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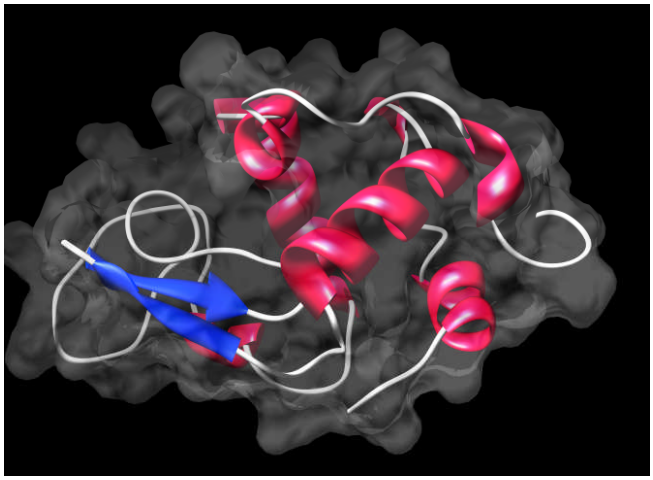
Bioactive dairy protein complexes - *in vitro* and *in vivo* digestion



Key external stakeholders:

Food, feed and pharmaceutical industry

Practical implications for stakeholders:

- Whey proteins can act as delivery vehicles of small molecules such as fatty acids, thereby changing their biological activity
- *In vitro* and *in vivo* tools are available within Teagasc to assess digestibility, bioaccessibility and bioavailability of food compounds

Main results:

The key results were:

- α-lactalbumin (α-la) and β-lactoglobulin (β-lg), both whey proteins, can bind small hydrophobic molecules and act as delivery vehicles to cells
- α-la and β-lg can alter the solubility of fatty acids, thereby affecting their biological activity e.g. increasing or decreasing their anti-tumour activity or delay the uptake of fatty acids
- *In vivo* gastric digestion of α-lactalbumin in adults (n=10) provided valuable and novel insight into the mechanism and kinetics of protein breakdown

Opportunity / Benefit:

The research team in Teagasc Moorepark has developed *in vitro* and *in vivo* tools to assess the digestive mechanism of food components. Assays such as bioaccessibility and bioavailability are now available to interested end users.

Collaborating Institutions:

Trinity College Dublin
University College Dublin
Alimentary Pharmabiotic Centre
Mercy University Hospital, Cork
INRA, France
Agrocampus Rennes, France
University of California at Los Angeles, USA

Contact André Brodkorb Email: andre.brodkorb@teagasc.ie

Teagasc project team: André Brodkorb (PI), Linda Giblin, Joseph Kehoe, Solène Le Maux and Louise Sullivan (all TFRC Moorepark)

External collaborators: Ken H. Mok (Trinity College Dublin), Nora O'Brien (University College Dublin), Fergus Shanahan (APC), Martin J. M. Buckley (Mercy University Hospital, Cork) Saïd Bouhallab, Didier Dupont (both INRA, France), Thomas Croguennec (Agrocampus Rennes, France), Dorothy J. Wiley (University of California at Los Angeles, USA), Collaborators in COST action INFOGEST FA1005

1. Project background:

The past decade has seen a large increase in interest from consumers and industry in functional foods and ingredients; these ingredients can provide the consumer with other health benefits beyond nutrition.

α-lactalbumin (α-la) is the main whey protein in human milk and is also present in bovine milk. It has been known that a cytotoxic complex can be formed when α-la associates with a major fatty acid, oleic acid. Studies on this complex have, until recently, primarily focused on its cytotoxic mode of action and the type of mammalian cells against which it was active. While it has been suggested that α-la/oleic acid complexes could be formed during the digestion of dairy products, no studies have been carried-out to verify this.

2. Questions addressed by the project:

The main questions prior to 2008 were whether α-la/oleic acid–like complexes have any relevance for food and more specifically whether they could be formed *in vivo* during gastro-intestinal digestion. In addition, there was little or no information of the role of the individual components, protein vs. fatty acid, and whether other proteins and fatty acids can induce similar effects.

3. The experimental studies:

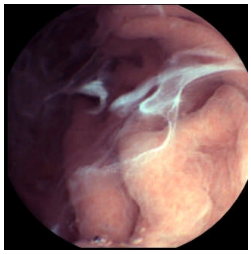
A semi-dynamic *in vitro* gastric digestion model was established using computer controlled titration to mimic the pH gradient in an infant's stomach during digestion. The α-la/oleic acid complexes were digested, analysed for structural changes and tested with cell lines such as HL60, U937, PC12 and Caco2.

A human *in vivo* study was carried-out in the Mercy University Hospital in collaboration with the Alimentary Pharmabiotic Centre (APC) to follow the fate of α-la during digestion in the stomach of healthy adults.

A variety of novel complexes were formed from the proteins α-la, β-lactoglobulin (β-lg) and bovine serum albumin combined with a range of fatty acids. The critical reaction parameters were identified. One detailed study was carried out on the bioavailability of β-lg – linoleic acid complexes. Bioaccessibility, delivery and uptake of linoleic acid was tested in enterocyte-like Caco-2 monolayers. Changes in the production or secretion of the enteroendocrine satiety hormone, cholecystokinin, were investigated.

International links were established with the leading groups working on food digestion as part of an EU-funded COST action INFOGEST. The PI André Brodkorb led a group to harmonise existing food digestion methods.

4. Main results:



- α-la/oleic acid complexes could be formed during *in vitro* gastric digestion
- *In vivo* gastric digestion of α-la, using naso-gastric tube aspiration revealed a great insight into structural changes and the kinetics of protein hydrolysis. Capsule endoscopy provided real time images of gastric digestion of α-la, see image.
- Extensive physico-chemical characterization of a variety of α-la/oleic acid complexes was performed and substantial structural heterogeneities were discovered
- Complexes of α-la and β-lg with a number of fatty acids revealed a clear trend: the amount of fatty acid is correlated to the cytotoxicity of the complex, i.e. the protein is the "mule", the fatty acid is the "drug"

Contact André Brodkorb Email: andre.brodkorb@teagasc.ie

- β-lg can significantly delay the uptake of linoleic acid in intestinal cells.
- In collaboration with the COST action INFOGEST, an international consensus was established for a harmonised static *in vitro* digestion method suitable for food, coordinated by the PI André Brodkorb

5. Opportunity/Benefit:

Teagasc can provide expertise and/or service (assays and models) for testing food and food ingredients for their *in vitro* and/or *in vivo* digestibility, bioaccessibility and bioavailability.

6. Dissemination:

Main peer-reviewed publications:

*Le Maux, S., Giblin, L., Croguennec, T., Bouhallab, S., & Brodkorb, A. (**2012**). β-lactoglobulin as a molecular carrier of linoleate: characterisation and effects on intestinal epithelial cells in vitro. Journal of Agricultural and Food Chemistry, 60(37), 9476–9483. doi: 10.1021/jf3028396

*Brinkmann, C. R., Brodkorb, A., Thiel, S., & Kehoe, J. J. (**2013**). The cytotoxicity of fatty acid/α-lactalbumin complexes depends on the amount and type of fatty acid. European Journal of Lipid Science and Technology, 115(6), 591-600. doi: 10.1002/ejlt.201200165

*Le Maux, S., Bouhallab, S., Giblin, L., Brodkorb, A., & Croguennec, T. (**2013**). Complexes between linoleate and native or aggregated β-lactoglobulin: Interaction parameters and in vitro cytotoxic effect. Food Chemistry, 141(3), 2305-2313. doi: 10.1016/j.foodchem.2013.05.031

*Le Maux, S., Brodkorb, A., Croguennec, T., Hennessy, A. A., Bouhallab, S., & Giblin, L. (**2013**). β-Lactoglobulin-linoleate complexes: In vitro digestion and the role of protein in fatty acids uptake. Journal of Dairy Science, 96(7), 4258-4268. doi: 10.3168/jds.2013-6682

*Sullivan, L., Mok, K. H., & Brodkorb, A. (**2013**). The Formation of an Anti-cancer Complex Under Simulated Gastric Conditions. Food Digestion, 4(1), 7-18. doi: 10.1007/s13228-012-0030-0

De Azambuja, K., P. Barman, J. Toyama, D. Elashoff, G. W. Lawson, L. K. Williams, K. Chua, D. Lee, J. J. Kehoe, A. Brodkorb, R. Schwiebert, S. Kitchen, A. Bhimani and D. J. Wile (**2014**). Validation of an HPV16-mediated Carcinogenesis Mouse Model. In Vivo, 28(5), 761-767.

*Kehoe, J. J., & Brodkorb, A. (**2014**). Interactions between sodium oleate and α-lactalbumin: the effect of temperature and concentration on complex formation. Food Hydrocolloids, 34, 217-226. doi: 10.1016/j.foodhyd.2012.09.009

*Kehoe, J. J., Lišková, K., Mok, K. H., O'Brien, N., Kelly, A. L., & Brodkorb, A. (**2014**). Formation of cytotoxic α-lactalbumin / sodium oleate complexes: Concentration and temperature effects. International Dairy Journal, 38(1), 65-73. doi: http://dx.doi.org/10.1016/j.idairyj.2014.04.005

Le Maux, S., Bouhallab, S., Giblin, L., Brodkorb, A., & Croguennec, T. (2014). Bovine β-lactoglobulin/fatty acid complexes: binding, structural, and biological properties. Dairy Science & Technology, 1-18. doi: 10.1007/s13594-014-0160-y

*Minekus, M., M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carriere, R. Boutrou, M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. McClements, O. Menard, I. Recio, C. N. Santos, R. P. Singh, G. E. Vegarud, M. S. J. Wickham, W. Weitschies and A. Brodkorb (**2014**). A standardised static in vitro digestion method suitable for food - an international consensus. Food & Function, 5(6), 1113-1124. doi: 10.1039/C3FO60702J

*Sullivan, L. M., Kehoe, J. J., Barry, L., Buckley, M. J. M., Shanahan, F., Mok, K. H., & Brodkorb, A. (**2014**). Gastric digestion of α-lactalbumin in adult human subjects using capsule endoscopy and nasogastric tube sampling. British Journal of Nutrition, 112, 638–646. doi: 10.1017/S0007114514001196

* Teagasc researcher as corresponding author

Popular publications:

Reactivating HAMLET, Science Spin – Issue 56; Page 22-23 – January 2013,
http://issuu.com/spin35/docs/supplement_2013/10

When the experimenters become the experiment, Irish Times (2 January 2014).
http://www.irishtimes.com/news/science/when-the-experimenters-become-the-experiment-1.1634800

7. Compiled by: André Brodkorb

Contact André Brodkorb Email: andre.brodkorb@teagasc.ie