08028 Barcelona (Spain); Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), UB.

<sup>2</sup>Institute of Pathophysiology and Allergy Research, Centre for Pathophysiology Infectiology and Immunology, Medical University Vienna, Währinger Güerte 18-20I, A-1090 Vienna (Austria).

\*Presenting author

### **Background and objectives:**

Cocoa powder contains over 500 different compounds with polyphenols and methylxanthines as the most recognized active ones. The human adenocarcinoma cell line (Caco-2) has been widely used as model of the intestinal epithelial layer for barrier investigations. The aim of this study was to establish the effect of cocoa powder, a cocoa polyphenol extract (CPE), theobromine and hesperidin on the gene expression of tight junction proteins in the Caco-2 cell model.

### **Methodology:**

The Caco-2 clone TC7 was grown in DMEM medium supplemented with non-essential amino acids, HEPES, heat inactivated foetal calf serum, glutamine and penicillin-streptomycin. After 21 days, Caco-2 cells were incubated either with cocoa powder (10 µg/mL), CPE (10 µg/mL), theobromine (10 µM- 100 µM) or hesperidin (10 µM- 100 µM) for 30, 60 and 120 min. Total RNA was isolated using Trizol and the RNeasy RNA isolation kit including a DNase digestion. Later, cDNA was obtained by the High Capacity cDNA Reverse Transcription Kit and a PCR quantitative assay was performed to determine gene expression of claudin-1, claudin-4, occludin, zonula occludens (ZO)-1 and ZO-2.

### **Results and conclusions:**

The incubation of cocoa powder for 120 min increased claudin-1 gene expression of Caco-2 cells. No differences were obtained for claudin-4, occludin, ZO-1 and ZO-2 in the three studied time points and for all tested compounds.

In conclusion, the present data suggest that cocoa polyphenols, as well as the polyphenol hesperidin and the alkaloid theobromine do not compromise the intestinal epithelial barrier *in vitro*. Further studies are needed to in depth evaluate the effect of these food compounds in the intestinal layer.

***Acknowledgements:***

The authors would like to thank Denise Heiden and Teresa Manhart for her excellence technical assistance.