Project number: 5942
Funding source: Enterprise Ireland
Date: Oct, 2014
Project dates: Nov 2009-Oct 2013

Food Solutions for Weight Management

FOOD FOR HEALTH IRELAND

Key external stakeholders:
Dairy Industry, Food manufactures, Consumer

Practical implications for stakeholders:
Teagasc, under the Food for Health Ireland (FHI) umbrella, is striving to deliver foods that enhance satiety.

- Ingestion of such foods may reduce portion size and/or frequency leading to a reduction in food intake over time.
- The aim is to identify ingredients that 'make you feel fuller for longer'

Main results:
- One thousand dairy fractions were screened in high throughout satiety assays *in vitro.*
- Of these, eleven lead functional compounds were identified; eight activating satiety receptors in the brain and three increasing satiety hormone secretion in the intestine.
- Three of these 'leads' have been proven to reduce food intake over time in animal trials.
- Successful scale up to 100 litres has occurred with at least one lead functional compound

Opportunity / Benefit:
- We have identified eleven milk derived ingredients that enhance satiety.
- These ingredients can potentially be used by the food industry in weight management/slimming products.
- An economical viable scaled up satiety enhancing ingredient with strong scientific data benefits the consumer in the 'battle of the bulge'.
- A major achievement has been the establishment of high throughput bioassays for satiety and adiposity which are now available as a compound screening platform to industry.

Collaborating Institutions:
UCC, UCD, UL, DCU under the FHI umbrella www.fhi.ie.

Contact Linda Giblin Email: linda.giblin@teagasc.ie.

Teagasc project team: Dr. Linda Giblin (PI)
Dr. Fiona O'Halloran
Dr. Christine Bruen

External collaborators: Prof. John Cryan, UCC
Prof. Ted Dinan, UCC
Prof. Alan Kelly UCC
FHI academic and industrial partners

1. Project background:

Obesity is a global health concern. In Europe alone, obesity and obesity related illness are responsible for over 1 million deaths each year. By 2015 almost 1.5 billion consumers worldwide will be overweight or obese. The diet and weight management market is, therefore, worth over €13.9 billion. An increase in portion size has been identified as a major contributing factor to the obesity epidemic. Several of the drug-based therapies currently on the market to treat obesity either lack efficacy or have adverse side-effects. Food-based solutions can be more easily adapted into daily life. The weight management/slimming market primarily focuses on boosting satiety or heat generation. Satiety is the feeling of fullness. The presence of food in the gut causes secretion of several satiety hormones that control food intake.
Protein is generally regarded as the most potent stimulus of satiety. Bovine milk protein is of particular interest as several studies have correlated increased milk and dairy consumption with positive effects on body weight, metabolic control and glycaemia.
The aim of this project was to screen milk derived fractions for their ability to enhance satiety. Ingestion of foods containing such ingredients may reduce portion size and/or frequency leading to a reduction in food intake.

2. Questions addressed by the project:

- Can milk fractions increase satiety secretion from intestinal cells in vitro?
- If so, can these lead functional compounds reduce food intake in animal trials?
- What is the effective dose?
- Can these lead functional compounds survive gut transit?
- Can production of these lead functional compounds be scaled up and is this scale-up economical?
- What is the bioactive and what is it's mechanism of action?

3. The experimental studies:

We employed two different cell-based high-throughput assays to screen milk fractions for satiety and fat burning activities. These assays used the murine enteroendocrine cell line, STC-1 as the *in vitro* gut model and the differentiated 3T3-L1 cell line as the *in vitro* adipocyte model. The high throughput satiety assays were based on HTRF technology and measured cAMP and calcium flux. 'Hits' were then funneled into satiety-specific bioassays that measure levels of satiety signals (GLP-1, PYY and CCK) using qRT-PCR and MSD technology. Positives from here were labeled as lead functional compounds and were progressed into animal studies and scale-up.
An in-house scale-up team (led by Dr Phil Kelly) focused on the economic potential of pre-commercial scale up of the lead functional compounds. Lead functional compounds were given to mice via oral gavage or intra-peritoneal injection and food intake levels recorded over time. Follow-up, and arguably more accurate, studies were then performed in pigs. A lead functional compound at different concentrations in a food matrix was fed to pigs. Blood samples were taken every 15 minutes over a 2 hour period and levels of satiety signals in the blood measured. Although researchers at Teagasc have focused on screening milk fractions, the screening assays developed are applicable to screen any food fraction for promoting satiety or fat burning.

4. Main results:

Dr. Linda Giblin's team screened > 1000 milk fractions in high throughput satiety assays. Of these, approximately 15% were funneled into specific satiety assays. We identified three 'hits', capable of dose dependently increasing GLP-1 secretion from the STC-1 intestinal cells. Two of these were progressed to rodent studies. With our collaborators at UCC, the team performed oral gavage experiments and simulated upper gastric digestions with one lead functional compound. This gave us some anecdotal evidence of bioactivity survival during gut transit.
Parallel work focused on modification of production protocols so that 100 litres scale-up was achieved economically. The optimized protocol (change in % solids, enzyme concentration, pH, freeze drying V spray

Contact Linda Giblin Email: linda.giblin@teagasc.ie.

drying survival) resulted in increased biofunctionality.
To determine bioactive peptides, the lead functional compound was fractionated and tested in cellular bioassays. 'Best guess' bioactive peptides were synthesized and tested.
With our front runner, we performed a post-prandial pig trial to look at effective dosage, food matrix and allow for blood sample collection post-ingestion. Satiety hormones in blood were measured every 15 mins for 4 hours post-ingestion, The team at Moorepark concluded that this lead functional compound needed protection from the harsh conditions of the gut to maintain bioactivity. The project has entered a very exciting phase with a human trial now underway.
During the lifetime of this project we developed a large number of bioassays (high medium and low throughput) to screen compounds (food or chemicals) for their ability to modify satiety and adiposity. As such a spin off from this project was a number of confidential industrial projects.

5. Opportunity/Benefit:

The satiety enhancing ingredients identified can potentially be incorporated into weight management/slimming products by the food industry.

Discovering ingredients that target satiety are of public good as these ingredients will aid the consumer manage their weight.

A major achievement has been the establishment of high throughput bioassays for satiety and adiposity which are now available as a compound screening platform to industry.

6. Dissemination:

Technology transfer will be managed via FHI www.fhi.ie

Main publications:

Book Chapters:
Giblin, L., McCarthy, T., Gil-Lozano, M., Gagnon, J.D.and Brubaker, P.L. (2014) 'Enteroendocrine Cell Models for Screening Food Bioactives..' *INFOGEST*, Springer (in press).

McCarthy, T., Green, B.D., Calderwood, D., Gillespie, A., Cryan, J.F. and Giblin, L. (2014) 'STC-1 cells.' *INFOGEST*, Springer (in press).

Journal Articles:
Bruen, C.M., O'Halloran, F., Cashman, K.D. and Giblin, L. (2012) 'The effects of food components on hormonal signalling in gastrointestinal enteroendocrine cells.' *Food and Function,* 3(11): 1131-43.

Bruen, C.M., Kett, A.P., O'Halloran, F., Chaurin, V., Fenelon, M.A., Cashman, K.D. and Giblin, L. (2012) 'Effect of gelatinisation of starch with casein proteins on incretin hormones and glucose transporters *in vitro*.' *British Journal of Nutrition* 107: 155–163.

4 scientific papers submitted to peer reviewed international journals.

Conference Presentations:
Diacetyl (2,3 butanedione), increases GPR120 production and cell surface expression *in vitro*. Triona McCarthy, T., Bruen, C., Schellekens, H., Cryan, J.F. and Giblin, L. (2013) *Young Life Scientists' Symposium 2013 - Cell Signalling*, Cork, Ireland 11th Sept.

Bruen, C.M., Schellekens, H., Simpson, P., Cryan, J.F., Dinan, T.G. and Giblin, L. (2013) 'Identification and characterisation of a milk protein hydrolysate that increases satiety signalling *in vitro* and reduces feed intake *in vivo*.' *European Congress on Obesity* 2013 Liverpool, U.K. 12-15 May.

'Dairy components and satiety- a taste of things to come?' McCarthy, T., Cryan., J.F. and Giblin , L. (2012) *UCC Doctoral Showcase*, Cork, Ireland March.

Bruen C., Hannon J., O'Halloran F., Cashman K. and Giblin L. (2011) 'Mechanistic investigations into the effect of volatile dairy compounds on in vitro satiety signaling'. *40th Annual UCC Food Research Conference*, Cork, Ireland April.

Contact Linda Giblin Email: linda.giblin@teagasc.ie.

Effects of dairy protein hydrolysates on satiety Bruen, C., O'Halloran, F., Kett, A., Chaurin, V., Fenelon, M., Cashman, K. and Giblin, L. (2010) *International Congress on Obesity*, Stockholm, Sweden11-15 July

Bruen C., Hannon J., O'Halloran F., Cashman K. and Giblin L. (2011) 'The effects of aromatic compounds on taste transduction and gastrointestinal satiety signalling.' *Teagasc Walsh Fellowship Conference*. Dublin, Ireland Nov.

Bruen, C., O'Halloran, F., Kett, A., Fenelon, M., Cashman, K. and Giblin, L. (2009) 'Gut hormonal signaling in response to protein-carbohydrate food components.' *European Congress on Obesity* Amsterdam, The Netherlands, 5-8 May.

Popular publications Press Clippings:

'FHI program identifies new bioactive peptides in Milk Proteins.' (2010) *ADP1 American Dairy Products Institute*, May Release.
'FHI milk mining starts to strike gold.' (2010) *Nutriceutical Business and Technology*, June Release.
'FHI identifies new bioactive peptides.' (2010) Dairy Industries International, July Release.
'FHI programme identifies new bioactive peptides in milk proteins.' (2010) *Food ingredients*, Aug Release.
'Next satiety foods will contain cocktail of ingredients.' (2011) *Food navigator*, July Release.
'Milk protein could be sold as diet drink.' (2012) *Sunday Times,* 14 Oct.
'Food solutions for weight management - Satiety enhancing bioactives.' (2012) *T-Research*, 7(3) 16-17 ISSN1649-8917.

7. Compiled by: Dr. Linda Giblin

Contact Linda Giblin Email: linda.giblin@teagasc.ie.