Full4Health 266408 Final Report

4.1 Final Publishable Summary Report

Executive Summary

The Full4Health project was constructed with the intention of advancing knowledge of the mechanisms of hunger and satiety, including how food interacts with the gut-brain axis, in order to potentially inform future interventions targeting obesity and clinically significant undernutrition. The overall project was a mix of ground-breaking human volunteer studies addressing issues including multi-system responses to food across the life course, the importance and potential of dietary protein, and the detail of the relationship between physical activity and weight loss, allied to more mechanistic investigation of molecules, signalling systems and neural structures in appropriate pre-clinical models and cell-based systems. Key breakthroughs and major advances were made at the level of the gut, the brain and the connectivity between the two – the vagus nerve, microbiota, hormone secretion and blood metabolites.

These advances, examples of which are presented below, are relevant to a number of stakeholder groups: the scientific community, where internationally competitive research is recognised by publication in leading peer-reviewed journals and invitations to present work at prestigious scientific gatherings; medical and healthcare communities, where findings such as an alternative to bariatric surgery could have a major impact on obese patient groups for whom existing therapy is inappropriate; the pharmaceutical industry, where dissection of molecular signalling systems offers the potential to develop drugs that can exploit an endogenous physiological process in gut or brain to target obesity or diabetes, or replicate the effects of bariatric surgery in stimulating secretion of gut satiety hormones; the food and drink industry, where the move away from a 'one-size-fits-all' approach will have clear potential for targeted food reformulation and consumer engagement – this is exemplified by our pioneering comparison of responses to a dietary intervention in human volunteers across four age groups, two body phenotypes and both genders; public health policymakers and NGOs, where important messages have emerged, for example, relating to universal advantages of physical activity despite individual variation in body weight response.

At the core of the Full4Health project has been a common dietary intervention applied to population sub-groups by age, BMI and gender. We have collected a unique dataset with comprehensive measures comparing hunger and satiety, and hormone responses to protein-energy dietary challenge. This includes the first comparison of food-related brain responses between age groups. Physiological and behavioural factors influencing appetite across the lifespan will be useful to target specific interventions in the future, such as in the elderly who, at opposite ends of the BMI range, may be frail and at risk of sarcopenia or obese and needing to reduce caloric intake. Brain responses to food cues in the environment may provide insights into how to promote healthier choices to specific target groups, e.g. prevent snacking behaviour, such as in children and teenagers.

In a breakthrough in understanding individual variability, we have defined population groups that respond differently to exercise-induced energy deficit - responders and non-responders - based on exercise-induced changes in body fat. This indicates differences in energy compensation. We have generated important public health messages around exercise training induced improvements in appetite control and metabolic health independent of changes in body composition, and the observation that gender is not a barrier to exercise for improvements in body composition.

At a more mechanistic level, but with clear translational significance, we show that the gut hormone, ghrelin, is required for normal development of brain energy balance circuits in very early life, and ghrelin function is inhibited by neonatal overnutrition. With large numbers of women entering pregnancy overweight or obese, and childhood obesity at epidemic levels, new therapeutic opportunities need to be developed to address the metabolic abnormalities associated with early life overnutrition.
In another application of pre-clinical models, we have examined the mode of action of established obesity surgeries and possible treatments targeting the vagus nerve, and have taken endoscopic gastric injection of Botulinum toxin A (Botox) into the clinic for the adult obese. Invasive gastric bypass surgery is inappropriate for children and adolescents who are still growing, and Botox injection may be a viable option in these patient groups.

Summary Description of Project Context and Objectives

PROJECT CONTEXT

Full4Health is a multidisciplinary European collaboration of internationally renowned laboratories investigating the mechanisms of hunger, satiety and feeding behaviour, the effects of dietary components and food structure on these processes, and their possible exploitation in addressing obesity, chronic diet-related disease and under-nutrition. The project integrates investigation of human volunteers (dietary/exercise intervention studies and administration of encapsulated nutrients) and pre-clinical animal research with emphasis on neuronal, hormonal, molecular, physiological and psychological responses to food at different stages of the life course. It applies imaging and other cutting edge technologies in both humans and experimental animals to answer critical research questions at different levels of the food-gut-brain axis.

In human volunteers, responses to diet are being investigated from childhood through to the elderly, whereas wide-ranging innovative rodent studies are investigating related issues such as early developmental programming of the food-gut-brain axis, multiple feedback signalling interactions, and inflammation-induced anorexia. The project examines the interaction of food and dietary components with the gastrointestinal tract, and is characterising the role of gut endocrine secretions, the vagus nerve, and hindbrain, hypothalamic and forebrain structures in signalling and integration of hunger and satiety. Physiological and psychological responses to food may change as we develop and age, with impact on food choices and preferences. This is a critical issue in the battle against food intake-related chronic disease, most commonly driven by over-consumption, but also in consideration of relative under-nutrition in the elderly and clinically compromised.

The main research objectives of the Full4Health project are focused on food-gut-brain mechanisms in hunger and satiety. We are examining responses to dietary manipulation across the lifespan, assessing psychological and behavioural parameters, and food preferences, and quantifying hunger and satiety hormones produced in the gut, and activity responses in the brain. Very early life nutritional events are also being examined in animal models for impact on the development of, and sensitivity to, gut-brain signals, and translation into life-long feeding behaviour and energy balance. Responses to energy deficit are also being investigated, along with the relative importance of dietary protein content in determining energy intake. The studies in human volunteers under nutritional or energetic intervention are underpinned by the mechanistic study of food-gut-brain links using animal models, *in vitro* systems and human trials. At the level of the gut we are examining the impact of bioactive compounds from food or gut origin on synthesis and secretion of gut hormones relevant to food intake and energy balance mechanisms, and the impact of the gut microbiota in relation to food composition on central mechanisms of hunger and satiety. How hormonal and neural signals arising in the gut are transmitted to the brain is poorly understood. We are investigating the integration of food-related signals in hindbrain relay structures and their role in hunger and satiety, along with the characterisation of neural circuits and molecular mechanisms that contribute to the regulation of hunger, satiety and feeding behaviour elsewhere in the brain, and especially in the hypothalamus energy balance centres and forebrain reward centres.

LIMITS TO OUR UNDERSTANDING

Our understanding of gut-brain signalling pathways has gained in sophistication in recent years, but little attention has been given to the potential for manipulating these pathways via diet. There is a particular need for greater understanding in the following areas:

1. **We have a relatively sophisticated map of the networks of neural circuits within the brain that are involved in hunger and satiety, and knowledge of the molecular components of these processes is also growing**. *But we do not have a good understanding of how areas of the brain involved in homeostasis and hedonic evaluation are integrated with gut responses to food through peripheral hormones and the caudal brainstem to regulate food intake.*

2. **There is evidence of integratory capacity in the mammalian hindbrain and of relaying of signals forward to hypothalamic and higher brain centres.** *But there is poorer understanding of the response of hindbrain structures to food-related signals, the relative importance of blood-borne vs neural (vagal) signalling in the mechanism of hunger/satiety, the potential to manipulate these signals, and identify dietary components/food structure that can help to control food intake.*

3. **Satiety hormones are released from the gut during meal processing, and the resultant signalling to the brain leads to meal termination and suppresses future food intake.** *However, there is poor understanding of the mechanisms by which nutrients arriving in the intestinal tract act to release the gut hormones that contribute to satiety.*

4. **The interaction between the gut microbiota and dietary composition is an area of burgeoning interest with effects on energy balance being of particular interest.** *But we do not know how the diet/microbiota interaction influences the balance between hunger and satiety, and the activity of gut hormone and brain peptide signalling systems.*

5. **Early life nutritional experience can programme individuals' susceptibility to chronic disease in later life, and there is evidence of developmental programming of neuroendocrine systems related to energy balance.** *Little is known of the role of gut-derived signals in the nutritional programming of brain circuits involved in feeding and body weight regulation. Disruptions in gut-brain communication during critical periods of life may prompt substantial changes in the development of hunger and satiety networks.*

6. **Dietary interventions based on caloric restriction or macronutrient manipulation can promote weight loss, as can exercise-induced energy deficit.** *However, the psychological, behavioural and endocrine/neurological bases of these effects and their applicability across age, gender and body phenotype remain to be determined. We do not understand the variability in psychological and behavioural parameters of hunger/satiety and food preference during energy deficit (exercise or diet induced) across the life course, how these manipulations relate to gut hormones, neural activation and energy metabolism, or how these responses might vary in European populations.*

7. **Most emphasis in attempts to regulate food intake has been on changing patterns of fat and carbohydrate consumption**, *By contrast, the role of protein has largely been ignored. Intake of protein appears to be very tightly regulated in laboratory rodents*, *but we do not know whether dietary protein also exerts leverage to drive food and energy intake in humans*.

PROJECT OBJECTIVES

In the project objectives we addressed major gaps in knowledge:

We do not have a good understanding of how areas of the brain involved in homeostasis and hedonic evaluation are integrated with gut responses to food through peripheral hormones and the caudal brainstem to regulate food intake

Objective 1: To identify and characterise the neural circuits and molecular mechanisms that are integrated in the central (brain) regulation of hunger, satiety and feeding behaviour.

There is poorer understanding of the response of hindbrain structures to food-related signals, the relative importance of blood-borne vs neural (vagal) signalling in the mechanism of hunger/satiety, the potential to manipulate these signals, and identify dietary components/food structure that can help to control food intake

Objective 2: To investigate the integration of food-related, gut hormone and neural (vagal) signals in hindbrain relay structures and their role in hunger and satiety.

There is poor understanding of the mechanisms by which nutrients arriving in the intestinal tract act to release the gut hormones that contribute to satiety

Objective 3: To establish the impact of bioactive compounds from food or gut origin on synthesis and secretion of gut hormones relevant to food intake and energy balance, and underlying molecular mechanisms.

We do not know how the diet/microbiota interaction influences the balance between hunger and satiety, and the activity of gut hormone and brain peptide signalling systems

Objective 4: To determine the impact of the gut microbiota in relation to food composition on central mechanisms of hunger and satiety.

Little is known of the role of gut-derived signals in the nutritional programming of brain circuits involved in feeding and body weight regulation - disruptions in gut-brain communication during critical periods of life may prompt substantial changes in the development of hunger and satiety networks

Objective 5: To investigate the involvement of early post-natal nutrition on the development of gut-brain signalling systems, and the impact of hypothalamic sensitivity to gut-derived signals on life-long feeding behaviour and energy balance.

The psychological, behavioural and endocrine/neurological bases of these effects and their applicability across age, gender and body phenotype remain to be determined. We do not understand the variability in psychological and behavioural parameters of hunger/satiety and food preference during energy deficit (exercise or diet induced) across the life course, how these manipulations relate to gut hormones, neural activation and energy metabolism, or how these responses might vary in European populations.

Objective 6: To relate psychological and behavioural parameters of hunger/satiety and food preference to gut hormones, neural activation and energy metabolism during energy deficit and dietary manipulation, and across the lifespan.

The role of protein has largely been ignored. Intake of protein appears to be very tightly regulated in laboratory rodents, but we do not know whether dietary protein also exerts leverage to drive food and energy intake in humans.

Objective 7: To establish the relative importance of dietary protein content in determining energy intake.

Description of Main S & T Results and Foregrounds

(This document was also uploaded as a separate PDF).

General Objective 1: To identify and characterise the neural circuits and molecular mechanisms that are integrated in the central (brain) regulation of hunger, satiety and feeding behaviour.

Under this Objective is reported the main outcomes of a number of state-of-the-art pre-clinical studies. These focus on the mechanisms (molecules, neural structures and signalling pathways) related to hunger, satiety and feeding, and in particular the involvement of the brain in these processes. Experimental approaches range from investigation of the roles and interactions of novel and known candidate molecules, the neural structures in the brainstem, hypothalamus and forebrain reward centres involved in stimulating or inhibiting feeding, and the regulation of these energy balance components by dietary manipulation and *in vivo* feeding behaviours, and in pathological states such as obesity and cancer anorexia.

Key finding 1A: Meal feeding initiates a complex profile of timed gene expression changes.

We investigated longitudinal gene expression changes in hypothalamic and brainstem energy balance centres in response to food intake delivered in a carefully constructed scheduled feeding regime. 243 transcripts showed a significant two-fold change at a minimum of one of the meal related time-points in the hypothalamic arcuate nucleus (ARC), 192 transcripts in the paraventricular nucleus (PVN), 301 transcripts in the ventromedial nucleus (VMN), and 411 transcripts in the nucleus of the solitary tract (NTS). This is a much more subtle energetic intervention than the fasting-refeeding challenges that are frequently applied in the context of transcriptomics analysis, and should yield important information relevant to meal feeding. Further work is required to establish the involvement of all these gene expression changes in relation to each other and to satiation and satiety. In the longer term, new targets may emerge for therapeutic interventions or we may be able to place into context gene or regulatory sequence polymorphisms which affect hunger and satiety.

Key finding 1B: The ventral tegmental area (VTA) has a key role in responses to gut hormones.

GLP-1 inhibits reward behaviour for food by direct actions at the level of the ventral tegmental area (VTA). We have identified the VTA as a novel target for ghrelin to alter reward behaviour for food. The new insight that classic homeostatic regulators such as ghrelin and GLP-1 also impact reward behaviour related to food has had a large impact on our overall understanding of the complexity of human energy balance regulation and how it is linked to feeding behaviour. It is important to fully understand basic regulation of feeding behaviour in order to develop novel strategies to prevent and treat obesity.

Key finding 1C: Optogenetic technologies can be used to control neurones involved in both homeostatic and reward processes.

We have successfully developed and applied optical neuroengineering technologies to advance our understanding of gut-brain interactions, with the objective “to analyse and manipulate the behavioural consequence of controlling ghrelin-regulated neuronal circuitry in response to ghrelin and in anticipation/ingestion of food”. We achieved the objective by deploying a diverse range of novel optogenetic applications. We developed a set of minimal promoters to drive viral expression globally in neurones and also in specific ghrelin-sensitive neuronal populations involved in appetite control. We refined this approach by using a transgenic rat model where viral expression was confined to neurones known to be critically involved in reward and motivated behaviour. Using these technologies, we achieved *in vivo* optical control in anaesthetised rats of ghrelin-sensitive hypothalamic neurones and dopamine neurones of the midbrain reward pathway. We also introduced canine adenovirus as a tool to deliver a recombinase enzyme (Cre) retrogradely (1). This allows us to bring specific neurons projecting to a specific site under the control of chemo- or optogenetics.

Key finding 1D*:* Neuronal activity in the homeostatic hypothalamus in conscious rats during food anticipation and consumption reveals a role for ghrelin in these behaviours.

We manipulated neuronal activity in ghrelin-sensitive dopamine neurons and showed that long-lasting inhibition of these neurons is aversive in a behavioural assay but does not affect food consumption, showing that feeding behaviour is not dependent on time-locked activity of these neurones. Furthermore, we showed the effects of hormonal signalling on the processing of reward-related information by the dopamine neurones, thus providing a neuronal substrate of satiety-induced changes in motivated behaviour. We went on to optogenetically inhibit ghrelin-sensitive dopamine neurons during a behavioural task and showed that inhibition of these neurons has long-lasting effects on perception of food-predicting cues. Importantly, we also showed that neural responses to reward-predicting cues are dependent on metabolic state. We extended these findings by recording from ghrelin-sensitive dopaminergic neurons during food seeking behaviour. Specifically, we combined optical, imaging, and viral techniques to record somatic calcium transients from genetically identified neurons in freely moving animals. This technique allows visualisation of calcium activity *in vivo* and permits a better understanding of the real-time activity of specific neurones during eating behaviours.

Key finding 1E: The supramammillary (SuM) nucleus is involved in food reward.

Our work on dopamine neurones also led to the discovery of a brain region not previously reported to be involved in food reward. The SuM nucleus is a posterior hypothalamic structure that contains a rich population of cells that express the key enzyme in dopamine synthesis (tyrosine hydroxylase; TH). We showed that SuM cells were activated by palatable food consumption and that these cells project to other brain regions involved in homeostatic and reward-related appetite control. We also characterised SuM cells using *in vivo* electrophysiology and determined their sensitivity to ghrelin. We went on to use genetic approaches to map the neuroanatomical connections of SuM TH cells.

In terms of impact, we have demonstrated that these genetic technologies can be implemented in key brain systems involved in appetite and reward. We have shown that modulating these systems can affect behaviour. Furthermore, we also identified a brain region previously unrecognised as having a role in food reward. This was an unanticipated outcome, but one that was a direct consequence of the programme of work. These ground-breaking studies open new avenues in translational research – by better understanding the complex neuronal and hormonal mechanisms underlying eating behaviours, we increase the potential for exploiting these mechanisms to therapeutic benefit.

Genome-wide association study (GWAS) screens have identified a number of genes related to human obesity. Three of these have been investigated further here - Fto, Tmem18 and Negr1. These and other studies have led to much discussion as to which of the genes close to the loci identified by the initial wave of GWAS are responsible, either wholly or partially, for association with a range of human phenotypic characteristics. What this body of work illustrates is the complex biology that can emerge from the deeper exploration of the basis for genomic variants robustly associated with human adiposity.

Key finding 1F: Fto may have a critical role in the control of lean mass, independent of its effect on food intake.

Adult onset hypothalamic Fto loss has a small, but significant, effect on body weight due to decreased food intake. Although hypothalamic Fto can impact feeding, the effect of loss of Fto on body composition is brought about by its actions at sites elsewhere. Our data suggest that Fto may have a critical role in the control of lean mass, independent of its effect on food intake.

Key finding 1G*:* Fto influences the metabolic outcomes of high fat diet (HFD) feeding through alteration of hypothalamic NFκB signalling.

The effects of ingestion of different diets on brain signalling was examined in mice of different genetic backgrounds; with and without the *Fto* gene. *Fto*$^{+/-}$ and *Fto*$^{-/-}$ mice remained sensitive to the anorexigenic effects of leptin, both after exposure to a HFD or after acute central application of palmitate. Genes encoding components of the NFκB signalling pathway were down-regulated in the hypothalami of *Fto*-deficient mice following a HFD. When this pathway was reactivated in *Fto*-deficient mice with a single low central dose of TNFα, the mice became less sensitive to the effect of leptin. We identified a transcriptional coactivator of NFκB, TRIP4, as a binding partner of FTO and a molecule that is required for TRIP4 dependent transactivation of NFκB. This supports the notion that pharmacological modulation of FTO activity might have the potential for therapeutic benefit in improving leptin sensitivity, in a manner that is influenced by the nutritional environment.

Identifying FTO as being able to functionally interact with the NFκB signalling pathway adds another role to an increasing list of putative functions for this molecule. Fto has been characterised as a demethylase with the ability to remove 3 methyl groups from single stranded nucleic acid, and we have also shown that Fto plays a role in the sensing of amino acids, which potentially provides an explanation for the significant interaction observed between FTO genetic variation and dietary protein on appetite and food cravings. How and if these mechanisms link back into the original observations of associating genomic variance around the *FTO* locus with human pathophysiology remains to be fully determined. The most parsimonious explanation is that a number of genes in this region play an important role in determining body composition.

Key finding 1H: Both *Tmem18* and *Negr1* have a role in murine energy homeostasis.

Overexpression of Tmem18 within the paraventricular nucleus (PVN) ameliorates the feeding response to a fast, reduces food intake and increases energy expenditure in *ad libitum* fed mice, increases expression of hypothalamic anorectic factors and increases markers of thermogenesis in brown adipose tissue (BAT). We have generated 'whole-body' Negr1 KO mice and PVN-specific Negr1 KO mice, and shown that deletion of Negr1 results in a leaner mouse because of a reduction in food intake.

Key finding 1I: Hypothalamic serotonin signalling plays a major role in the decrease in food intake during cancer, and serotonin signalling is modulated by inflammatory mediators.

Appetite is often reduced in patients with chronic illness, including cancer. Inflammatory processes in the hypothalamus are considered to play a crucial role in the development of disease-related anorexia. To investigate mechanisms specifically involved in cancer anorexia, we set up two tumour mouse models with opposing food intake behaviours: a C26-colon adenocarcinoma model with increased food intake and a Lewis lung carcinoma model with decreased food intake. The contrast in food intake behaviour between tumour-bearing (TB) mice in response to growth of the two different tumours was used to distinguish processes involved in cachexia from those specifically involved in anorexia. The hypothalamus was used for transcriptomic analysis (Affymetrix chips). We found expression of genes involved in serotonin signalling in the hypothalamus to be differentially regulated between the two tumour models. Furthermore, transcriptional activity of genes involved in serotonin signalling were inversely associated with food intake behaviour. We also found a strong increase in gene expression of NPY and AgRP, potent orexigenic neuropeptides, in both models, which may have reflected weight loss, which was severe in both tumour models. Inflammatory markers IL-6 and TNFα in plasma were different in the two tumour models, and these differences in inflammatory response could be implicated in the differences in feeding behaviour and serotonin signalling.

General Objective 2: To investigate the integration of food-related, gut hormone and neural (vagal) signals in hindbrain relay structures and their role in hunger and satiety.

This Objective primarily addresses the signalling upstream of the hypothalamus and forebrain reward centres (i.e. afferent signals), focusing on signals of food/nutrient, metabolite, and peripheral nervous system (especially vagus nerve) origin, and their integration in the less-well-studied brainstem nuclei. Synergistic interactions between metabolic and hormonal signals are also considered along with the mitochondrion as a new integration point for nutrient and hormone signals coming from the periphery to the hypothalamus. Although mainly conducted in pre-clinical models, many of the findings have obvious translational potential, especially the consideration of surgical (bariatric) approaches and alternatives to these that might be more appropriate in certain patient groups.

Key finding 2A: VBLOC activates vagal signalling to the brainstem and hippocampus but blocks vagal signalling to the stomach.

To extend knowledge of the role of the vagus nerve in neural signalling, studies were undertaken of the mechanism-of-action of VBLOC. VBLOC is a pacemaker-like neuroregulatory device that is implanted laparoscopically into the lateral chest wall with a flexible lead placed around the vagus nerve. Electrical stimulation which sends intermittent blocking signals to disrupt the vagal signal. In January 2015, the U.S. Food and Drug Administration approved "vagal blocking therapy (VBLOC therapy)" for obese adults (2). The concept behind the design of VBLOC is consistent with the important role of the vagus nerve in the gut-brain axis, in satiety control and in the progression of obesity (3, 4). In animal studies, we found that VBLOC activated vagal signalling to the brainstem and hippocampus but blocked vagal signalling to the stomach, leading to increased satiety, reduced food intake and eventually weight loss (<10%TWL). The action of VBLOC was independent of the hypothalamus-gut hormone pathway (Johannessen *et al*. 2016 in prep).

Key finding 2B: Gastric Botox injection could be a viable obesity treatment, especially for adolescents.

Another focus was to understand how best to control satiety, focusing on comparisons between gastric bypass, VBLOC and Botulinum toxin A (BTxA) injection. A new therapeutic concept was developed using a modified method of BTxA injection to block the gastric vagal nerve input to the brain. When BTxA is injected into the antrum either at the sub-mucosal or sub-serosal layer and more importantly to cover the whole area of the antrum, it binds to SNAP 25 (and synaptobrevin) and blocks the release of acetylcholine from the vagal efferent fibres. As a result of muscular paralysis and extension only in this part of the stomach, the vagal afferent fibres send a satiety signal to the brain. In order to develop this treatment for adolescents, we performed a series studies in animals and a pilot clinical trial. Young rats became obese when fed a high-fat diet, probably due to increased meal size (5). We found that gastric injection of BTxA in the young rats induced a higher satiety ratio and lower food intake than in those received saline, leading to body weight loss. The repurposing of endoscopic injection of Botulinum toxin A for obesity treatment (EIBO) in adolescents is currently the subject of a proposal for funding for a multi-centre clinical trial.

Key finding 2C*:* A liquid diet in a pre-clinical scheduled feeding model results in reduced body mass.

We investigated whether or not the physical form in which food is consumed affects body mass. A scheduled feeding paradigm was developed as an effective means of inducing coordinated food intake consumption and creating a meal type access to, and consumption of, food. Diets tested were high fat, high protein or high carbohydrate. A liquid diet of the same composition and caloric value as solid diet consistently resulted in lower body mass. Body mass loss was composed entirely of fat loss. This is the first data in an animal model to compare the effect of the physical form of a diet composed of exactly the same ingredients presented in the solid or liquid state on long term body weight and gene expression response in key areas of the brain involved in appetite and energy balance - the ARC and NTS.

Key finding 2D*:* Both solid and liquid diets activate c-fos in hindbrain neurones.

Both solid and liquid diet forms were equally effective at increasing c-fos expression in the hindbrain when fed *ad libitum*. When given as a fixed caloric intake, a liquid fat diet may evoke a slightly larger induction of c-fos. Together with a gavage study in which gavage of saline did not evoke NTS activation, this data supports current evidence for the NTS as the primary relay station for registration of food intake and initiation of satiation. Using the schedule feeding protocol with rats consuming a solid diet, we found that gavaging a solution of equal caloric value containing any one of the macronutrients, was equally effective at reducing intake at a second meal, and to a greater level than the caloric value of the gavaged solution.

Key finding 2E: Dietary protein cross-linking enhances satiety.

A protein crosslinked diet was shown to induce satiation/satiety at a lower caloric intake than a liquid diet of the same caloric density and composition or a solid diet of the same composition but of a higher caloric density. Analysis of metabolic parameters showed that the response to the crosslinked diet was more similar to a solid diet than to a liquid diet. Hypothalamic gene expression of orexigenic neuropeptides NPY and AgRP in the ARC was similar between a solid and crosslinked diet, but elevated in rats consuming a liquid diet. These data are consistent with limited studies in humans indicating enhancement of satiation by protein crosslinking, and would support incorporation of protein crosslinking into liquid weight loss diets as a means of increasing the feeling of fullness achieved.

Key finding 2F: Leptin and glucose act synergistically to suppress food intake.

Injection of leptin and glucose together resulted in a stronger reduction of food intake 4 hours after icv injection than that observed when leptin or glucose were injected by themselves. Only injection of both molecules resulted in suppression of food intake 24 hours after central administration. We further investigated the molecular basis for the interaction of leptin, glucose and PYY to suppress feeding.

Key finding 2G: DNA damage accumulating in the neuronal populations of the hypothalamus may result in metabolic deterioration with advancing age.

We also investigated whether age-related changes in the above mechanisms alter leptin, glucose and PYY's ability to synergize in suppressing feeding. To investigate how ageing, as an internal deterioration process, affects structural and/or electrophysiological properties of POMC and NPY neurons, we assessed the accumulation of DNA damage foci in the nuclei of both NPY and POMC neurons at 12 months of age compared to 2 months of age. We observed accumulation of DNA damage foci in NPY as well as in POMC neurons at 12 months of age.

Key finding 2H*:* Mitochondrial function in POMC neurons is a new integration point for nutrient and hormone signals coming from the periphery to the hypothalamus.

Inactivation of a key mitochondrial factor, namely AIF (apoptosis inducing factor, which is essential for the assembly and stability of the complex I in the electron transport chain), resulted in improved insulin sensitivity after high fat diet feeding by improved suppression of hepatic glucose production. The observed hepatic response to insulin is very likely mediated by AMPK signalling. We wanted to answer questions on how gut peptide signalling interacted with macronutrient depletion at the level of the hindbrain and what was the hindbrain synergistic action of ghrelin and specific macronutrient depletion on feeding behaviour.

Key Finding 2I*:* Glucose sensing is impaired in AIF-deficient POMC neurons, whereas responsiveness to oleic acid is increased under high fat diet conditions.

As POMC neurons are critical targets for the integration of fuel sensing signals such as glucose and free fatty acids, we further studied the POMC neuron electrophysiology responses to specific nutrients on a

cellular level. We detected that glucose sensing was impaired in AIF-deficient POMC neurons. At the same time, we observed that AIF-deficient POMC neurons showed an increased responsiveness to oleic acid under high fat diet conditions, suggesting that the pre-existing glucose sensing impairment protects POMC neurons in terms of fatty acid sensing after a diet switch.

Key Finding 2J: Lack of AIF protects against the deleterious effects of high fat diet, and mitochondria can act as central sites for integration of multiple homeostasis signals.

Defining the functionality of AIF-deficient POMC neurons by characterizing the electrophysiological response and analysing mitochondria density and shape, as well as cristae morphology electron-microscopically, under normal chow and high fat diet conditions, we saw that lack of AIF resulted in hyperpolarization, reduced firing and mitochondrial elongation in POMC neurons of animals fed a normal chow diet. The absence of any metabolic phenotype suggested that the POMC neurons remained responsive to nutrient and hormonal signals under these conditions. High fat diet feeding profoundly affected firing of wild type POMC neurons, together with changes in mitochondria and cristae shape, and reduced endoplasmic-mitochondria contacts. Most importantly, AIF-deficient POMC neurons had improved firing and all measured mitochondria parameters were comparable to the wild-type, normal chow diet situation.

Key finding 2K*:* The NTS contains ghrelin-responsive cells that are both sufficient and necessary for eliciting a feeding response.

The important orexigenic hormone ghrelin interacts with responsive cells in the NTS to affect food intake. The NTS also contains cells that are responsive to a lack of utilizable glucose. However, the combination of the two signals does not seem to act synergistically. We also observed an orexigenic effect of direct ghrelin delivery to the PBN (parabrachial nucleus), mediated in part by ghrelin's direct action on PBN GHSR1A.

Key finding 2L*:* The lateral PBN is a neural substrate for the feeding suppression effect of GLP-1.

Having demonstrated that both nutrient depletion and endogenous ghrelin can signal directly within the NTS to change energy expenditure and food intake, and that ghrelin also affects the PBN, administration of the GLP-1 analogue, Exendin-4, was shown to activate c-Fos in the PBN. This mechanism of action may be relevant to patients receiving Ex-4, or other GLP-1 analogues, given that these pharmaceuticals can cross the blood-brain barrier after peripheral application.

General Objective 3: To establish the impact of bioactive compounds from food or gut origin on synthesis and secretion of gut hormones relevant to food intake and energy balance, and underlying molecular mechanisms.

This Objective focused on the enteroendocrine cells in the gastrointestinal tract that secrete key peptide hormones relating to meal processing and satiety signalling, including their distribution, sensitivity to food composition and nutrients, and their phenotypes in terms of the hormones secreted. The report encompasses complementary approaches to addressing the nutrient-gut hormone interface including cell-based investigation, pre-clinical models and organ culture, allied to a proof-of-principle clinical study delivering encapsulated nutrients to the intestine. The sensitivity of cells secreting peptide hormones such as GLP-1, PYY and GIP to nutrients and western-type diets and the mechanisms involved have translational relevance at a population level in terms of our contemporary diets and to the pharmaceutical and nutraceutical industries seeking to address the problems of over-consumption and metabolic complications through enhanced hormone secretion.

Key finding 3A*:* Short chain fatty acids, triglyceride digestion products, di/tri/oligopeptides and amino acids stimulate gut endocrine cells.

We investigated the molecular mechanisms underlying signalling triggered by nutrient-related stimuli. Pathways underlying gut hormone secretion were monitored initially by fura2 imaging of primary enteroendocrine cells to monitor intracellular Ca2+, and by FRET-based imaging of GLUTag cells using the Epac2-camps sensor to monitor cAMP. During the project we developed new murine models expressing genetically encoded Ca2+ and cAMP sensors specifically in enteroendocrine cell populations, which enabled imaging of cAMP in primary cells and improved our imaging of Ca2+. The following signalling pathways were identified: short chain fatty acids elevated gut hormone secretion via elevation of Ca2+, mediated by activation of the Gq-coupled receptor GPR43 (FFAR2), and Ca2+ release from intracellular stores; lipid micelles stimulated hormone secretion through a variety of mechanisms - most important are likely to be GPR40 (FFAR1) and GPR120 (FFAR4), which are coupled to store-mediated Ca2+ release and a downstream pathway involving TRP channels; mono-acyl glycerols activate GPR119, found in approximately 50% of enteroendocrine cells, and linked to elevation of cAMP - knockout of GPR119 from L-cells abolished lipid gavage-triggered GLP-1 secretion in living mice; protein digestion products – di, tri and oligopeptides and amino acids – recruited a range of signalling pathways in enteroendocrine cells, linked to elevation of hormone secretion. PEPT1 is an electrogenic di/tri peptide transporter, activation of which in L-cells was linked to increased voltage-dependent Ca2+ entry and hormone secretion; the calcium sensing receptor CaSR was found to exhibit broad substrate specificity, and underlies a substantial component of protein triggered hormone release; arginine vasopressin activates AVPR1b, with elevation of Ca2+ and cAMP, and suggests a novel role of gut hormones in contributing to fluid balance regulation.

Key finding 3B*:* GLP-1, PYY and GIP are released from an overlapping population of enteroendocrine cells.

Transcriptomic analysis of L and K cells from mice on chow diet revealed that GLP-1, PYY and GIP are released from an overlapping population of enteroendocrine cells with common molecular machinery.

Key finding 3C*:* High fat diet affects the mouse L-cell transcriptome, reducing gut hormone secretion.

High fat diet had a small effect on L-cell number, but exhibited its greatest effect on the L-cell transcriptome. Small intestinal L-cells from high fat diet-fed mice exhibited a globally reduced pattern of L-cell characteristics, notably a reduction in production of gut hormones and reduced expression of machinery involved in gut hormone secretion. These studies have revealed a range of signalling pathways specifically expressed in enteroendocrine cells, which contribute to the detection of ingested nutrients. A number of these pathways are of translational interest and are currently under evaluation as potential drug targets for new treatments for obesity and diabetes. The analysis of L-cells from mice fed on high fat diet revealed the negative impact of chronic dietary habits on gut hormone secretion, which if translatable to humans would suggest that consumption of a high fat Western diet would impair post-prandial satiety mechanisms and contribute to over-eating and weight gain.

Key finding 3D*:* GLP-1 and GIP are not co-secreted in small intestine.

It has been observed that after Roux-en-Y Gastric Bypass there is dramatically increased secretion of GLP-1, PYY, and oxyntomodulin. The hypothesis is that these hormones could be responsible for the resolution of diabetes that occurs quickly after surgery and weight loss. Thus, increasing endogenous GLP-1 secretion could be possible as a cure for diabetes without surgery. Using the perfused rat intestine we have investigated the co-secretion and co-localisation of metabolism regulating hormones from the intestine, including GLP-1, PYY, neurotensin, GIP and CCK. It was initially found that GLP-1 and neurotensin were secreted in equal amounts from the proximal and distal small intestine whereas PYY was only secreted from the distal small intestine in response to bombesin and IBMX. Furthermore, it has been shown that the same secretion pattern applies when using a more physiological stimulus, the meat hydrolysate peptone which proved to be an excellent stimulator of gut hormone secretion (6). Studies in the perfused mouse

small intestine and *in vitro* showed some degree of co-localisation between GLP-1 and GIP. However, we have found that while bombesin provides a robust stimulus of GLP-1 secretion there is no effect on GIP secretion. These findings are further supported by expression analysis showing that the bombesin receptor is highly expressed in GLP-1 positive cells but not in GIP positive cells. This demonstrates that GLP-1 and GIP are not co-secreted (7).

***Key finding 3E**:* GPR40, GPR119, GPR41/43 and bile constituents (MS33+34) stimulate GLP-1 secretion in perfused intestine.

We have successfully developed models for perfusion of rat and mouse intestine as a supplement to pig studies. This will allow us to perform more experiments with the simpler setup using rodents, and allow results to be compared to other *in vitro* and *in vivo* studies often performed using rodents. We have used our models to investigate the secretion pattern of GLP-1 and other metabolism-regulating hormones.

***Key finding 3F**:* There is a dense neural GLP-1 receptor expression in the gut.

An investigation of the mechanisms of action of appetite and metabolism regulating hormones from the gut with special emphasis on afferent sensory neuronal pathways was performed in *in vivo* models of rats and pigs. There are indications that neural transmission may be demonstrable in pigs, and this work will continue. The recent rat studies have demonstrated a dense neural GLP-1 receptor expression in the gut, strongly supporting our hypothesis of important neural effects of GLP-1.

In parallel with the *in vitro* and perfused intestine work, we performed a number of studies in healthy human volunteers to assess the impact on circulating concentrations of peptide hormones of encapsulating nutrients formulated to target specific areas of the intestine.

***Key finding 3G**:* Encapsulated nutrients are a viable delivery route, once stability and shelf life are established.

After preliminary testing which identified no serious adverse events, we found that administration of 10 capsules of glutamine (6g) produced a small measurable increase in GLP-1. Further work with healthy lean and obese volunteers investigated the dose dependence of encapsulated nutrient on gut hormone secretion, hunger and voluntary food intake. The primary outcome was the ability of glutamine capsules to cause an increase in circulating concentrations of GLP-1. However, there was some inter-individual variation in response but the reasons for this are unclear. Groups of responders and non-responders were identified. The effect of encapsulated glutamine on glucose tolerance in lean and obese humans was investigated: there was no effect on peak glucose concentrations, although when participants received the maximum dose of glutamine, the time of peak glucose was delayed by 30 minutes. The area under the curve of insulin concentrations during the OGTT was similar between groups. However, there was evidence that insulin levels were higher at time point 90 minutes, prior to the OGTT commencing, in patients who had received the glutamine capsules compared to placebo. Additionally, healthy volunteers were recruited to assess the effect of glutamine capsules on meal size. Unexpectedly, the group receiving 10 capsules of glutamine ate significantly more food during the *ad libitum* meal compared to the placebo group. Analysis of hunger questionnaires showed a small but significant increase in hunger levels immediately prior to the meal following ingestion of the glutamine compared to placebo.

General Objective 4: To determine the impact of the gut microbiota in relation to food composition on central mechanisms of hunger and satiety.

The gut microbiota are increasingly being seen as an important component of the food-gut interface and to contribute to metabolism and health at a variety of levels, including a likely role in the development of obesity. The makeup of the gut microbiota and gut metabolism are sensitive to dietary composition. The impact of the gut microbiota on the energy balance systems in the brain is less well understood, and this

Objective investigates the relationship between the gut microbiota and the hypothalamic and brainstem body fat regulating circuits, comparing gene expression in germ free and conventionally raised mice.

***Key finding 4A**:* Expression of energy balance related neuropeptide genes in brainstem and hypothalamus differs in germ-free and conventional mice.

We found that conventionally raised mice had lower expression of the anorexigenic neuropeptide glucagon-like peptide-1 (GLP-1) precursor proglucagon (*Gcg*) in the brainstem. Moreover, in both the hypothalamus and the brainstem, conventional mice had lower expression of the anorexigenic neuropeptide brain-derived neurotrophic factor (*Bdnf)*. Conventionally raised mice had lower expression of the orexigenic peptides neuropeptide-Y (*Npy*) and agouti-related protein (*Agrp*), and higher expression of the anorexigenic peptides proopiomelanocortin (*Pomc*) and cocaine and amphetamine regulated transcript (*Cart*) in the hypothalamus. These differences in neuropeptide expression could be secondary to higher fat mass in conventionally raised mice. Leptin treatment caused less weight reduction and less suppression of orexigenic *Npy* and *Agrp* expression in conventional mice compared with germ free mice. The hypothalamic expression of leptin resistance associated suppressor of cytokine signalling 3 (*Socs-3*) was increased in conventionally raised mice (8).

***Key finding 4B**:* Gene expression differences between germ-free and conventional mice precede differences in body weight.

It was found that conventionally raised young mice had lower expression of the anorexigenic neuropeptides GLP-1 and *Bdnf*. These differences preceded the development of differential body weight, consistent with a hypothesis that the decrease in GLP-1 and *Bdnf* may contribute to the effects on body fat that occur at an older age (9). Previously germ free mice that were recolonized with gut microbiota for 3 weeks from 8 weeks of age showed a markedly decreased expression of GLP-1 and *Bdnf*, and these mice also show reduced body weight. These findings suggest that the continuing presence of gut microbiota during adult life is enough to suppress the expression of GLP-1 and *Bdnf*. Interestingly, germ free mice that were recolonized with gut microbiota during adulthood also showed a markedly decreased expression of interleukin-6 and interleukin-1β. This is of interest given that we have reported that both IL-6 and IL-1β suppress obesity at the level of the CNS, and thus seem to act as anti-obesity neuropeptides in healthy animals (10, 11).

Partly based on the finding that introduction of gut microbiota enhanced the expression three anorexigenic neuropeptides, we investigated their possibly interactions.

***Key finding 4C**:* The clinical GLP-1 analogue, exendin-4, induces expression of both IL-6 and IL-1.

We found that the anorexigenic GLP-1 analogue, exendin-4, which is in use in the clinic, induced expression of both IL-6 and IL-1. We also observed that IL-6 and IL-1 mediate the anti-obesity effect of the GLP-1 analogue exendin-4 (12). Others have provided evidence that IL-6 also mediates the anti-obesity effect of amylin analogues (which also are used clinically (13, 14).

***Key finding 4D**:* Preliminary data indicate that the effects of gut microbiota are independent of diet.

All the effects of gut microbiota described so far were observed in mice fed normal chow - a diet consisting mostly of protein and complex carbohydrates. However, similar effects of gut microbiota were obtained in mice on various other diets containing higher levels of fat and more sucrose.

General Objective 5: To investigate the involvement of early post-natal nutrition on the development of gut-brain signalling systems, and the impact of hypothalamic sensitivity to gut-derived signals on life-long feeding behaviour and energy balance.

A recent major advance in early life nutritional programming has been the demonstration that the adipose tissue hormone, leptin, has a neurodevelopmental role during neonatal life in pre-clinical models. This is manifest as a trophic role on developing axonal projections from and between hypothalamic nuclei which are establishing their normal adult pattern and density during this critical period. This has obvious implications for the developing brain in the human foetus, where much of the equivalent development takes place *in utero*, and where many women now enter pregnancy overweight or obese. This provides an over-rich nutrient environment for the developing foetus. In this Objective we have added a further layer of complexity to this scenario by demonstrating a role for ghrelin in the developing brain in mice.

Key finding 5A*:* Ghrelin is developmentally regulated and acts on hypothalamic "feeding" neurons during early postnatal life.

Neonatal ghrelin blockade in mice causes lifelong metabolic disturbances and structural alterations in the developing hypothalamus. Correct timing and amplitude of ghrelin levels are required for the normal developmental pattern of hypothalamic feeding circuits. The neurodevelopmental activity of ghrelin is essentially restricted to the neonatal period, and has overall effects that are the opposite of those observed with the adipose tissue hormone, leptin. Adult injections with an anti-ghrelin compound did not affect metabolic regulation and did not alter hypothalamic neural projections (15).

Key finding 5B*:* Neonatal overnutrition causes early alterations in the central response to peripheral ghrelin.

We have obtained novel information about how the brain responds to the gut-derived signal, ghrelin, in the context of early life overnutrition. Neonatal overnutrition achieved through manipulation of litter sizes in the mouse alters ghrelin levels during postnatal life. Postnatal overfeeding causes persistent overweight and increased sensitivity to diet-induced obesity. We demonstrated that this postnatal overfeeding also causes central (brain) resistance to peripheral ghrelin during important periods of hypothalamic development, and impairs ghrelin transport into the hypothalamus (16). The results also reveal that early postnatal overnutrition causes metabolic and neurodevelopmental alterations that likely involve changes in leptin levels and hypothalamic leptin resistance during important periods of development. Together, these findings point out potential new therapeutic opportunities to ameliorate the metabolic abnormalities associated with neonatal overnutrition.

General Objective 6: To relate cerebral, physiological, psychological and behavioural parameters of hunger/satiety and food preference to gut hormones, neural activation and energy metabolism during energy deficit and dietary manipulation, and across the lifespan.

This Objective reports on the results from three ground-breaking clinical studies. In some cases the outcomes of the clinical studies are necessarily preliminary due to the late completion of the data collection phases of the relevant investigations, as detailed in previous reports, and their double blinded design. Nevertheless, the breadth of the comprehensive dietary intervention studies that traverse 4 age groups (children to elderly), gender and BMI, with assessment of physiological, endocrine, psychological and behavioural factors influencing and responding to appetite and food intake are unmatched to our knowledge. The dataset that has been collected is unique in the different types of measures that are combined and in terms of the age groups examined together, extending from children to elderly, and the comparison of hunger and satiety. For example, to date, food-related brain responses have not been compared systematically between age groups. Thus, the initial rationale for these interventions in the Full4Health project is still highly relevant. The outcomes of these studies will identify key mechanistic and

phenotypic parameters in population sub-groups and help inform specific interventions across the lifespan in the future. Similarly, the study of supervised physical activity and heterogeneity in energy balance response provides critical mechanistic insight into the individual variability behind the relationship between energy intake and energy expenditure and generates important messages of public health relevance.

WP1: Neuro-gut interactions in humans across the lifecourse

A two site trial examined dietary protein-energy interactions (high or normal protein content drinks fed at maintenance or weight loss energy content) for effects on short-term appetite, and the influence of eating behaviour, body phenotype and psychology in lean or obese volunteers of both genders from 4 age groups on this interaction.

Key finding 6A*:* Calorie load in the test meal had, in general, more influence than diet composition on motivation to eat and subsequent food intake.

Analysis of responses to the four meal challenges across the four age groups revealed that the calorie content of the test meal had, in general, more influence than diet composition on motivation to eat and subsequent food intake. A rich dataset on the effect of dietary macronutrients and energy on short-term human appetite, across the lifespan, has been collected that will provide an evidence base for understanding the role of breakfast intake on short-term subsequent food, energy and nutrient intake.

Key finding 6B: Individual subject characteristics ('phenotype') are as important as diet composition in influencing gut hormone concentrations.

The most relevant gut hormones (ghrelin, PYY, GLP-1) were measured prior to and during the 2 hours after each of four challenge meals. This work will provide insight into the mechanisms by which nutrients arriving in the intestinal tract act to release the gut hormones that contribute to hunger and satiety, specifically the role of dietary protein and calorie load, across the life course. Hormone profiles appeared valid in terms of physiological response, with differential effects on hormone secretion after a meal - ghrelin levels decrease to reduce hunger sensations and anorectic hormones increase to promote satiety. Postprandial differences in the secretion patterns of these appetite-related hormones between healthy-weight and obese adults were demonstrated, and subject phenotype appeared to be as important as diet composition in influencing hormone concentrations. Such altered appetite hormone responses may have an impact on satiety, potentially contributing towards increased food intake and energy imbalance. The combination of fMRI data with the gut hormone profile will allow further investigation of peripheral and central mechanisms.

These data suggest that different countries (cultures) and people (gender, body mass, age) need to be considered when defining and designing foods to promote within-meal satiation and between-meal satiety. This is a fundamental finding of these novel data, where similar protocols have been applied across the lifespan. Our current limited understanding of how to alter appetite to avoid or treat obesity does not currently address these issues. Clearly a 'one diet approach' does not fit all people. Public health advice and food strategies need to be tailored for specific phenotypes to generate a sustainable and healthy approach for appetite control. Our new data suggest that our physiology and psychology (liking/wanting) are key areas that can be manipulated by the food or diet we eat. Collaborative with the food and drink industry, we can further use these data to design and test food products to generate modulation of appetite control.

WP3: How does age affect the power of hunger, gut signals and food cognitions in the brain?

To investigate the effects of hunger and satiety on the cerebral responses to food presentation and food choice, a protocol for making standardized food images was developed. With the use of this protocol a large set of low- and high food images was produced, including both widely accepted as well as country-specific images for the Netherlands, Greece and Scotland. The protocol for making these images and comparison of subjective ratings of the food images across The Netherlands, Scotland, UK and Greece was

published (17) and the image set has been made available online for other researchers. Food viewing is the most widely used paradigm to study food-related brain responses with functional MRI. We examined how responses to low- and high calorie foods change with age, and how they are affected by hunger state and BMI.

Key finding 6C*:* Age-related differences in the neural response to high (versus low) calorie food cues suggest a greater susceptibility of children, in particular teens, to food cues.

Hunger: Our first analyses show age-related differences in the neural response to high (versus low) calorie food cues, which may suggest a greater susceptibility of children, in particular teens, to food cues. This may be linked to the fact that their prefrontal cortex and thereby their capacity to withhold responses (inhibitory control) is still developing. This underscores that to limit overconsumption different strategies or policies may be needed for different age groups.
Satiation: Across the lifespan there is a difference in activation by high vs low calorie food between hunger and satiety in the medial prefrontal cortex (superior frontal gyrus) of normal weight participants; high-minus low calorie food viewing activation during hunger is greater than that during satiety across all age groups. This may reflect the greater biological value of high-calorie food in a fasted state.

Further analyses will elucidate how weight status affects food cue responsivity in the different age groups and which individuals are most prone to engage in eating in the absence of hunger. The food choice task developed and used in Full4Health is unique because low- and high calorie foods are individually matched on liking. Thus, any differences in brain activation are really due to caloric content and not to differences in preference. We have shown in a first publication, based on the first data obtained during the creation of the fMRI protocol that during satiety, food choice compared with non-food choice elicits stronger activation in several brain regions implicated in appetitive processes, such as the insula (18). This suggests that the food stimuli were more salient despite the subject's low motivation to eat. However, low- and high calorie food choices did not differ prominently, due to the matching on liking. The right superior temporal sulcus (STS) was the only region that exhibited greater activation for high versus low calorie food choices between foods matched on liking. Together with previous studies, this suggests that STS activation during food evaluation and choice may reflect the food's biological relevance independent of food preference.

Key finding 6D*:* Fasted overweight subjects hyperactivate visual processing areas during food choice, even when choice options are matched for individual preference.

In a first analysis of the fMRI data, significant activation for food versus non-food choices in a hungry state was found. Moreover, while making food choices in a hungry state, several areas, including visual processing areas, were more strongly activated in overweight compared to normal weight adults. Thus, fasted overweight subjects hyperactivate visual processing areas during food choice, even when choice options are matched for individual preference. This may reflect heightened attention for food and suggests a higher food ‘wanting’ in overweight individuals, regardless of the type of food choice made (low/high calorie) and thus a greater sensitivity for food reward. The unique food choice fMRI data that we have collected will allow us to establish how food choice is modulated by hunger, weight status and age, independent of liking. This will advance our understanding of the implicit neural drivers of food choice, which may provide knowledge on how to promote healthier food choices in different populations.

Key finding 6E*:* Overweight and normal weight adults differ in striatal downregulation after satiation during monetary reward anticipation.

We added an established monetary reward task to the fMRI study, to be able to investigate reward system functioning in the different subgroups. To our knowledge we are the first to have investigated the effects of hunger state and weight status (BMI) on monetary reward anticipation in normal weight and overweight adults. We found a lack of striatal downregulation after satiation during monetary reward anticipation in

overweight but not normal weight adults: striatum activation was equal for the groups when fasted. However, during reward anticipation, striatum activation decreased with satiation in the normal-weight but not the overweight group. This lack of downregulation in overweight adults might explain eating in the absence of hunger since their reward system is still in hungry-mode despite being sated. These results are highly relevant for understanding why some individuals are prone to overconsumption and highlight the importance of hunger state for reward sensitivity. The next step will be to use the Full4Health data to investigate age effects on reward processing and how these may be affected by hunger state and BMI.

The first results pave the way for follow-up publications on how age, hunger state, BMI and appetite hormones impact on low- and high caloric food choice in the brain. Complementary data are available for food viewing-related brain responses, which will deepen our understanding of neural differences in food anticipation. The potential of these data is confirmed by the first results, summarized above. They are highly relevant given the abundance of food cues in our environment and may provide insights into how to prevent snacking behaviour in specific target groups such as children and teenagers. This is complemented by data on the monetary reward task performed during hunger and satiety, which taps into 'general' reward system functioning.

Through analysis of our complete data set, we will advance the understanding of 'hedonic' vs 'energostatic' control of eating in adults, across middle age and elderly, by the combination of fMRI data, gut hormone concentrations and explicit liking/wanting to identify these influences in appetite during a fed (satiated) and fasted state. Further, in the children (with no biological samples), we will investigate if there is a 'hedonically susceptible' phenotype characterised by liking and wanting of food, and if this relates to enhanced brain activation during fMRI scanning. Further, we will investigate whether ageing has an impact on brain activation or if appetite/satiety cues remain dynamic and intact, and responsive to eating.

WP2: Food hedonics, hunger/satiety and appetite regulation in exercised-induced compensatory eating

Exercise training gives rise to a variable degree of body weight and fat mass loss, and is associated with individual differences in appetite control (subjective hunger, food choice and energy intake). Such variable outcomes can undermine the public health message that stresses the importance of physical activity as part of a healthy lifestyle, and a number of unhelpful 'myths' have grown up around the relationship between exercise and weight change, which may discourage participation. Gut hormones released during and after eating are known to influence appetite but their role in exercise-induced compensatory eating was previously unknown.

***Key finding 6F**:* Exercise-induced changes in fat and fat-free mass define 'Responders' and 'Non-Responders' – indicating differences in energy compensation.

Overweight and obese individuals completed a 12 week aerobic exercise programme and were classified as Responders or Non-Responders depending on net energy balance from observed compared to expected body composition changes from measured energy expenditure from exercise. Responders and Non-Responders were distinguished by hedonic response to high fat/low carb food: Non-Responders had greater liking and wanting for high fat/low carb vs low fat/high carb before exercise training and reduced after exercise; and Responders showed equivocal liking and wanting for high fat/low carb or low fat/high carb foods before exercise and increased liking but decreased wanting after exercise – this is a possible novel marker of improved appetite control.

***Key finding 6G**:* No gender effect on compensatory changes in appetite, food hedonics or gut peptides in response to exercise-induced energy deficit.

Men eat more than women but this is fully accounted for by differences in resting energy requirements. There were no differences in compensatory changes in appetite, food hedonics or gut peptides in response

to exercise-induced energy deficit according to gender. Gender is not a barrier to exercise for improvements in body composition.

***Key finding 6H**:* Responders exhibit a more sensitive appetite control system than Non-Responders, pre- and post- exercise intervention.

Exercise training improved fasting insulin and acylated ghrelin response irrespective of weight loss/body composition response to exercise response. Leptin, total ghrelin and insulin also appear to be biomarkers for changes in body composition in response to prolonged exercise, but are markers for changes in body composition rather than exercise training per se. Responders showed several markers of a more sensitive appetite control system than the Non-Responders pre and post intervention. Therefore episodic postprandial peptide profiles appear to form part of the pre-existing physiology of Responders compared to Non-Responders, and may explain the differences in satiety potential underlying exercise-induced compensatory eating.

Exercise training is associated with improvements in appetite control and metabolic health independent of changes in body composition. Early identification and support for those with enhanced hedonic preference for high energy dense food and reduced postprandial peptide responses to food could be necessary to facilitate exercise-induced fat loss in these individuals.

General Objective 7: To establish the relative importance of dietary protein content in determining energy intake.

Protein is the most satiating macronutrient and high protein diets are widely recognised as being effective in supporting weight loss due to the consumer being able to eat to appetite but at a lower caloric intake. High protein diets are also effective in regimes designed to sustain reduced body mass achieved through caloric restriction (e.g. Diogenes FP6 study). A protein content manipulation is at the heart of the multiple age group study described under Objective 6, and dietary protein is also addressed in this Objective in a series of related studies that begin with consideration of the protein leverage hypothesis. The effect of a high protein meal on the undernourished elderly is also considered.

***Key finding 7A**:* The protein leverage hypothesis was not confirmed.

Independent of age, gender, BMI, and type of protein, only under-eating on an *ad libitum* high protein diet was confirmed, but overeating on an *ad libitum* low protein diet did not occur over 12 days in a controlled situation, nor over 1 week in the free-living situation. Over 12 weeks, habituation to the satiating properties of protein occurred: neither over-eating nor under-eating was observed on a high protein diet in the field situation. At a stable body-weight, the initial satiating effect of a high protein diet is transient after 12 weeks, after which dietary protein content does not determine energy intake. Consequently, the hypothesis that protein intake is regulated more strongly than energy intake was not confirmed. Although evidence was found for individuals under-eating relative to energy balance from diets containing a higher protein-to-carbohydrate + fat ratio, there was no evidence of over-eating from diets containing a lower protein-to-carbohydrate + fat ratio. These observations were independent of age (between 18 and 70 y), gender and BMI (between 18 and 34 kg/m^2), and type of protein (19, 20).

***Key finding 7B**:* A low-protein diet for 12 days does not result in negative nitrogen balance, and there is no compensation for insufficient indispensable amino acid (IAA) intake.

The background of the protein leverage hypothesis, especially the hypothesized overeating on a low-protein diet, is anticipated compensation for a negative nitrogen balance, or for indispensable amino-acids. We found that, independent of age, gender, and BMI, nitrogen balance was maintained on the 5% protein diet, and was positive on the 15% and 30% protein diets over 12 days. The 5% protein diets did not provide

the amount required to meet the calculated minimal IAA requirements, but IAA were sufficient in the 15% and 30% protein diets. Lack of compensation for a low-protein diet coincides with a maintained nitrogen balance and with lack of compensation for IAA (21).

***Key finding 7C**:* Maintenance of energy expenditure on high-protein vs. high-carbohydrate diets at a constant body weight may prevent a positive energy balance.

Relatively high-protein diets are effective for body weight loss, and subsequent weight maintenance, yet it remained to be shown whether high-protein diets would prevent a positive energy balance. Over 12 weeks at a constant body-weight, total energy expenditure, sleeping metabolic rate, and diet-induced thermogenesis appeared to be higher on a high protein:low carb diet than a high carb:low protein diet. Energy balance was maintained on the high protein:low carb diet but became positive on the high carb:low protein diet. Maintenance vs. decrease of energy expenditure on a high-protein vs. high-carbohydrate diet at a constant body weight may prevent a positive energy balance (22).

***Key finding 7D**:* A high protein-low carbohydrate diet may preserve intrahepatic triglyceride content in healthy humans.

Since protein supplementation has been shown to reduce the increases in intrahepatic triglyceride (IHTG) content induced by acute hypercaloric high-fat and high-fructose diets in humans, we assessed the effect of a 12-wk iso-energetic high protein-low carbohydrate (HPLC) diet compared with an iso-energetic high carbohydrate-low protein (HCLP) diet on IHTG content in healthy non-obese subjects, at a constant body weight. IHTG content changed in different directions with the HPLC compared with the HCLP diet, which resulted in a lower IHTG content in the HPLC compared with the HCLP diet group after 12 weeks (23). These findings suggest that a HPLC vs. a HCLP diet has the potential to preserve vs. enlarge IHTG content in healthy non-obese subjects at a constant body weight.

***Key finding 7E**:* Prolonged adaptation to a low or high protein diet does not modulate basal muscle protein synthesis rates.

A higher dietary protein intake causes a positive protein balance and a negative fat balance. A positive net protein balance may support fat free mass accrual. To assess the impact of prolonged changes in habitual protein intake we measured changes in whole-body protein turnover and basal muscle protein synthesis rates following 12 weeks of adaptation to a low versus high dietary protein intake. After 12 weeks, whole-body protein balance in the fasted state was more negative in the high protein treatment than in the low protein treatment. Whole-body protein breakdown and synthesis were significantly higher in the high vs low protein group. Basal muscle protein synthesis rates were maintained on a low vs high protein diet (24). In the overnight fasted state, adaptation to a low-protein intake (0.4 g/kg/d) does not result in a more negative whole-body protein balance and does not lower basal muscle protein synthesis rates when compared to a high-protein intake. The positive protein balance on a high-protein diet is only due to the postprandial positive protein balance, in the fed state.

***Key finding 7F**:* The effects of dietary protein are dependent on energy balance.

The favorable effects of relatively high protein diets in negative energy balance, are due to sustained satiety, sustained energy expenditure, and preserved fat free body-mass. This effect is independent of a dietary 'low-carb' content (25). In a positive energy balance, a high-protein diet contributes to risk of weight gain (25). The potential impact of these findings is that although a low-protein diet is unfavourable, the body can cope with it for at least 3 months. This is primarily based on the protein-sparing effect of a very accurate protein balance. Then, with little protein synthesis, protein breakdown is kept at a minimum. Moreover, during the first 12 days, no negative nitrogen balance occurs. Contrary to expectation, a low-protein diet does not cause overeating per se. On the other hand, a slightly elevated protein diet in a condition of constant body-weight is favourable for prevention of overweight and prevention of liver fat accumulation. This is due to the effect

of protein metabolism, causing at least a maintained, or slightly higher energy expenditure, and not due to the expected effect of a higher satiety. The latter short-term effect is transient in time scale, which explains the prevalence of obesity in countries with high habitual protein intakes, such as Australia.

Key finding 7G: A high protein breakfast could be especially beneficial to the undernourished elderly.

Data collected in elderly subjects address a key nutrition area on undernourished elderly, where optimizing protein and calorie intake can be a challenge with poor appetite associated with ageing. We observed that undernourished elderly do not detect a protein-calorie surplus as a breakfast meal and this had no impact on subsequent calorie intake later the same day. Thus, a high protein, high calorie breakfast meal, in a diary liquid format, was beneficial at maximizing nutrition quality in a group of undernourished elderly.

Full4Health ROADMAP

(This document was also uploaded as a separate PDF).

We have put together a summary of highlights of Full4Health, indicating key outcomes and future perspectives.

Full4Health OUTCOME: **Early life and gut hormones**: We show that the gut hormone, ghrelin, is required for normal development of brain energy balance circuits in very early life, and ghrelin function is inhibited by neonatal overnutrition.

PERSPECTIVE: With large numbers of women entering pregnancy overweight or obese, and childhood obesity at epidemic levels, new therapeutic opportunities need to be developed to ameliorate the metabolic abnormalities associated with neonatal overnutrition.

Full4Health OUTCOME: **Food-gut-brain and life course**: We have collected a unique dataset with comprehensive measures across age groups, body mass index and gender, comparing hunger and satiety, and hormone responses to protein-energy dietary challenge. This includes the first comparison of food-related brain responses between age groups.

PERSPECTIVE: Physiological and behavioural factors influencing appetite across the lifespan will be useful to target specific interventions in the future, such as in the elderly. Brain responses to food cues in the environment may provide insights into how to promote healthier choices to specific target groups, e.g. prevent snacking behaviour, such as in children and teenagers.

Full4Health OUTCOME: **Alternative to obesity surgery**: Using pre-clinical models, we have examined the mode of action of established obesity surgeries and possible treatments targeting the vagus nerve, such as vagal blocking therapy, and have taken endoscopic gastric injection of Botulinum toxin A into the clinic for the adult obese.

PERSPECTIVE: Invasive gastric bypass surgery is inappropriate for children and adolescents who are still growing. There are currently no viable therapeutic options to address this need, and a clinical trial of Botulinum toxin A injection in obese adolescents is proposed.

Full4Health OUTCOME: **Exercise and energy balance**: In a breakthrough in understanding individual variability, we have defined population groups that respond differently to exercise - responders and non-responders - based on exercise-induced changes in body fat. This indicates differences in energy compensation. There were no gender related differences in response to exercise-induced energy deficit.

PERSPECTIVE: We have generated important public health messages:

- Exercise training is associated with improvements in appetite control and metabolic health independent of changes in body composition.
- Gender is not a barrier to exercise for improvements in body composition.

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Potential Impact and main dissemination activities and exploitation results

(This document was also uploaded as a separate PDF).

Strategic impact

Impacts

At the outset of the project, it was anticipated that the over-arching **high-level impact** of the Full4Health project would be in the application of the additional insight that would be gained into the "***mechanisms of the neurological and neuroendocrine pathways regulating hunger/satiety***" across the integrated food-gut-brain signalling system. The expansion of the knowledge base in this area would contribute to translational opportunities for the food and dietary supplement industries, and potentially for the pharmaceutical sector also, and would have clear implications for **public health policy**, and **wealth creation**.

Update: The Full4Health consortium has made substantial progress in increasing the evidence base in food-gut-brain signalling as it relates to hunger and satiety mechanisms. We have sought to maximise the impact of this research through a range of dissemination and exploitation activities targeted at a range of stakeholders and end users. The coordinator and members of the consortium have been active in promoting the plans of the project (in its early stages) and (latterly) its scientific outcomes to fellow scientists, members of the food and drink and pharmaceutical industries and to policy colleagues and relevant NGOs, such as at the Platform for Action on Diet, Physical Activity and Health. These activities will continue beyond the end of the project duration, which will be important as the final data analysis becomes available from the bigger human intervention studies, which have clear translational potential. Much of the dissemination activity has been conducted in close collaboration with the aligned FP7 project, SATIN, which is pursuing food reformulation for enhanced satiety using exclusively clinical and *in vitro* approaches. The S&T report identifies areas where improved mechanistic insight into hunger and satiety would be of potential value to different sectors of industry, and these are relevant to this update: pharmaceutical/nutraceutical – where knowledge of signalling and receptor systems may identify drug targets in the brain or gut of relevance to homeostatic or hedonic feeding, or for induction of enhanced satiety hormone secretion; food and drink – where characterisation of hunger and appetite processes in identifiable sub-groups of the wider population could be the stimulus for targeted food reformulation for enhanced satiety or sustained appetite. Similarly, although it is recognised that much of the research in the project is too technical and mechanistic to be directly applicable to public health issues, there are outcomes, mainly clustered around the human intervention trials which will generate relevant public health messages. These include the improved public buy-in which is likely to result from more phenotype-specific dietary and behavioural advice, and the physical activity messages.

A food based approach: Full4Health should make a significant contribution to **"*preventative strategies for the European population*"** to limit further growth in the prevalence of obesity and its related complications, and through preventative and therapeutic development contribute to a ***"reduction in the chronic disease burden"***. Its potential to ***"support European public health policy"*** will be predominantly via food reformulation and consumer-based, rather than clinical, approaches to the detrimental effects of excessive food consumption on long-term health, allowing consumers to take responsibility for their own health. Simultaneously, it will generate opportunity for **"*increased competitiveness of European food industry through the development of new food products*"** and dietary supplements.

Update: The consortium recognises that viable prevention strategies to reduce the chronic disease burden (mainly due to over-consumption of food) require a degree of tailoring of approach to the individual that has been lacking hitherto. One size or 'one diet' will not fit all, and requirements and approaches will be very different in children and adolescents and in the elderly. This has been addressed head-on in our multi-centre dietary intervention, where similar protocols have been applied across the lifespan, with food culture and individual phenotype emerging as being important considerations when defining and designing

foods to promote within-meal satiation and between-meal satiety. This is a fundamental finding of these novel data. Improved understanding of how to manipulate appetite to avoid or treat obesity, or respond to clinical malnutrition, will not only inform public health policy but also support innovative NPD in the food and drink industry. Public health advice and food strategies need to be tailored for specific phenotypes to generate a sustainable and healthy approach for appetite control. With this in mind, several consortium members already have a strong track record of interaction with the food industry, and the foundations are therefore in place to promote the findings of Full4Health and maximise the societal benefit. Full4Health data will be showcased at the Vitafoods meeting in May 2016.

(i) Critical mass to strengthen European research capacity

Tackling a large scale multi-faceted integrating project on "mechanisms of hunger, satiety and gut behaviour" requires a truly **multi-disciplinary** collaborative team approach, which we have achieved, that combines specific expertises and skill sets from across a spectrum of bioscience and medical disciplines.
Full4Health will **strengthen European research capacity** and project the consortium into a position of international prominence. In so doing, we will recruit, mentor and nurture a cohort of outstanding young bioscientists from different backgrounds into the Full4Health project.

Update: The consortium assembled to address the original funding call on mechanisms of hunger and satiety brought together researchers who had worked together in earlier projects and thus had prior experience of working in large multi-disciplinary groups but also others for whom it was a first experience of such collaboration. Accordingly, we have strengthened European capability in this area, and provided lead scientists with the opportunity to interact with scientists from other disciplines and other institutions. This has already resulted in a number of additional multi-disciplinary research funding applications involving consortium labs that had not collaborated prior to Full4Health. Throughout the programme we have also recruited and nurtured a cohort of Early Career Researchers (ECRs) at pre-doctoral and post-doctoral level, and provided them with an unrivalled opportunity to be part of and interact within a consortium containing a number of internationally prominent scientists from complementary research fields, thereby widening horizons. From the outset we invested considerable time and thought into making the ECR experience within Full4Health as interesting and worthwhile as possible, and included an ECR rep in the Project Steering Group in order that any concerns or ideas could be formally fed back to the project management. Support for ECR development included facilitating a very successful summer school "Food for Thought" close to the mid-point in the project and supporting mobility between labs both within and beyond the consortium. Details of training and mobility for Full4Health ECRs can be found below, along with feedback from the ECRs themselves.

(ii) Advance in application of cutting-edge technology

Full4Health is designed to actively pursue key areas where mechanistic understanding is, at present, deficient, utilising cutting edge technologies. Experimental refinement of such techniques and application in mechanistic studies of hunger and satiety will advance science within the wider scientific community. A particular focus will be the integration of **functional neuroimaging** (BOLD fMRI) with **measures of food hedonics** using procedures to measure liking and wanting in response to a battery of visual food stimuli systematically varied in their nutrient and sensory characteristics. **Stimulus administration in the scanner environment** will enable Full4Health scientists to establish how the brain's responses to foods changes with age.

Update: As set out in the original proposal, the Full4Health project deployed state-of-the-art methodologies routinely, and cutting-edge technology as appropriate in its clinical and pre-clinical studies. Examples of such field-leading approaches are given below.
To investigate the effects of hunger and satiety on the cerebral responses to food presentation and food choice across three geographic locations, a large set of standardized food images was produced for use as a food choice task by subjects in the fMRI scanner. Food viewing is the most widely used paradigm to study food-related brain responses with functional MRI. The food choice task developed and used in Full4Health

is unique because low- and high calorie foods are individually matched on liking. Thus, any differences in brain activation are really due to caloric content and not to differences in preference. The unique food choice fMRI data that has been collected within Full4Health will allow us to establish in more detail how food choice is modulated by hunger, weight status and age, independent of liking. This will advance our understanding of the implicit neural drivers of food choice, which may provide knowledge on how to promote healthier food choices in different populations. To our knowledge we are the first to have investigated the effects of hunger state and weight status (BMI) on monetary reward anticipation in normal weight and overweight adults. The dataset is unique in the different types of measures that are combined and in terms of the age groups examined together, extending from children to elderly, and the comparison of hunger and satiety. To date, food-related brain responses have not been compared systematically between age groups.

Technical innovation was also applied in pre-clinical studies, where optogenetics gave us the ability to observe the effects of neuronal modulation on complex behaviours such as appetite control and reward-related behaviour, and understand the interplay between neural and hormonal control of these behaviours. We used optical neuroengineering technologies to understanding gut-brain interactions, developing a set of minimal promoters to drive viral expression globally in neurones and also in specific ghrelin-sensitive neuronal populations involved in appetite control. We refined this approach by using a transgenic rat model where viral expression was confined to neurones known to be critically involved in reward and motivated behaviour. Using these technologies, we achieved *in vivo* optical control in anaesthetised rats of ghrelin-sensitive hypothalamic neurones and dopamine neurones of the midbrain reward pathway. We also introduced canine adenovirus as a tool to deliver a recombinase enzyme (cre) retrogradely (1). This allows neurons projecting to a specific site to be brought under the control of chemo- or optogenetics. We extended our findings by recording from ghrelin-sensitive dopaminergic neurons during food seeking behaviour. Specifically, we combined optical, imaging, and viral techniques to record somatic calcium transients from genetically identified neurons in freely moving animals. This technique allows visualisation of calcium activity *in vivo* and permits a better understanding of the real-time activity of specific neurones during eating behaviours. Our work on dopamine neurones also led to the discovery of a brain region not previously reported to be involved in food reward, the supramammillary nucleus.

(iii) Inter-disciplinary/cross-departmental linkages

The mechanisms of hunger and satiety and their downstream consequences for energy balance, body weight and chronic disease interact at a number of different levels in biological terms and at the level of the individual, but also have obvious social, economic and medical implications that are all interrelated. Consequently, it will be important that the Full4Health scientists and their advisors take a consciously **'joined-up' approach** to the project at all points in its evolution and actively engage with stakeholder and policy groupings wherever their interests intersect with the negative repercussions of over- or under-consumption, or the potential benefits of interventions of all sorts, be they dietary recommendations, novel foods, supplements etc.

Update: Periodically during the project we discussed with members of our Advisory Board and with key stakeholders such as European parliamentarians how best to promote the project and ensure that outputs reach the best audience and are communicated effectively. From this engagement, and recognition that research outcomes that might be of most interest to one stakeholder group might not be of interest to another, we developed a strategy of promoting the project to identified stakeholder groups by taking the project to meetings where the desired audience would already be in attendance or targeting information to these audiences through publication in sector media outlets. Utilising this strategy, we have engaged with and plan further engagement with policy makers and consumer representatives, food and drink industry representatives and different scientific audiences, enabling us to reach across disciplinary boundaries.

<u>Training and exchange of Early Career Researchers</u>

To facilitate career development, Full4Health has sought to help young researchers develop to their full potential. Part of this activity has involved a considerable amount of training. The research environment of all participating groups has supported this process via local training activities, courses and direct supervision from highly skilled and trained scientists associated with the project. Moreover, we have supported exchange visits for young researchers to other research groups involved in the project and beyond. A list of these exchange activities is included below. This not only provided added value for the project through increased collaboration but also provided increased training in new techniques and know-how from leading researchers in the field. Full4Health engaged successfully with the mobility aspect of ECR training, as evidenced by the activities below.

An ECR from UCAM, together with the UNIABDN Quality Assurance (QA) Manager conducted two QA audits of the capsule production facilities at Encap in Livingston, Scotland in 2013 and 2015. This ECR also visited UNIABDN to discuss specimen analysis. Two ECRs from UNIABDN visited HUA, Athens in 2012 to discuss the human study in WP1. An ECR from UMCU also visited Athens in 2013 to see the fMRI facilities and to carry out pilot scans. An ECR from WUR visited the University of Barcelona (outside the Full4Health consortium) for 2 months to gain expert training in conducting *in vivo* experiments. An ECR from UKK visited UCAM for 3 months' training in gut peptide analysis and an ESR from NTNU visited UMCU for 1 week's training in *in vivo* electrophysiology and vagal nerve stimulation.

A very successful Full4Health Workshop: "Measuring Gut Hormones" was organised in Copenhagen in August 2012. Professor Jens Holst of the Panum Institute (Beneficiary 13 UCPH), a world leading expert in the field of gut hormones, introduced the field and its problems. His presentation was expertly complemented by PhD student Monika Bak from his group, who shared her extensive studies of commercially available gut hormone measuring kits. Dr Dan Crabtree of the Rowett Institute of Nutrition and Health, University of Aberdeen (Beneficiary 1 UNIABDN) gave an overview of the blood and saliva sampling protocol then about to be initiated in the Full4Health project. The workshop allowed researchers who work on different aspects of the project to recognize putative problems in sample generation, measurement and analysis, and organize procedures accordingly. Through the interdisciplinary exchange the team resolved a strategy to accommodate the scientific and logistic problems posed by the substantial numbers of samples to be generated during the lifetime of the project. The workshop furthermore provided an excellent opportunity for early stage researchers to network, initiate and develop working contacts and exchange ideas.

At the final consortium meeting held in Chania, Crete in September 2015, we asked the ECRs to reflect on the overall management of the project and training opportunities that had been available to them during the course of Full4Health and to provid feedback on this.

They reported that the best aspects of the project included training visits to other laboratories, the Alison Douglas Summer School (discussed in more detail in Dissemination), being able to contribute to report writing and research briefs in newsletters, gaining insight into the workings of the EU, with a view to applying for future funding, and the fact that it was a well organised project.

Some of the other aspects which were less appreciated by them included the fact that the PhD students felt they had less flexibility in their projects because of the need to fulfil specific predefined deliverables. They also felt there was a lack of ECR leadership and that communication was not so good, especially for those ECRs who came to the project some time after it had started. For example, some of the ECRs arriving later in the project did not know there was a regular newsletter, and although a second ECR conference was proposed, it did not materialise. The problems due to lack of ECR leadership may have been mitigated by the fact that there were other career development opportunities for young scientists within their own countries.

DISSEMINATION

A broad range of dissemination activities have been undertaken in Full4Health, with input from all partners, using a variety of media to engage different stakeholder groups. These numerous activities have been highlighted as News on the project website and are included in the dissemination log in the ECAS database. The number of activities recorded stands at 572, including presentations, interviews, TV and radio broadcasts, blogs, posters, media briefings and articles in the popular press.

Website

full4health.eu

The Full4Health website was quickly established at the start of the project, with two interfaces. The Consortium were provided with project-related information via a password-protected intranet and a public site was available for different stakeholders to access appropriately targeted project information. The website has evolved throughout the project and been regularly updated with Newsletters, Publications, News, including news of the successful defence of PhD theses, meeting reports and highlighting public engagement activities. We recently added a new "Science News" section to highlight project outputs as Full4Health comes to a close. The Consortium benefits from the intranet, which is an essential project repository containing uploaded Periodic Reports, Meeting Reports, Publications, Deliverables and Template Documents.

Research Briefs

Research Briefs were written by a number of consortium members on a wide range of subjects within the remit of Full4Health. These are available to download from the Full4Health website, as standalone documents, but were also incorporated into the regular Newsletters which were produced during the course of the project. They covered different areas within Full4Health and were written to be accessible to scientific and policy colleagues, media, interested members of the public and other stakeholders.

The five Research Briefs were entitled:

- "You are what your mum ate" – by Dr Sebastien Bouret (UL2)
- "Why is eating so rewarding?" – by Dr John Menzies (UEDIN)
- "Eating on the run" – by Dr Graham Finlayson (UNIVLEEDS)
- "Do the bugs in your gut influence the hunger responses of your brain?" – by Professor John-Olov Jansson (UGOT)
- "Gut feeling" by Nikki Cassie (UNIABDN)

"Food for Thought" - Alison Douglas Summer School

In July 2013, the 1st Alison Douglas Summer School was held in Bavaria, Germany at the Frauenwörth Abbey. Professor Alison Douglas was Chair of the British Society of Neuroendocrinology (BSN) for two years until her untimely death, and was hugely committed to the career development of ECRs, also an important component of EU-funded grants. The Alison Douglas Summer School was instigated to commemorate her life and commitment and it was entirely fitting that the event was funded by the BSN and EU-funded projects including Full4Health, NeuroFAST, EUROCHIP and MAITRE. The event, whose overall science theme was "Hunger and Satiety" was attended by 32 ECRs from across Europe (not all of whom were involved in EU projects, and some of whom had discovered the Summer School through their own on-line research, and were self-funded), 16 members of faculty (FP7 project members) and 2 journalists from FP7 project MAITRE, who ran a media training workshop. A mix of networking events, interactive workshops based around topics including future EU funding, the future of publishing and an industry perspective made for a diverse and hugely enjoyable event. The School ended on a high note with a "Dragon's Den" style event based on funding proposals prepared by groups of ECRs, and judged by the

faculty. The Summer School was hailed a great success by all those who attended and set a high standard for future Summer Schools.

Conference presentations

Full4Health scientists at all levels have presented their research findings at a wide range of national and international scientific conferences throughout the 5 years, covering topic areas including obesity, food-gut-brain interactions, appetite regulation and gut hormones, bariatric surgery, satiety and appetite control, and how it may be affected by exercise, psychological aspects of feeding behaviour and food reward to name but a few. Oral and poster presentations, especially those highlighting ground-breaking findings, often appeared hand-in-hand with press releases, radio and TV interviews and podcasts, enabling wide dissemination to a variety of stakeholders.

Public engagement

The consortium as a whole has been enormously active in the area of Public Engagement throughout the 5 years of the project, including participation in international events such as the British Science Festival and European Researchers' Night, including the very successful Explorathon 2014. The diversity of activity is reflected in the following selected examples: a PhD student taking part in the Edinburgh Fringe Festival mixing research with comedy; a TV programme on how the genetics of one family put them at risk of excessive weight gain; a PhD student in a national research contest describing the reduction in appetite and thus body weight achieved by blocking the vagus nerve; a TV series based on profiling gut hormones in different individuals in order to personalise diets to minimise weight gain. As a consequence the science of Full4Health reached millions.

Policy Engagement

The EU Platform for Action on Diet, Physical Activity and Health was identified in the original funding call as one of the main stakeholder groups with which the project should seek to engage. This is a forum for European-level organisations, ranging from food industry representatives to consumer protection NGOs, committed to tackling issues in diet and physical activity. Its emphasis is on prevention of chronic disease through dietary and lifestyle change. Contact between the Full4Health coordinator and the Commission, over a few months led to an invitation to produce a briefing document for the 'Platform' meeting in September 2012. This document promoted the Full4Health, NeuroFAST and SATIN projects, and was incorporated as a research highlight into the 'What is new?' section delivered by Philippe Roux, Deputy Head of Unit, European Commission, DG Health and Consumers, Health Determinants at the meeting. Subsequently, following an intervention from the Full4Health Project Officer at the Commission, Ms Isabelle de Froidmont-Görtz, the Full4Health co-ordinator was invited to address both the EU Platform for Action on Diet, Physical Activity and Health and the High Level Group of Member States on Nutrition and Physical Activity (European Government Representatives) at their adjacent meetings in February 2013 Again, the presentations were on behalf of the three projects, but were more focused towards Full4Health and SATIN, and had the title 'Research targeting food reformulation in the regulation of hunger and satiety'.

Full4Health sought to re-engage with the Platform at a meeting in September 2015 and although the meeting was focused on food reformulation, Full4Health was not included and only SATIN was able to take part. However the intention is still that Full4Health will present to the Platform in 2016, although this will be beyond the duration of the project.

Industry Engagement

Individual laboratories and PIs within the Full4Health consortium already had effective relationships with industry contacts in appropriate sectors, be they pharma, food and drink or nutrition based, prior to the start of the project. The presence of industry partners within the consortium and PIs with experience of working in industry further strengthened communication channels, and the Full4Health project was promoted directly or indirectly to industrial scientists in a variety of fora, be they specifically targeting this stakeholder group e.g. Food Matters Live, Vitafoods, or more generic meetings where industry colleagues would also be present e.g. World and European Nutrition meetings. Accordingly, and as noted in Periodic Reports and Deliverables reports, there has been active but appropriate dissemination of project findings to industry stakeholders at both specific and general levels throughout the project and in planned dissemination events.

Other

Scientific audiences were engaged through a conference jointly sponsored by Full4Health and SATIN – Association for the Study of Obesity conference, "Satiety – From Origins to Application" in March 2015. A Full4Health symposium, "Gut-brain lessons for energy balance", will form part of the British Society for Neuroendocrinology Annual Meeting in August 2016. Dissemination at the latter events has been and will be supported by a number of journal articles published in the final months of the project, and designed for both general and specialist audiences – *Food Science and Technology* (the journal of the Institute of Food Science and Technology), *Nutrition Bulletin*, *Current Obesity Reports*, and a collection of original articles and reviews in a Special Issue of the mainstream journal, *Peptides* (Full4Health).

EXPLOITATION

Full4Health has generated a significant amount of new knowledge but as with most research projects based around fundamental mechanistic research, the lead-in time to fully exploit this could be considerable. It is hoped that our range of contributions to the evidence base in hunger, satiety and feeding behaviour at various levels will influence policy and practice and inform new drug targets and surgical or dietary therapies. Four broad categories of potential exploitation are outlined below.

Dietary approaches

A 'one diet approach' to the problems of overconsumption and resultant weight gain will not work. Differences between people (gender, body mass, age), and between countries, with their different food cultures, need to be taken into account in the identification and assessment of foods and diets designed to promote satiation and satiety. Our application of similar protocols across the lifespan in sixteen defined population study groups in contrasting European countries will underpin opportunity for tailoring food strategies to specific phenotypes with the goal of sustainable and healthy approaches to appetite control. We will actively engage with the food industry, using contacts that we have developed prior to and during the lifetime of Full4Health, and under the umbrella of the SATIN project, to assess how we can co-operate to design and test appropriate food products. The analysis of L-cells from mice fed on a high fat diet revealed the potentially negative impact of chronic dietary habits on gut hormone secretion, which if translatable to humans would suggest that consumption of a high fat Western diet would impair post-prandial satiety mechanisms and contribute to over-eating and weight gain. These findings could potentially find application as a pre-clinical screen of novel diets or dietary components.

Obesity 'surgery'

Gastric bypass is the gold standard treatment for morbid obesity in adults, but may not be appropriate for obese children and adolescents who are still growing. In assessing less invasive interventions that can achieve similar weight loss and clinical outcome based on blocking the gastric vagus nerve input to the brain, we have assessed use of Botulinum Toxin A (Botox). Injection of Botox into the stomach to block the vagus nerve was successful in preclinical studies and was tested with success in a clinical trial in adults. The

procedure only requires an out-patient appointment for injection by means of gastroscopy so the potential of this as a new obesity therapy is significant due to its lower cost in both clinical and financial terms. Transfer of this technology, which is naturally reversible with time, into child and adolescent patients could represent a major breakthrough, and funding is being sought for a multi-centre clinical trial in this area. We would envisage that uptake of this technology would be global if proven to be effective over reasonable time scales.

Drug targets

Our research has revealed much more of the fine detail of a range of signalling pathways specifically expressed in gut enteroendocrine cells, which contribute to the detection of ingested nutrients, including the inter-relationships of different cellular phenotypes and the molecular biology of their regulation. A number of these pathways are of translational interest and are currently under evaluation as potential drug targets for new treatments for obesity and diabetes. This sector is of considerable economic significance and is an active 'lead discovery' area for many pharmaceutical companies. The central integration of hunger and satiety signals and the reward based appetite systems also represent areas where ground-breaking findings could translate into drug targets, and several Full4Health 'CNS' scientists have strong links with pharmaceutical companies, to ensure this take-up where appropriate.

Public health policy

Within Full4Health we have collected a unique dataset with comprehensive measures across age groups, body mass index and gender, comparing hunger and satiety, and hormone responses to protein-energy dietary challenge. This includes the first comparison of food-related brain responses between age groups. A better understanding of physiological and behavioural factors influencing appetite across the lifespan will be invaluable in targeting specific interventions in the future, such as in the elderly. It will be important, for example, to assess whether ageing has an impact on brain activation or if appetite/satiety cues remain dynamic and intact and responsive to eating. Such outcomes from the Full4Health project should have a major impact on public health policy. Similarly, brain responses to food cues in the environment may provide insights into how to promote healthier choices to specific target groups, e.g. prevent snacking behaviour, such as in children and teenagers. Development of more bespoke interventions for specific population groups will likely increase buy-in from the consumer.

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