New Research Project Application 2021

Project Leader Name: Linda Giblin

Full Project Title: Infant Intestinal Barrier Model

Project Code: 1370

Project Start Date: 02/10/2021 **Project End Date:** 30/09/2025

Final Report Delivery Date: 30/12/2025

Categorisation

General Project Overview

Programme: Food Programme

Sub-Programme: Food & Health

Department: Food Bioscience

Location: Moorepark

Type of Proposal: Targeted

Please specify the primary Teagasc Goal this project contributes towards

Improve the competitiveness of agriculture, food and the wider bio-economy

Please specify the secondary Teagasc Goal(s) this project contributes towards

Support sustainable farming and the environment

Please indicate the National Research Prioritisation Area to which this project relates

H. Food for Health

TRL Level

TRL 2 Applied Research: technology concept and/or application formulated

Strategic Priority Area

Foods for health

Please indicate the appropriate type of research to describe this project

Applied/Pre-commercial

Description

Lay Abstract

The digestive system of a newborn baby is immature with low activity of digestive enzymes, the absence of gut bacteria and a leaky gut barrier. A leaky gut barrier can allow bacteria and large food components to escape the gut. The gut barrier then becomes inflamed and, in extreme cases, a portion of the bowel dies. This is termed necrotizing enterocolitis (NEC) and is a life threatening condition. The risk of developing NEC is much higher for formula fed infants than breast fed babies. INFBar will develop an in vitro model of an infant leaky gut. It will then use this model to select infant formula products that promote gut cells to form tight seals. The infant gut barrier will be grown in the laboratory by treating gut cells with short chain fatty acids. This leaky gut will then be tested with Infant formulae that differ in their processing method (heat treatment or filtration treatment). INFBar has the potential to radically improve the next generation Infant formula produced in Ireland.

Impact Statement (*Max 300 Words*)

Benefit to Industry:
INFBar delivers an in vitro model of the immature infant gut barrier. This is urgently needed to assess the ability of Infant Formula (IF) products to promote gut maturity. At present, laboratories routinely use adult gut barriers that are simply not physiologically relevant. Ireland manufactures upto 20% of the worldwide supply of IF. As such it must be a leader in producing next generation IF. INFBar will offer its toolkit to industry as a Teagasc technical expertise and via 2 Teagasc Gateways and 1 Moorepark Openday events. In addition, Ireland's economic development will be supported by the training of a skilful, well-educated PhD graduate in a research area that can easily translate to the dairy industry.
Benefit to society/consumers:
INFBar will help prevent necrotizing enterocolitis in babies that are formula fed, by testing IF products for their ability to promote gut barrier maturity. It will also reduce the use of parenteral nutrition for low weight pre-term infants.
Improvement to Irish scientific capacity:
INFBar will add critical mass to Teagasc's food digestion platform which is unparalleled in Ireland. INFBar aims to strengthen a recent Invention Disclosure by LGiblin. However it is also our intention (with some time delay due to IP protection) to deliver 4 publications in scientific journals of impact factor >3, 4 conference presentations and 2 presentations at meetings of the NutRedOx Cost Action and the INFOGEST network. INFBar will also have an active online presence (2 uploads per month) via LinkedIn, Twitter, ResearchGate. INFBar educational outreach will include 1 popular press article (TResearch) and 2 school demonstrations.
Gender balance and encouraging women in science:
INFBar team is led by an established female scientist and 2 early carer female scientists. These early career scientists will gain valuable experience in post-graduate supervision via INFBar under LGiblin tutelage.

Scientific Abstract

INFBar delivers an in vitro model of the intestinal barrier in the infant gut. A simple and reliable model of the infant intestinal barrier is urgently needed to study nutrient absorption, confirm safety and investigate possible health benefits of infant

formulae. The intestinal barrier of newborns is immature and ‘leaky’. An immature intestinal barrier can lead to nutrient malabsorption, enterocyte apoptosis, inflammation and necrotizing enterocolitis. Developing necrotizing enterocolitis is much higher in formula fed than breastfed infants. To test the ability of infant formulae to promote intestinal barrier maturity, an in vitro model of the immature infant barrier is urgently needed. At present laboratories routinely use intestinal barriers (Caco2, Caco2-HT29MTX co-cultures) that resemble adult physiology. A real strength of INFBar is to go beyond this ‘state of the art’ to develop a model that best represents the infant gut. To do this, Caco2 monolayers will be treated with short chain fatty acids. Monolayer integrity, cytotoxicity, permeability and inflammatory responses will be tracked. The ‘best in its class’ immature monolayer will be exposed to Infant formulae that differ in processing maps (cascade membrane filtration or high thermal load) to assess promotion of tight junction maturity.

Project Team

Full Collaborator List

Researcher/Specialist/Advisor	Institution	Programme	Department	Country
Dr. Linda Giblin	Teagasc	Food Programme	Food Bioscience	Ireland
Dr. Elena Arranz	UCC (University College Cork)		Food and Nutrtional Sciences	Ireland
Dr. Eva Rath	Technical University of Munich		Institute of Food and Health	Germany

Walsh scholarship

Does the completion of this project depend on securing a Walsh Scholar in the Call?

Yes

Suggested Reviewers

Title	Name	Email	Institution
Dr	Beatriz Miralles	beatriz.miralles@csic.es	CSIC
Dr	Anita Feraretto	anita.ferraretto@unimi.it	University of Milan

SECTION 2: PROJECT INFORMATION

Project Context

Relevance to current Teagasc and national strategies (e.g. Teagasc Statement of Strategy, Teagasc Technology Foresight 2035, Food Wise 2025, Sustainable Healthy Agri-Food Research Plan, Innovation 2020).

INFBar embodies INNOVATION 2020 as it is innovative PhD research proposal (Invention disclosure submitted) in a strategically important area (Infant formula) for the Irish economy. INFBar fits within Food and Health research in the TEAGASC FOOD PROGRAMME ROAD MAP 2025. It addresses TEAGASC FOOD HARVEST 2025 ’Lifestage nutrition’- since it aims to develop robust models of the infant intestinal barrier to study health effects of early nutritional products.

INFBar meets the challenges of Theme 4: New Technologies for Food Processing, identified in TEAGASC TECHNOLOGY FORESIGHT REPORT 2035 as it focuses on the 'need to establish the relationship between process-induced compositional changes in food' 'and its 'fate in the human body as regards the digestive process and delivery of nutrients'. INFBar also fits within the priority 'Food for nutrition and Health'. Specifically, INFBar will 'add value to food' in the production of Infant Formula by developing a technical platform to test nutritional products for 'infants in the first six months of life', 'functional foods' and 'medicinal foods intended to ameliorate disease risk, allergies and food intolerance'. Under the NATIONAL RESEARCH PRIORITIZATION, INFBar is aligned to Priority area H (Food for Health) and I (Sustainable Food Production and Processing) as it provides a method to test next generation infant formula for promotion of gut health. In addition, INFBar addresses 2 goals within the SUSTAINABLE HEALTHY AGRI-FOOD RESEARCH PLAN ie 'to develop and manufacture high value nutrition product' and 'to strengthen existing knowledge base in the key strategic area of the IMF sector'.

Existing research by other groups (Ireland & abroad) and how your research will build on this

In vivo infant models of the intestinal barrier simply employ neonatal animals. For diseased inflammed states such as colitis, increased intestinal permeability has been successfully achieved in animals with administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) or dextran sulfate sodium (DSS). Immaturity of a newborn's 'leaky' intestinal barrier is not characterized by inflammation but rather low proliferation and differentiation rates of intestinal epithelial cells, underexpression of tight junction proteins and paltry localization arrangement.
In vitro gut barrier models (Caco2, Caco2-HT29MTX) resemble adult physiology with fully formed tight junctions. Permeability of these monolayers reliably resembles conditions for adults, but are unsuitable to test food bioavailability or drug permeability in babies. To create a 'leaky' but healthy infant barrier with reduced expression of tight junction proteins is challenging, and simply adjusting Caco2 seeding density and/or differentiation period yields inconsistent results. A number of chemical treatments can increase permeability of intestinal monolayers. DSS (1-10%) can significantly increase permeability in Caco2 monolayers after 6d exposure (Nielsen 2006). TNBS (200 µg/ml) disturbs and inflammes Caco2 monolayers after 24h (Lu 2017). The short chain fatty acid (SCFA), butyrate, at low concentrations appears to promote differentiation of epithelial cells but at higher concentrations it appears destructive, increasing paracellular permeability of rat distal colon (Peng 2007). SCFA are present in our diet and are a by-product of gut microbiota so they are a good starting point to develop a leaky but healthy gut barrier.
Lu 2017 BMC Comple.Altern.Med. 17(1)35.
Peng 2007. Pediat.Res. 72(6),560.
Nielsen 2006. J.Ani.Sci., 94(3) 467-471

Recently completed and on-going research projects

Code	Title
MDBY6610	WheyGSH
MDBY0321	FoodBIBS
MDBY0531	Amino Acid Analogues
MDBY6288	CheeseBoard
MDBY6792	FHI-Cheese for Satiety

Introduction to the Research

Hypothesis to be tested

Our hypothesis is that at a certain concentration, short chain fatty acids can increase permeability of a mature Caco2 intestinal barrier without causing an inflammatory response. Therefore this treatment will generate an in vitro intestinal barrier that most closely resembles the newborn gut.

Overall Project Objective(s)

This Objectives of this PhD project are:
1. To create a Caco2 model of the infant intestinal barrier
2. To validate this model using clinical assays of permeability
3. To measure inflammatory response of Caco2 monolayers to short chain fatty acids
3. To transfer intestinal organoids methodology to Teagasc so we can continue to build and expand Teagasc's food digestion platform
4. To test Caco2 infant barrier model with infant formula products

Rationale/Methodology

This PhD project is divided into 5 Tasks:
Task 1: Exposure of Caco2 monolayers to short chain fatty acids
Task 2: Permeability assessed via Lactulose:Mannitol
Task 3: Inflammatory response of Caco2 monolayers to short chain fatty acids
Task 4: Tight junction protein response in Caco2 monolayers to short chain fatty acids
Task 5: Validation of infant intestinal barrier model using dairy proteins and infant formulae
All of the lab infrastructure and technical know-how to perform the experiments are already available in LGiblin's lab (Corrochano et al. 2019). Lab infrastructure includes Biosafety hoods, CO2 incubators, Centrifugation equipment, cyropreservation, X-celligence (TEER), aMillicell-ERS, Olympus CKX31 Inverted Microscope, standard PCRs, Micro-titre plate readers, refrigerated microcentrifuge, orbital shaker, Nanodrop, Luminex MagPx, Lightcycler 96 and horizontal and vertical gel electrophoresis equipment. GCs, HPLCs, FPLC, Amino Acid Analyser, Light microscope, Nikon C1Si laser scanning confocal imaging system.
Statistical analysis: Cytotoxicity, inflammatory response and permeability data will be from 3 experiments on different days with 2 technical replicates each. A general linear model repeated measurements, combined with a one-way Analysis of variance (ANOVA) followed by the appropriate post-hoc tests will be employed to compare results using the SPSS and MiniTab software.
Corrochano 2019 Food Chem. 288: 306-314.

Impact of the Research

Identify the end-users of the research

The end users of the project are

Food Scientists in both academia and private enterprise

Pharmaceutical Scientists in both academia and private enterprise

Infant Formula manufacturers and ingredient suppliers

Pharma Industry

Nutritionists

Teagasc Researchers

What is the expected benefit to the end-users? (do not deal with dissemination here – that is dealt with in a separate Task description)

The benefit to the end users is that INFBar offers an in vitro model of the immature infant gut barrier. Currently scientists use an ADULT intestinal barrier in vitro to test baby foods or pediatric drug permeability. This is not ideal. As distinct from adults, the intestinal barrier of newborns is immature and 'leaky'. A simple and reliable model of the infant intestinal barrier is urgently needed to study drug or nutrient absorption, confirm safety and investigate health effects.
For foods, an immature intestinal barrier can lead to nutrient malabsorption, enterocyte apoptosis, inflammation and the life threatening condition of necrotizing enterocolitis. Developing necrotizing enterocolitis is much higher in formula fed than breastfed infants. An in vitro model of the immature infant barrier will allow food scientists to test the ability of infant formulae to promote barrier maturity. This will ultimately lead to a reduction in the incidence of necrotizing enterocolitis.

How will the research contribute to meeting one or more of the industry impact indicators in the Teagasc Level 1 Business Plan?

INFBar fits within Teagasc's organizational goal 'to improve the competitiveness of food' by creating an infant intestinal barrier so that IMF manufacturers can assess their products for promotion gut health. INFBar adds critical mass to and expands capability of Teagasc's food digestion research platform which is a stated research focus within Teagasc Level I Business Plan. Expanding capacity(ie culturing intestinal organoids) will give Teagasc a competitive edge, allowing LGiblin to partner and lead EU projects. INFBar contributes to Level 1 Business plan targets for referred publications, number of Walsh scholars, invention disclosure and patents filed. INFBar establishes a formal link with Technical University of Munich. INFBar's toolkit will be employed across a number of existing Food digestion projects (VistaMilk, InfoTech, UProtein) in LGiblin's lab so these projects will contribute to lab consumable costs of INFBar ensuring exceptional value for money.

What is the scientific impact of the research?

INFBar will develop a simple robust reliable in vitro model of the immature infant intestinal barrier. If accepted by the scientific community it would become the in vitro gold standard for testing gut permeability of compounds in infants. This would revolutionize the pharma industry allowing them to test the permeability of drug compounds for this life stage. At present the pharma industry uses (in vitro) the adult Caco2 monolayer model and (in vivo) infant animals. For the infant formula manufacter, this infant model would allow them to test infant formula for its ability to promote gut maturity and therefore reduce the incidence of necrotizing enterocolitis. At present there are limited methods available to asses promotion of gut barrier. maturity.

What is the expected scientific output of the project (no. of peer-reviewed papers and target journals)?

LGiblin has recently submitted an Invention Disclosure to Teagasc TTO. INFBar aims to strengthen this Invention Disclosure. Although there may be some time delay due to IP protection, INFBar will also deliver 4 publications in scientific journals of impact factor>3 (ie Food chemistry, Molecular Food and Nutrition, Gut, Journal of Dairy Science), 2 national conference presentations (Irish Annual Food Conference, Irish Section of Nutrition Society) and 2 international conference presentation (7th International Conference on Food Digestion-Cork, ICDGMHH 2022 Australia).
INFBar will also be presented as a webinar to INFOGEST network of which Linda Giblin is working group 3 leader and EU-COST Action CA16112 NutRedOx of which Linda Giblin is working group 4 leader.
INFBar will result in one PhD dissertation.

SECTION 3: PROJECT TASK LIST

Tasks

Tasks and Workplan

Task Number	01
Start Month/Year	05/10/2021
Finish Month/Year	04/10/2022
Person responsible for the task:	Linda Giblin

Objective(s):

To decrease TEER values in differentiated Caco2 monolayers without affecting cell viability by short chain fatty acid treatment

Description of work to be undertaken:

Caco-2 cell line will be from the American Tissue Culture Collection (ATCC code HTB-37), and routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM). Caco2 Monolayers will be cultured in 12-well 12 mm Transwell plates with 0.4 µm Pore Polyester Membrane Inserts. Transepithelial electrical resistance (TEER) will be measured using a Millicell®-ERS voltammeter (EMD Millipore, USA). Initially Caco2 will be allowed to differentiate for 20 days until TEER values reach>500 Ωcm2.This is a robust starting point to create an infant barrier. These 20 day old monolayers will be treated with varying concentrations (0-250mM) of short chain fatty acids (SCFA) (formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate) in glutamine-free DMEM media for 24 hours with TEER values measured at 1, 2, 4, 6 and 24h. To ascertain if SCFA decreases TEER simply because these compounds are cytotoxic, basalateral and apical compartment will be collected and tested for the cytoplasmic enzyme, Lactose dehydrogenase (LDH). Release of LDH occurs in a leaky phenotype at 12% apoptosis. Preliminary work in our lab has indicated that butyrate may dose dependently decrease TEER without resulting in LDH leakage. Certainly Peng et al observed that treatment of Caco-2 monolayer with 8 mM butyrate resulted in significantly lower TEER after 48h and higher permeability to inulin-Fluorescein isothiocyanate (FITC) after 68h (Peng et al., 2007). However, 2 mM of butyrate suppressed Caco-2 monolayer permeability to inulin-FITC and instead improved TEER values over 4 days monitoring (Mariadason et al. 1997; Peng et al., 2007). Monitoring of the barrier permeability will also be performed with 4 kDa Na+-Fluorescein and 40 kDa Fluorescein isothiocyanate-dextran tracers.
Peng et al. 2007. Pediat.Res. 72(6),560
Mariadason et al. 1997. Am.J.Physio.Gas. & Liver Physio.272(4); G705-G712.

Task Details:

People

Name or Category	No. Days Year 1	No. Days Year 2	No. Days Year 3	No. Days Year 4	No. Days Year 5
Linda Giblin	10	0	0	0	0
Walsh Scholar	200	0	0	0	0
Elena Arranz	5	0	0	0	0

Laboratory (Bench Space)

No. of people	Name/location of laboratory	Brief Justification	Year	From Month	To Month
1	lab102	Instrumentation needed to perform this task is located in tissue culture lab or in Lab 102: for example Biosafety hoods, CO2 incubators, cyropreservation, X-celligence (TEER), aMillicell-ERS, Olympus CKX31 Inverted Microscope, standard PCRs, Micro-titre plate readers, Centrifugation equipment, refrigerated microcentrifuge, Luminex MagPx, Lightcycler 96 and gel electrophoresis equipment.	2021	1	12
1	tissue culture lab	Instrumentation needed to perform this task is located in tissue culture lab or in Lab 102 (listed above).	2021	1	12

Consumables

Indicate the type of consumables required	Brief Justification
Cell culture costs: tissue culture flasks	

vented caps €99.80 x 8), 12mm transwell plates €712.42 x 8), antibiotics (€20 x4), DMEM culture media (€57.80 x 8), Fetal bovine serum(€159.50 x 6), fluorescein sodium salt tracer (€152.55 x 2), myotoxin kit (350 x 4), glucose and essential amino acids (€37.9 x 4), 0.4microfilters (€72 x 8), LDH assay €385, Standard laboratory plastic ware: Filter tips (p10 €103.60/case x 4, p20 €109.71/case x4, p200 €109.71/case x x4 and p1000 €68.84/case x 4), pipettes (5ml €83/case x 4, 10ml €83/case x 4, 25ml €83/case x 4), 96 well microtitre plates (€163.50/box x 4), eppendorf tubes (€39.75/case x 8), Centrifuge tubes (15ml €227.60/box x 4, 50ml €268.60/box x 4), liquid nitrogen €50 x 4, protective disposable gloves (€39.5 x x4); laboratory reagents €500 ie formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate.	to expose Caco2 monolayers to short chain fatty acids

Milestones

Number	Short Description	End Date
1.	Caco2 monolayers cultured for 21 days	29/09/2022
2.	Caco2 monolayers exposed to individual short chain fatty acids	30/09/2022

Deliverables

Number	Short Description	End Date
1.	A report on the TEER values for Caco2 monolayers exposed 5 concentration of 4 short chain fatty acids over a 24 hour period	30/09/2022

Task Number	02
Start Month/Year	01/03/2022
Finish Month/Year	01/10/2022
Person responsible for the task:	Linda Giblin

Objective(s):

To investigate the permeability of SCFA treated monolayer using lactulose:mannitol test

Description of work to be undertaken:

The routine clinical test to estimate permeability of infant's intestinal barrier and thereby predisposition to development of intestinal inflammation involves administration of the mixture of non-absorbable lactulose and mannitol, where the larger disaccharide lactulose (Mw=342 g/mol, Mr=0.62 nm) is transported paracellularly across the intestinal barrier and the smaller simple sugar alcohol mannitol (Mw=182 g/mol, Mr=0.38 nm) is transported transcellularly (Kosek et al. 2017). As both compounds are not metabolized, the ratio of Lactulose:Mannitol (L:M) excreted in infant urine samples is a reliable measure of intestinal permeability. In pre-term infants ratio of L:M recovery in the urine ranges from 0.6:1 to 3.3:1, with higher ratios for <34 weeks gestation infants (Weaver et al., 1984). Absorption from ingested preload occurs for <1% of lactulose and <5% of mannitol (Weaver et al., 1984). To study transcellular and paraceulluar transport rates in the lab, Caco-2 monolayers treated with short chain fatty acids from Task 1 will be 3X washed with HBSS buffer and then exposed to lactulose and mannitol at a ratio of 5:1 for 4h. Lactulose and mannitol will be quantified in apical and basolateral compartments by reverse phase HPLC. Lactulose: mannitol ratios and % transport rates will be calculated.

Kosek et al. 2017. J. Ped. Gastroent.& Nut. 65(1), 31-39.
Weaver et al., 1984. Archives Dis Childhood. 59(3), 236-241

Task Details:

People

Name or Category	No. Days Year 1	No. Days Year 2	No. Days Year 3	No. Days Year 4	No. Days Year 5
Linda Giblin	5	0	0	0	0
Walsh Scholar	40	0	0	0	0

Elena Arranz	2	0	0	0	0

Laboratory (Bench Space)

No. of people	Name/location of laboratory	Brief Justification	Year	From Month	To Month
1	lab102	Instrumentation needed to perform this task is located in tissue culture and Lab 102			
1	tissue culture	Instrumentation needed to perform this task is located in tissue culture and Lab 102			

Consumables

Indicate the type of consumables required	Brief Justification
Cell culture costs as described in Task 1 plus : lactulose €279, mannitol €37.10, Standard laboratory reagents €500 (buffers, acids, bases, salts)	to perform lactulose mannitol tests on monolayers

Milestones

Number	Short Description	End Date
1.	Caco2 monolayers treated with lactulose and mannitol	30/09/2022

Deliverables

Number	Short Description	End Date
1.	A report on the permeability of Caco2 monolayers	30/09/2022

Task Number	03
Start Month/Year	02/10/2022
Finish Month/Year	01/10/2023
Person responsible for the task:	Linda Giblin

Objective(s):

To investigate the inflammatory status of Caco2 monolayers treated with short chain fatty acids

Description of work to be undertaken:

Intestine barrier permeability is closely connected to inflammation (Van De Walle et al. 2010). In inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, permeability of the intestine is increased and circulating levels of pro-inflammatory cytokines are raised compared to the levels in healthy subjects (for Crohn's disease IL-6: 8.24 vs. 1.59 pg/mL; IL-8: 53.70 vs. 9.77 pg/mL; TNF-α: 3.12 vs. 0.61 pg/mL) (Szkaradkiewicz et al. 2009). To study if short chain fatty acids treatment causes an inflammatory response in Caco2 monolayers, levels of inflammation biomarkers IL-6, IL-8 and TNF-α will be assayed with Milliplex Map Kit (HSTCMAG-28SK, Human High Sensitivity T cell Magnetic Bead Panel, Merck, Ireland) and MagPix fluorescent detection system (Luminex, The Netherlands). These Caco2 infant monolayers will not be treated with LPS or equivalent prior to supernatant cytokine quantification. Our results will be compared with others that have observed basal levels of IL-8 (96.7 pg/mL), IL-6 (1.01 pg/mL) (Van De Walle et al., 2010) and TNF-α (3.72 pg/mL) (Araki et al. 2006) in differentiated Caco-2 cells.

Van De Walle et al. 2010. Toxic. In Vitro 24(5) 1441-1449.
Szkaradkiewicz et al. 2009. Arch.immunologiae et therapiae experimentalis, 57(4) 291.
Araki et al. 2006. Oncology reports, 16(6), 1357-1362.

Task Details:

People

Name or Category	No. Days Year 1	No. Days Year 2	No. Days Year 3	No. Days Year 4	No. Days Year 5
Linda Giblin	0	15	0	0	0
Walsh Scholar	0	200	0	0	0

Elena Arranz	0	3	0	0	0

Laboratory (Bench Space)

No. of people	Name/location of laboratory	Brief Justification	Year	From Month	To Month
1	Lab102	instrumentation required for this task is located in either Lab102 or tissue culture lab and is listed in Task 1.	2022	1	12
1	tissue culture	instrumentation required to perform this task is located in Lab102 (luminex) or in tissue culture lab and is listed in Task 1.	2022	1	12

Consumables

Indicate the type of consumables required	Brief Justification
Cell culture costs as described in Task 1 plus Milliplex Map Kit (HSTCMAG-28SK, Human High Sensitivity T cell Magnetic Bead Panel €650)	to perform cytokine assays

Milestones

Number	Short Description	End Date
1.	Cytokine assays performed on apical and basolateral samples from Caco2 monolayers	30/09/2023

Deliverables

Number	Short Description	End Date
1.	A report on the inflammatory response of Caco2 monolayers to short chain fatty acids	30/09/2023

Task Number	04
Start Month/Year	01/05/2023
Finish Month/Year	01/10/2024
Person responsible for the task:	Linda Giblin

Objective(s):

To track tight junction biomarkers in Caco2 monolayers treated with short chain fatty acids
To treat 3D intestinal organoids with short chain fatty acids

Description of work to be undertaken:

Caco-2 mRNA transcript levels of tight junction proteins after 24 h treatment with short chain fatty acids will be quantified to investigate whether these compounds alter tight junction (TJ) gene expression. Claudin-4 is known for its involvement in the repair of TJs after damage, so upregulation of its mRNA transcript post treatment may indicate that the monolayer integrity has been compromised. Other biomarkers involved in tight junction scaffold will also be tracked by Real time PCR including Claudin-1, Actin and JAM1. For example Claudin-1 is an integral membrane protein, crucial for the development of TJs and maintaining epithelial barrier function (Gonzalez-Mariscal et al. 2003). Actin is indirectly involved in TJ formation, as transmembrane proteins occludin and claudins bind to the actin cytoskeleton and regulate its distribution and expression (Hartsock & Nelson 2008). Interestingly, Wang et al. (2012) have observed that very low concentrations of short chain fatty acids (2-4mM) were capable of significantly enhancing CLDN1 mRNA levels resulting in increased TEER values (Wang et al. 2012). Total RNA will be quantified spectrophotometrically with Nanodrop 1000 (Thermo Fisher Scientific, USA) and cDNA will be prepared from 1 µg of RNA with SensiFAST cDNA Synthesis Kit (Medical Supply Company, Ireland). Real-time PCR will be performed with Light Cycler SYBR GREEN I Master kit using a Light Cycler 96 instrument (Roche Diagnostics, Germany). Primers for ZO1, OCLN, CLDN1, CLDN2, CLDN4, ACTb, JAM-A and RPLP0 genes will be designed using GenBank across intron-exon boundaries, where possible.
For follow on experiments, a dose will be selected for each fatty acid which (a) can reduce TEER values temporarily but allow them to recover by 24hrs, (b) is not cytotoxic, (c) has a minimal if any cytokine response, (d) increases L:M ratio similar to in vivo data for pre-term infants and (e) alters mRNA transcript levels of tight junction markers. With these ‘front runners’, monolayers will be collected and western blots and/or immunofluorescence staining will be performed to quantify/visualize the localization of TJ proteins (ie occludin and ZO-1). Preliminary data in LGiblin's lab suggest 100mM butyrate will meet the requirements of a front runner. Previously expression and re-arrangement of TJ proteins was studied in response to dietary compounds, bacteria and chemical treatments (Putt et al. 2017; Ulluwishewa et al. 2011). Overall, re-distribution of TJ proteins corresponded to the significant increase in paracellular permeability and decrease in monolayer resistance, measured by TEER (Seth et al. 2004). Western blot analysis will be performed in lysed cell samples. Briefly, proteins will be separated with NuPage 4-12% Bis-Tris

gel (NP0322), transferred to nitrocellulose membrane and probed with primary antibodies for ZO-1, claudin-1, occluding and other TJ proteins at optimised dilutions and incubation times. Following this, membranes will be incubated with secondary antibodies (HRP conjugates) and visualised with Fusion Solo S (Vilber) camera. Localisation of TJ proteins will be performed by immunofluorescence microscopy. Caco-2 monolayers will be fixed with 4% formaldehyde, permeabilized with 0.3% Triton X-100 and blocked with 2% BSA. Then cells will be incubated with primary polyclonal antibodies, followed by secondary antibodies at optimised dilutions and incubation times. The membrane will be dissected from the Transwell insert using a scalpel and mounted between glass slide and coverslip with DAPI-containing mounting media Fluoroshield (Sigma, Ireland). TJ proteins will be visualised with wide field fluorescence microscopy. TJ proteins will be visualised at 470 nm LED excitation with FITC filers 472/30 ex and 535/50 em; DAPI will be visualised at 390 nm LED excitation with 5060B-ZHE “DAPI-Semrock” filers.
Caco2 monolayers are 2D models of the intestinal barrier and represent only epithelial cells. This task will also look at developing 3D intestinal organoids for infants. Intestinal organoids will be cultured initially at TUM under the supervision of Dr. Eva Rath. Dr. Rath is an expert in culturing organoids (Zietek et al. 2015). Experimental design will include mice sacrifice by CO2 and collection of small intestine tissue. From this tissue crypts will be isolated and cultured in cell culture plates to produce organoids. This subtask will follow the 3Rs (replace, reduce, refine) with only one animal required to produce organoids per experiment and where possible the tissue will be sourced from other mouse trials ongoing in TUM. This work will be performed under the ethical approval and license of TUM. Similar to task 1, obtained organoids will be treated with short chain fatty acids. Similar to Caco2 monolayers, changes in the expression and localization of tight junction proteins will be analysed by western blots and qPCR as outlined above. It is not envisioned that organoids will be the infant barrier model of choice for screening food components but it does increase Teagasc's toolkit in food digestion and will have a role to play in bridging the physiological gap between in vitro and in vivo.
Gonzalez-Mariscal et al. 2003. Prog.Biophysics & Mol. Biol.81(1), 1-44.
Hartsock & Nelson, 2008. Biochimica et Biophysica Acta-Biomembranes, 1778(3), 660-669.
Wang et al. 2012. Digestive diseases and sciences, 57(12), 3126-3135.
Ulluwishewa et al. 2011. J.Nutrit. 141(5), 769-776.
Seth et al. 2004. Am. J. PhysioGastro. & Liver Physio.287(3), G510-G517.
Zietek et al.2015. Sci Rep 5:16831.
Putt et al. Food and Function 2017 8(1) 406-414.

Task Details:

People

Name or Category	No. Days Year 1	No. Days Year 2	No. Days Year 3	No. Days Year 4	No. Days Year 5
Linda Giblin	0	2	15	0	0
Eva Rath	0	0	10	0	0
Walsh Scholar	0	40	240	0	0
Elena Arranz	0	1	5	0	0

Laboratory (Bench Space)

No. of people	Name/location of laboratory	Brief Justification	Year	From Month	To Month
1	Lab102	instrumention required for this task is located in lab102 and tissue culture lab. Intestibnal organoids will be initially performed at TUM.	2024	1	12
1	tissue culture lab	instrumentation to perform experiments are located in Lab102 and tissue culture lab	2024	1	12

Consumables

Indicate the type of consumables required	Brief Justification
Cell culture costs as described in Task 1 plus SDS-PAGE (€375 x 4), tight junction antibodies €113/antibody, RNA extraction kit (€305), Lightcycler 480 kit (€394.80), Primers (approax €100.00) cDNA synthesis kit (€741).	to track tight junctions & culture organoids

Travel and Subsistence

Cost €	Brief Justification
€3,000.00	To learn how to culture organoids, the walsh scholar will visit Eva Rath's lab TUM for 3 months. Travel costs include return flights to Munich Germany, rental accommodation in Munich, living expenses in Munich. No bench fees will apply.

Milestones

Number	Short Description	End Date
1.	RT-PCR performed on Caco2 lysates	01/07/2024
2.	Western blots performed on Caco2 lysates	02/07/2024
3.	Immunoflouresence microscopy performed on fixed Caco2 monolayers	01/07/2024
4.	Intestinal organoids exposed to short chain fatty acids	29/09/2024

Deliverables

Number	Short Description	End Date
1.	A report on tight junction proteins response to Caco2 treated with short chain amino acids	29/09/2024
2.	Intestinal organoids knowledge transferred to Teagasc	30/09/2024

Task Number	05
Start Month/Year	01/10/2024
Finish Month/Year	21/09/2025
Person responsible for the task:	Linda Giblin

Objective(s):

To validate the Caco2 infant barrier using dairy proteins and IMF powders

Description of work to be undertaken:

To validate the Caco2 infant barrier with compounds relevant to infant nutrition and health, bovine dairy proteins α-LA and β-LG will initially be used. Transport rates of these proteins through the intestinal barrier are known to be strongly age-dependent. For example, pre-term infants with low birth weight had a 10 times higher concentrations of human α-LA in their serum post consumption of breast milk, compared to full-term infants (Axelsson et al. 1989). In exclusively breastfed full term infants, human α-LA was present in the serum up to 2 months of age. In infants consuming infant formula (IF), bovine β-LG could be detected in the serum up to 5 months of age (Kuitunen et al. 1994). 20 day old Caco2 monolayers will initially be treated with preferred doses of short chain fatty acids. Monolayers will then be washed and α-LA and β-LG at various conc will be applied to apical side for 4h. Basolateral and apical samples will be collected. ELISA assays will be performed to quantify the levels of α-LA and β-LG. Follow-on experiments will include 2 IF powders produced by different processing methods (high thermal load or cascade membrane filtration). Both of these powders have successfully been produced by L.Giblin lab at pilot plant scale. Recently L.Giblin ‘s team observed that IF produced by cascade membrane filtration increased Caco2 monolayer TEER values and influenced occludin protein levels (manuscript submitted). IFs will undergo simulated in vitro digestion modified for the infant conditions (Menard 2018). Resulting digesta will be inactivated with protease inhibitor, Pefabloc, to stop digestion and then snap-frozen. Degree of hydrolysis, free amino acids and peptide size ranges (size exclusion chromatography) will be determined for IF digesta samples. Caco2 infant monolayers, will be exposed for 4 h to IF digesta. To ensure detection of amino acids/peptides in the basolateral, 500-750 µg protein/cm2, (depending on cytotoxicity results) will be added to the apical side. Basolateral and apical samples will be analysed for free amino acids and peptide size ranges. The data will be compared to control Caco2 monolayers.
Axelsson et al. 1989. Acta.Paediatria. 78(4), 532-537.
Kuitunen et al. 1994. Allergy. 49(5) 354-360.
Menard 2018 Food Chem.240:338-354.

Task Details:

People

Name or Category	No. Days Year 1	No. Days Year 2	No. Days Year 3	No. Days Year 4	No. Days Year 5
Linda Giblin	0	0	0	15	0
Walsh Scholar	0	0	0	240	0
Elena Arranz	0	0	0	5	0

Laboratory (Bench Space)

No. of people	Name/location of laboratory	Brief Justification	Year	From Month	To Month
1	Lab102	instrumention required for this task is loacted in Lab102 or Tissue culture	2025	1	9
		instrumentation required for this task is located in			

1	Tissue culture	lab102 or tissue culture	2024	9	12

Consumables

Indicate the type of consumables required	Brief Justification
Cell culture costs as described in Task 1 plus gastrointestinal digestion costs: Pancreatin amylase and protease (€68.94), bile extract (€38.34), pepsin (€107.10), molecular weight cut off filters (€41), chemicals (HCL, NaOH, NaCl etc €875) Total amino acid analysis: 2 food samples at 4 data points (time zero, gastric, duodenal and basolateral) @ €75 per sample	to perform gastrointestinal digestions of IMF powders

Milestones

Number	Short Description	End Date
1.	Gastrointestinal digestion performed on two IF powders	01/03/2025
2.	Caco2 infant barrier exposed to dairy proteins	30/03/2025

Deliverables

Number	Short Description	End Date
1.	A report on the degree of hydrolysis and peptide sizes of 2 IF powders post intestinal digestion	02/09/2025
2.	A report on amino acid profile in the basolateral compartment of infant barrier model treated with IF	03/09/2025

Task Number	Dissemination
Start Month/Year	05/10/2021
Finish Month/Year	29/09/2025
Person responsible for the task:	Linda Giblin

Outline key stakeholders who will be informed of the research:

Key Stakeholders
1. IF manufactures and ingredient suppliers, Pharma Industry
2. General Public
3. Scientific community
4. Health care professionals
5. Teagasc

Methods of dissemination:

Stakeholder: IF manufactures and ingredient suppliers, Pharma Industry
INFBar will be offered to industry as a Teagasc Technical Expertise
It will presented at 2 Teagasc Gateways events and 1 Moorepark Openday (being mindful of IP protection)

Stakeholder: General Public/Education outreach
INFBar team will commit to
2 School lab demonstrations at Castlelyons Primary School
2 career events at UCC
1 Cork Discovery event/Cork Pub PhD Pint of Science
1 T-research article
Man a booth at BT Young Scientist Exhibition
1 You tube video
Active online presence: 2 uploads per month on LinkedIn/ResearchGate/Twitter

Stakeholder: Scientific Community:
As INFBar builds on an Invention Disclosure, publication will be cognizant of IP protection
4 publications in scientific journals of impact factor>3 (ie Food chemistry, Molecular Food and Nutrition, Gut, Journal of Dairy Science) where possible using open access journals or will be submitted to Teagasc T-Stor in 'accepted manuscript' format
2 national conference presentations (eg Irish Annual Food Conference, Irish Section of Nutrition Society
2 international conference presentation (eg 7th International Conference on Food Digestion, Cork, ICDGMHH 2022 on Diet, Gut Microbiology and Human Health, Sydney Australia, 2023 Women and Infant Health Congress: Europe, Nutrition Society Conference).

One webinar to INFOGEST network
One webinar to EU-COST Action NutRedOx CA16112
One PhD dissertation

Stakeholder: Health care professionals
One lecture to INFANT-Hub at Cork University Hospital for medical professions with an active research program.

Stakeholder: Teagasc
One end of project report (EOPR)
4 Annual Walsh Fellowship reports will be submitted ontime
1 presentation at Annual Walsh Scholar Conference
Walsh Scholar will give an oral presentation annually to Food Bioscience dept.
Walsh Scholar will train Teagasc students on Caco2 monolayers and intestinal organoids

SECTION 4: PROJECT MANAGEMENT

Project Management Plan

Please Upload Supporting Documentation

INFBar_gant.xlsx
29.1 KB - 08/09/2020 12:53

Total Files: 1

Co-ordination of the research/project team and work programme

INFBar management team consists of Linda Giblin and Elena Arrnaz. The **overall aim** of the management team is to ensure effective management of INFBar using best practice project-management tools. This PhD project team will meet each quarter. Teagasc' s TTO office will be updated on progress every 6 months.

Objectives of the Management Team:

To ensure that the necessary administrative documentation are in place at the outset of the project
To organize project meetings
To maintain project documentation and ensure appropriate accessibility
To monitor risks, or avoid delays to the project
To manage conflicts in the team
To manage IP rights in accordance with Teagasc and UCC TTOs
To manage budget
To submit progress reports ontime

Exploitation Plan and Intellectual Property (IP) Management

Examples of IP: software, databases, models, processes, products, strains etc…

This is an innovative project which builds on an Invention Disclosure by LGiblin. This invention disclosure will be brought into the project as background IP. The guidelines on how background (ie the existing invention disclosure)/foreground ownership and exploitation rights is agreed would be based on existing guidelines of Teagasc and UCC and may require a research agreement with TUM. This will be managed by Teagasc TTO in accordance with protocols outlined in Inspiring Partnership National IP protocols 2016. IP of INFBar will be discussed at quarterly project meetings. As

project manager Linda will inform Teagasc TTO of the outcomes of these discussions from an IP stance. Linda Giblin is experienced with licensing IP and client contracts under the guidance of Teagasc TTO. A patent search has revealed a freedom to operate.

Please outline an exploitation plan for the anticipated outputs (potential IP) identified above. What further engagements with industry, funding agencies, and/or collaborators may be required for further development, validation, and commercialisation of the potential IP? Outline any companies that may be interested in exploiting the anticipated outputs and any existing contacts with such companies.

The invention disclosure resulted from research activity within an Entreprise Ireland Parternship grant with 3 companies (Danone, Kerry and Dairygold). These 3 companies have been notified by Teagasc TTO of the invention. All 3 companies have expressed an interest in publishing the results as soon as possible. The value of this invention lies in the scientific community accepting and independantly verifying the method, much like Caco2 monolayers are accepted as the gold standard for adults. We envision that the method will be published and then Teagasc will offer the expertise to industry to test compounds for permeability across an infant gut model. The lead scientist Linda Giblin continues to be in contact with Teagasc TTO for IP protection and exploitation. In the meantime, INFBar serves to strengthen the invention disclosure to allow Teagasc (lead researcher and TTO) to make an informed decision about patent application, exploitation and dissemination.

SECTION 5: PROJECT PROOFING

Project Risk Register

Risk Management					
Risk	**Category**	**Impact**	**Probability**	**Rating**	**Response**
Short chain fatty acids kill Caco2 monolayers	Project	3	1	3	Unlikely, as LGiblins lab has some preliminary evidence that butyrate can reduce permeabiliy in Caco2 monolayers but these monolayers recover and thrive.
Caco2 monolayers have an inflammatory response to short chain fatty acids	Project	3	2	6	Preliminary evidence suggest the inflammatory response is within the range observed for non-LPS stimulated Caco2
TUM can not host the Walsh Scholar	Project	3	3	9	The project is not dependant on culturing intestinal organoids to meet its main deliverables. However intestinal organoids do expand Teagasc's in-house methodology. If TUM can not host the Walsh Scholar then Dr. Rath will be invited to Teagasc to demonstrate organoid cultures. Alternatively LGiblin will use her INFOGEST network connections to identify other labs with similar capabilities.

Definitions		
Scale	**Impact**	**Probability**
1	No significant impact on service, reputation or financial position	Not likely to occur, e.g. in a 10 year period
2	Minor impact	Rarely happens, e.g. likely to occur once in a 10 year period
3	Significant but containable impact	Possible, e.g. likely to occur once in a 2 year period.
4	Serious damage	Very Likely, e.g. likely in a 3-12 month time period
5	Catastrophic damage	Almost bound to happen e.g. likely over next 3 months
Category Code (Cat)	**Description**	

Business	Business Risk - A risk relating to the impact of the project on the business e.g. the success of this project adversely impacting on other areas of the business
Project	Project Risk - A risk to the success or failure of the project e.g. non-availability of testing resources causing the project to be cancelled

Budget

Expenditure

Item	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Placement / Casual Students:	€0.00	€0.00	€0.00	€0.00	€0.00	€0.00
Walsh Scholars:	€24,000.00	€24,000.00	€24,000.00	€24,000.00	€0.00	€96,000.00
Farm / Lab Supplies:	€2,000.00	€2,000.00	€1,000.00	€2,000.00	€0.00	€7,000.00
Vet, Consultancy and External Analysis:	€0.00	€0.00	€0.00	€0.00	€0.00	€0.00
Travel & Subsistence:	€0.00	€0.00	€3,000.00	€0.00	€0.00	€3,000.00
Equipment:	€0.00	€0.00	€0.00	€0.00	€0.00	€0.00
Rental:	€0.00	€0.00	€0.00	€0.00	€0.00	€0.00
Total Expenditure:	**€26,000.00**	**€26,000.00**	**€28,000.00**	**€26,000.00**	**€0.00**	**€106,000.00**

Income

Item	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Total Income:	**€0.00**	**€0.00**	**€0.00**	**€0.00**	**€0.00**	**€0.00**

Total Teagasc Funding Required

Year 1	Year 2	Year 3	Year 4	Year 5	Total
€26,000.00	**€26,000.00**	**€28,000.00**	**€26,000.00**	**€0.00**	**€106,000.00**

Industry Funding - Excluding Levies

Is there any industry funding contribution?: No

Is there any in-kind contribution?: No

Other

Does the completion of this project depend on securing a Walsh Scholar in the Call?

Yes

How many Walsh Scholars?

1

Walsh Scholarship Reference Number	Title	Teagasc Supervisor	External Supervisor	External Institution	Status
2021007	Infant Intestinal Barrier-INFBar	Linda Giblin	Elena Arranz	UCC (University College Cork)	Approved

SECTION 6: RELEVANT PUBLICATIONS

Publications

Relevant Publications

LGiblin has >60 publications impact factor>3, h-index = 25 (i10index=42) of which 28 are as senior author, 5 invited book chapters, orcid.org/0000-0002-9354-3121 ResearchID P-2436-2016. The 15 most recent relevant publications are:

1. Ariadna Gasa-Falcon A., Arranz E., Odriozola-Serrano I., Martín-Belloso O. and Giblin L. 202Delivery of β-carotene to the in vitro intestinal barrier using nanoemulsions with lecithin or sodium caseinate as emulsifiers. LWT-Food Science and Technology. 135: 110059.
2. Gilmartin S, O'Brien N, Giblin L. 2020. Whey for Sarcopenia; Can Whey Peptides, Hydrolysates or Proteins Play a Beneficial Role? Foods. 2020 Jun 5;9(6):750.
3. Kondrashina A., Brodkorb A., Giblin L. 2020. Dairy-derived peptides for satiety. Journal of Functional Foods. 66: 103801.
4. Arranz, E., A. R. Corrochano, C. Shanahan, M. Villalva, L. Jaime, S. Santoyo, M. J. Callanan, E. Murphy, and L. Giblin. 2019. Antioxidant activity and characterization of whey protein-based beverages: Effect of shelf life and gastrointestinal transit on bioactivity. Innovative Food Science and Emerging Technologies 57: 102209.
5. Tur, J. A., C. Jacob, P. Chaimbault, M. Tadayyon, E. Richling, N. Hermans, C. N. dos Santos, M. Diederich, L. Giblin, M. Elhabiri, C. Gaucher, P. Andreoletti, A. Fernandes, M. Davies, A. Bartoszek, M. Cherkaoui-Malki, and N. Investigators. 2019. Personalized nutrition in ageing society: redox control of major-age related diseases through the NutRedOx Network (COST Action CA16112). Free Radical Research. 22:1-8.
6. Chen, Z. F., A. Kondrashina, I. Greco, L. F. Gamon, M. N. Lund, L. Giblin, and M. J. Davies. 2019. Effects of Protein-Derived Amino Acid Modification Products Present in Infant Formula on Metabolic Function, Oxidative Stress, and Intestinal Permeability in Cell Models. Journal of Agricultural and Food Chemistry 67(19):5634-564
7. Giblin, L., A. S. Yalcin, G. Bicim, A. C. Kramer, Z. F. Chen, M. J. Callanan, E. Arranz, and M. J. Davies. 2019. Whey proteins: targets of oxidation, or mediators of redox protection. Free Radical Research. 12:1-1 doi: 10.1080/10715762.2019.1632445.
8. Knowles S, Gilmartin S, Arranz E, O'Brien N and Giblin L. Effect of Bioavailable Whey Peptides on C2C12 Muscle Cells. 2019. Proceedings. 11:1: 1-4
9. Corrochano, A. R., A. Ferraretto, E. Arranz, M. Stuknyte, M. Bottani, P. M. O'Connor, P. M. Kelly, I. De Noni, V. Buckin, and L. Giblin. 201 Bovine whey peptides transit the intestinal barrier to reduce oxidative stress in muscle cells. Food Chemistry 288:306-314.
10. Corrochano, A. R., Y. Saricay, E. Arranz, P. M. Kelly, V. Buckin, and L. Giblin. 2019. Comparison of antioxidant activities of bovine whey proteins before and after simulated gastrointestinal digestion. Journal of Dairy Science 102(1):54-67.
11. Corrochano, A. R., E. Arranz, I. De Noni, M. Stuknyte, A. Ferraretto, P. M. Kelly, V. Buckin, and L. Giblin. 2018. Intestinal health benefits of bovine whey proteins after simulated gastrointestinal digestion. Journal of Functional Foods 49:526-535.
12. Corrochano, A. R., V. Buckin, P. M. Kelly, and L. Giblin. 2018. Invited review: Whey proteins as antioxidants and

promoters of cellular antioxidant pathways. Journal of Dairy Science 101(6):4747-4761.
13. Kondrashina, A., C. Bruen, B. McGrath, B. Murray, T. McCarthy, H. Schellekens, S. Buzoianu, J. F. Cryan, A. L. Kelly, P. L. H. McSweeney, P. Lawlor, and L. Giblin. 2018. Satiating effect of a sodium caseinate hydrolysate and its fate in the upper gastrointestinal tract. Journal of Functional Foods 49:306-3
14. Kondrashina, A., D. Papkovsky, and L. Giblin. 2018. Physiological Gut Oxygenation Alters GLP-1 Secretion from the Enteroendocrine Cell Line STC-1. Molecular Nutrition & Food Research 62(3).
15. Kondrashina, A., S. Seratlic, D. Kandil, N. Treguier, K. Kilcawley, H. Schellekens, T. Beresford, and L. Giblin. 2018. Irish Cheddar cheese increases glucagon-like peptide-1 secretion in vitro but bioactivity is lost during gut transit. Food Chemistry 265:9-17.

Elena Arranz has 27 peer reviewed publications, 2 patents, 2 book chapters and a h index = 10 (Scopus). 10 Most recent relevant publications are

1. Gasa-Falcon A, Arranz E, Odriozola-Serrano I, Martín-Belloso O, Giblin L. (2020). Delivery of β-carotene to the in vitro intestinal barrier using nanoemulsions with lecithin or sodium caseinate as emulsifiers. LWT. 110059.
2. Villalva M, Jaime L, Arranz E, Zhao Z, Corredig M, Reglero G, Santoyo S. (2020). Nanoemulsions and acidified milk gels as a strategy for improving stability and antioxidant activity of yarrow phenolic compounds after gastrointestinal digestion. Food Research International, 130, 10892
3. Arranz E, Corrochano AR, Shanahan C, Villalva M, Jaime L, Santoyo S, Callanan MJ, Murphy E, Giblin L (2019). Antioxidant activity and characterization of whey protein-based beverages: effect of shelf life and gastrointestinal transit on bioactivity. Innovative Food Science and Emerging Technologies. 57:102209.
4. Giblin L, Yalç n AS, Biçim G, Kra mer AC, Chen Z, Callanan MJ, Arranz E, Davies MJ (2019). Whey proteins: targets of oxidation, or mediators of redox protection. Free Radical Research. 1-17.
5. Tari NR, Fan MZ, Archbold T, Arranz E, Corredig M (2019). Effect of milk protein composition and amount of β-casein on growth performance, gut hormones and inflammatory cytokines in an in vivo piglet model. Journal of Dairy Science. 102(10), 8604-8613.
6. Corrochano AR, Ferraretto A, Arranz E, Stuknytė M, Bottani M, O'Connor PM Kelly PM, De Noni I, Buckin V, Giblin L (2019). Bovine whey peptides transit the intestinal barrier to reduce oxidative stress in muscle cells. Food Chemistry. 288; 1, 306-314.
7. Tari NR, Arranz E, Corredig M (2019). Effect of protein composition of a model dairy matrix containing various levels of beta-casein on the structure and anti-inflammatory activity of in vitro digestates. Food & Function. 17; 10(4):1870-1879.
8. Arranz E, Villalva M, Guri A, Ortego Hernández E, Jaime L, Reglero G, Santoyo S, Corredig M (2019). Protein matrices ensure safe and functional delivery of rosmarinic acid from marjoram (Origanum majorana) extracts. Journal of the Science of Food and Agriculture. 99(5), 2629-2635.
9. Corrochano AR, Arranz E, De Noni I, Stuknytė M, Ferraretto A, Kelly PM, Buckin V, Giblin L (2018). Intestinal health benefits of bovine whey proteins after simulated gastrointestinal digestion. Journal of Functional Foods 49, 526-35.
10. Corrochano AR, Sariçay Y, Arranz E, Kelly PM, Buckin V, Giblin L (2018). Comparison of antioxidant activities of bovine whey proteins before and after simulated gastrointestinal digestion. Journal of Dairy Science. 102(1), 54-67.

Please describe how you have, or will, consider gender/sex in the planning, design and development of this proposal and during project implementation.

INFBar project is developed and will be led by an established female scientist and 2 early career female scientists. The project will aim at establishing and maintaining a gender-balanced and diverse team over the duration of the project, in line with 2020 Teagasc's Diversity and Inclusion Strategy. Also, the promotion of equal employment opportunities (e.g., flexible working arrangements that facilitate career development for parent researchers employed in this project) will be advanced within the frame of the proposed project. We will also arrange for project meetings and activities not to

disrupt a positive work-life balance. LGiblin sits on Teagasc's GenderSMART taskforce. She also has a particular interest and strong track record in encouraging women in a career in STEM with 6 of 8 completed PhDs are women, and 5 of 8 of her current team are females. She hosts at least 4 local educational outreach events per year and invites young girls to visit her lab. In 2019 she hosted 2 female secondary school students on TY placement, 1 female under graduate for 16 week research study and 1 visiting female PhD student for 3 months. Past female members from her lab hold senior positions in pharma such as Alcon, Merck Millipore, Mylan, in food industry (Glanbia, H&H global) and in permanent research positions in academia (UCC, CIT, Copenhagen University, CNR Italy).

ICT Resources

The purpose of these questions is to help anticipate and plan ICT support for your project.

During the lifetime of your project, will you require any new computer software or hardware, or do you envisage purchasing equipment which may contain or be linked to a computer?

Yes

Please Specify

standard computer for Walsh Scholar

Do you envisage needing to get any equipment connected to the internet or to the Teagasc computer network?

No

Declarations

Will the proposed research involve live animals?

No

Does the project require an environment assessment?

No

Do you have a data management plan?

Yes

Please provide some details on the expected data to be generated by this project (eg estimate of the size of the data; type of files, where it will be archived, storage or other relevant information).

This is a wet lab project. Data generated will be stored on projects2(\\mkvx341)(Q:) within Lab102 folder. Data is usually saved as Excel. Presentations are saved as powepoint files and reports as MicrosoftWord documents. Walsh Scholar will need Endnote and SAS on their computer. Similar projects in LGiblin lab's use approax 1.5GB of storage.

I declare that the information contained in this application is true to the best of my knowledge and belief

Yes

I have reviewed the PDF of my application and checked the layout and font

Yes

Communications

Preliminary Assessment Feedback

This project should proceed to a full proposal.

Reviewer Feedback for Applicant

Reviewer	Reviewer 1
Justification of ranking	
The idea is original and innovative. The proposed infant gut barrier model is already the object of an invention disclosure and this project will aid to strengthen the scientific background of the development. In current state-of-the art no models as the one proposed are available. The research team has a large capacity to develop the objectives considering the experience in cell culture models of absorption.	
Scientific background and technical quality of the application	
This project fits into the Food for health research area. Its relevance is demonstrated through the possibility to deliver an infant food barrier model for infant formula assay, a very direct applicability. The hypothesis is well based with regard to current development of models of gut barrier and reveals a deep knowledge on the area state-of-the art. The project is of high scientific quality and the methodology is very well suited to the objectives, as appropriate parameters in monolayer culture, permeability assays, inflammatory response and tight junction protein response will be employed. Finally, validation of the model using dairy proteins and infant formulae are proposed. A weak point is that the statistical input is not developed in the analysis of the results, especially the threshold between a leaky and non-leaky barrier could have been defined.	
Tasks	
The objectives are well specified and realistic. The proposed tasks respond to a well-organized plan considering the objectives. The objectives are clearly specified, they respond to a plan where model selection, ability of the model to assess permeability, physiological behavior determination together with assay of a superior model (organoids) and validation with two test products are included. Measurable parameters to assess the results are provided for each task. The proposed deliverables are achievable within the time frame proposed. In task 2 it would be interesting to compare the permeability of lactulose:mannitol in the new model with that of an adult gut monolayer, in order to better evaluate the results. It might also be interesting to determine the duration of the increased permeability expected by the applied treatment. This would help to define the time frame where experiments with the model would be possible.	
Expected benefits	
The expected benefits are achievable in the time proposed. The investigation of the inflammatory status of the Caco 2 monolayers treated with SCFA will expand the knowledge of this gut barrier model. Irish dairy industry is one of the targeted stakeholder groups identified, because the model would be a tool to develop next generation of infant formulas. Probably, further research in relation with the validation of the model with clinical studies will be necessary. The project adequately specifies that the new technology will be disseminated trough demonstrations, openday events.	
Collaborations & Resources	
The proposed project team has demonstrated capacity to deliver the project.There is an international collaboration with the TUM (Germany) to transfer knowledge on the organoids model, a superior model of the infant barrier although not ideally suited for transport assays at the moment. However, it could provide information on the inflammation state or tight junctions conditions in a more physiological way in comparison to Caco-2 monolayers. The three months of stay to gain this transfer knowledge should be sufficient provided a well planned stay. The project participants Linda Giblin and Elena Arranz have the qualifications and expertise to conduct the project. With regard to Eva Rath, there is little information provided to evaluate her qualification.The project represents a good value for money in view of the expected benefits for both end-users and industry, and the reduced budget as some existing projects will contribute to lab consumable costs.	

Scientific Assessment Feedback

The panel considers this to be a well-developed project of significant importance to the industry.

Applicant Modifications at Approved

Extension Requests

Number	Original Completion Date	Proposed new completion date	Status	

Progress Reports

Annual Report Schedule

Report Number	Status	Report Due Date	Report Year
No Results Found			

Final Report Schedule

Status	Report Due Date	Report Year
No Results Found		