Project Title: Identification and characterisation of novel bioactive marine peptides for management of metabolic disorders

Supervisors: Prof. Finbarr O’Harte Dr. Philip Allsopp

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Level: MRes/PhD PhD

Background to the project (200 words Max):
Marine sources, such as pelagic fish (e.g. blue whiting, boarfish) represent sustainable candidate raw materials for the mining of novel bioactive peptides for combatting common metabolic disorders. Fish protein hydrolysates will be supplied from our collaborators Prof. Dick FitzGerald at University of Limerick and/or Biomarine Ingredients Ireland (BII) Ltd., Killybegs, Co. Donegal. The ability of marine protein-derived peptides to modulate biomarkers associated with cardiometabolic risk, including obesity, diabetes and cardiovascular disease will be assessed using initial in vitro approaches. Pilot studies have indicated that fish protein hydrolysates elicit marked insulin releasing activity in cultured pancreatic β-cells as well as DPP-4 inhibitory actions. Initial screening of crude peptide hydrolysates or HPLC separated fraction components will be tested for insulin, GLP-1 or PYY/CCK releasing abilities in cultured pancreatic BRIN-BD11, GLUTag or STC-1 cells, respectively. The marine protein hydrolysates showing stability to simulated gastrointestinal digestion and significant insulitropic and potential appetite suppressing or cardiovascular benefits will be further assessed for dose-dependent effects in normal mice and therapeutic potential in high fat fed and/or db/db diabetic mice. This pre-clinical project aims to validate the hypothesis that marine peptides can improve the management of cardiometabolic risk.

Objectives of the research (400 words Max):
Following a period of laboratory training (0 to 4 months) the student will gain expertise in a range of screening assay techniques which are already available. The overall objective of the initial in vitro tasks (month 6 to 18) will be to investigate two different marine sources (e.g. blue whiting + boarfish or salmon) and employ various in vitro bioassays including assessment of appetite related peptides (using cellular models of ghrelin, PYY, CCK and GLP-1 secretion) and insulitropic cellular responses as well as antioxidant and anti-inflammatory responses for bioassay guided optimisation of hydrolysate generation and peptide enrichment and purification. Some of the bioactive fractions will have their peptides content characterised.
and identified using HPLC and MS analysis. Some of the identified peptides will be synthesised for further in vitro and in vivo testing. In years 2 and 3 acute in vivo testing will be performed in mice given orally ingested marine protein hydrolysates (as well as selected i.p. injected synthetic peptides) examining glucose tolerance, insulinotropic responses and inflammatory cytokine responses. In addition assessment of chronic hydrolysate administration on energy metabolism and appetite control will be assessed in diet induced high fat fed mice. Thus the initial assessment of marine hydrolysates for use in potential functional foods will be assessed. The most efficacious hydrolysates identified in the animal studies will inform potential efficacy of extracts to be incorporated in a future human trial.

**Methods to be used:**

*In vitro cellular assays*

A wide range of in vitro screening bioassays (month 6 to 18) will be used including toxicity testing by LDH assay (Phelan et al. 2009), insulinotropic cellular responses using pancreatic BRIN-BD11 cells (McClenaghan et al. 1996), GLP-1 secretion using GLUTag cells (Gribble et al. 2003). Appetite suppressing potential will be examined by determining the release of CCK-8 and PYY from STC-1 cells (Hand et al. 2010) and this will be complimented by the actions of hydrolysates on GLP-1 release above. The influence of the proteins/peptides on endothelial function will be assessed in HUVEC cells using Ransod and Randel assay kits which measure changes in intracellular reactive oxygen species, superoxide dismutase and gluthathione peroxidase, respectively (Li et al. 2010). The anti-inflammatory activity of the protein/peptides will be investigated in HUVEC and THP-1 cells through the measurement of a range of 10 selected cytokines prior to and following activation with TNF (HUVEC) (Yu et al. 2014).

**Bioactivity identification using chromatographic techniques**

In year 2 (months 14 to 20) the fractionation, enrichment and identification of protein hydrolysate will be undertaken in order to identify the peptides responsible for bioactivity. The two most promising hydrolysates (one from each source) will undergo fractionation using peptide fractionation techniques including solid phase extraction, ion exchange and semi-preparative/analytical RP-HPLC. Isolated fractions from the hydrolysates will undergo in vitro and cell based bioassay guided bioactivity screening in those assays where bioactivity was noted in the original hydrolysates as above. Identification of bioactive peptides will be performed by MS/MS (Zhu and FitzGerald 2010). Peptide sequencing by Edman degradation will confirm unequivocal structural identity (O'Harle et al. 2000) prior to peptide synthesis and testing of lead compounds in vitro and in vivo.

*In vivo animal studies*

*In vivo* acute studies will be performed (months 18 to 32) using orally administered marine hydrolysates (different doses) or synthetic peptides (identified above) in normal NIH Swiss and diabetic mice (db/db or high fat fed) (O'Harte et al. 2013). The effect of an OGTT with and without marine hydrolysates on glucose, insulin, PYY, ghrelin, GLP-1 and glucagon, as well as pro- and anti-inflammatory cytokines will be assessed over 120 min to determine acute effects of hydrolysates. A delayed oral glucose tolerance test (OGTT) will then be undertaken following 2, 6, 12 and 24 h hydrolysate consumption to obtain information on the pharmacokinetics of the hydrolysates or identified synthetic peptides (from above). Additionally the influence of hydrolysate gavage on food intake, and associated plasma glycaemic and hormonal responses (PYY, ghrelin, GLP-1, insulin, glucagon) will also be determined by measuring food intake at 30 min intervals up to 3 h in fasted conditioned mice. Difference in responses between groups will be assessed by ANOVA using a post hoc test and will enable the most effective concentration to be selected for subsequent chronic studies. The final thesis write up will be undertaken from months 32 to 36.

**Skills required of applicant (200 words Max):**

Key skills required will include considerable experience with in vitro laboratory work in cell culture with BRIN-BD11, GLUTag and STC-1 cells alongside analytical purification and identification techniques including RP-HPLC, ion-exchange and mass spectrometry. The ability to measure various analytes including cytokines and hormones using e.g. ELISA or insulin by RIA. A personal animal licence will be required and animal handling skills as well as competency with in vivo small animal studies.
References (Maximum of ten references):


Ethical Approval Required - **YES** / **NO** (Delete as appropriate)
If YES – please explain if this is currently in place or plans for obtaining such approval.

Not applicable.

Use of Core Facilities including BBRU - **YES** / **NO** (Delete as appropriate)
If yes, please ensure that appropriate costings are obtained from Dr Le Roy Dowey and provided below

Updated BBRU and metabolomics core facilities costings have been incorporated.

**THIS SECTION OF THE FORM IS FOR INTERNAL (i.e., BMSRI) USE ONLY**

Please identify how this project addresses/meets the research priorities of the Biomedical Sciences Research Institute/ Research Group:

This project forms part of a larger North/South collaborative project between Ulster University and University of Limerick (MaraPep) and commercial partners (BIi and Kerry Group) following on from the success of previous NutraMara projects. It seeks to exploit existing expertise on both sites to investigate the novel metabolic actions of marine sourced peptides as functional food ingredients with potential for the treatment and prevention of metabolic diseases including diabetes and obesity.

Please provide a list of the titles you submitted for this year’s round of undergraduate student projects (or provide details of extenuating circumstances which prevented the submission of titles):

**Project:**

First Supervisor – Finbarr O’Harte

Assessing the potential of apelin peptides for type 2 diabetes therapy (2 projects).
Marine derived protein hydrolysates for diabetes therapy (1 project).
Novel dual agonist peptide analogues: assessing their antidiabetic potential using cell culture models (1 project).
The effect of low-calorie sweeteners on insulin secretion from cultured pancreatic beta-cells (2 projects).

Please provide a list of the externally funded grants you have received within the last 3 years (print out from RO Required to be appended):

- Invest NI - Proof of Concept (Prof FPM O’Harte. Prof. PR Flatt, 2016-2017) £105,999.00 Validation of apelin peptide analogues for a new therapeutic approach to diabetes.
- Irish Endocrine Society - Small Grant Scheme (Prof. FPM O’Harte £15,562) Pre-clinical development of novel apelin-13 drug candidates for diabetes therapy.
- Department of Agriculture & Food – FIRM (Prof. FPM O’Harte, Dr E Brown, Dr E McSorley, Dr P Allsopp, 2013-2017) £249,100.00 Marine sourced peptides for glycaemic management
- HSC R&D Public Health Agency – TRG (Prof. VE Coates and Prof FPM O’Harte 2013-2014) £15,000.00 Diabetes, endocrinology and nutrition TRG 2013-2014
- Western Trust / BMSRI joint funding. (Dr. PJ Allsopp Co-App et al. 2014). £10,000 Assessing the bone mineral density, calcium intake and vitamin D status of patients who have underwent an ileostomy.
- Moy Park-Invest NI grant. (PI, Dr. PJ Allsopp 2013) £16,000 Scientific review of Chicken and Turkey to highlight nutritional opportunities for Moy Park.
- Enterprise Ireland innovation voucher (ROI) (PI, Dr. PJ Allsopp 2013) £5,000 Trace element analysis of seaweeds.

**Project Costing:**

Please identify the cost of undertaking the project and highlight current externally funded projects that align with this proposed project [Project Title, funding source, amount and effective dates].

<table>
<thead>
<tr>
<th>Cost Categories &amp; (Brief Details)</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
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<tbody>
<tr>
<td>Brief Consumables List</td>
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<td>Year 1: <em>In vitro</em> tissue culture, HPLC solvents, insulin RIA, hormone ELISA assays, cytokines, etc.</td>
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<td>£5,000</td>
<td>£3,000</td>
<td>£12,000</td>
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<td>Year 2: <em>In vivo</em> animal studies.</td>
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<td>Year 3: Human intervention studies.</td>
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<td>Travel &amp; Subsistence Details</td>
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<td>£500</td>
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<td>Exceptional Items Details</td>
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<td>Sub Total</td>
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<td>£5,500</td>
<td>£3,500</td>
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**Identify Source of Funding for the project and Confirmation that funds are available:**

The DEL student allocation of £1,000 per annum will contribute to the cost of the project. Some of the remaining consumable funding will be obtained from and existing 3 year FIRM grant 2015-2017. Additional grant funding and collaborative agreements from commercial sources who have an interest in this area BII and Kerry Group will be sought.

Signed: 

______________________________
(Lead Supervisor)

**Anticipated Project Funding [please tick relevant box(es)]:**

- DEL X Approved by Director at time of application
- VCRS X
- DARD
- CAST
- Self funding

**NOTE: Self funded students:**

It is intended to advertise as a many PhD projects on the web as possible (suitable for overseas self-funded students). Please note that all PhD projects for the 2014-2015 intake (including all projects to be offered to overseas self-funded students) should be included in this submission process.

**Research Group Leader:**

Research Group Leaders are required to rank this project in order of priority within the Research Group and ensure that they have discussed this ranking with the applicant (please insert the Ranked Position at the top of the application in the appropriate box):

I confirm that this application meets the research strategy of the Research Group and has my support:
Signature: ____________________________
(Appropriate Research Group Leader)

Date: ________________________ 10/11/16