

APPLICATIONS FUNDED THROUGH DRINC 2nd CALL AUGUST 2008

The Role of Plant Cell Walls in Regulating Starch and Lipid Bioaccessibility from Plant Foods: In Silico, In Vitro and In Vivo Studies		
Peter Ellis	King's College London	£394,663.44
Martin Wickham	Institute of Food Research	£205,730.67
<p>Most people commonly eat plant foods rich in starch, notably cereal products (e.g. bread, rice), and also some that are rich in fat (e.g. tree nuts). However, little is known about how such foods release starch and fat in the human gut and how, in turn, this may influence digestion and ultimately the absorption of nutrients into the body. Improving our understanding of these processes is important for basic scientists studying the behaviour of foods in the gut and their effects on metabolism. It is also important for health professionals and policy makers that are worried about excessive food consumption and the growing problem of obesity and associated problems of heart disease and diabetes. Moreover, the rate and extent of starch and fat digestion and absorption into the blood stream are important factors in altering the risk of heart disease.</p> <p>The release of fat and starch from plant foods and the digestion and absorption of these nutrients by the body are highly complex processes. Our progress in understanding these processes is impeded by the hugely complex structure and properties of plant foods and individual nutrients. Our project proposal brings together a unique combination of world experts from different institutions and disciplines. These experts have formed a large team in order to improve our knowledge of how edible plants behave in the gut and how the gut reacts to the starch and fat available for digestion. For example, it is important to know about the rate at which nutrients are released from plant foods as they move along the gut, since this will affect the time course of digestion and absorption. This in turn will influence the way the nutrients are metabolised within the body.</p> <p>We currently study almond nuts and cereals, e.g. wheat, to see how fat and starch are released from plant tissues. Starch, fat and other nutrients are found inside numerous cells that make up the plant tissue, e.g. an almond seed contains about 50 million cells. Such cells are very small in size, often with a diameter of less than about one tenth of a mm. One significant factor that seems to affect nutrient release from plant cells is the presence of cell walls, more commonly referred to in nutrition as 'dietary fibre'. How starch and fat are released from these cells is poorly understood. Initial studies will involve examining the role of cell walls as physical barriers in controlling the release and digestion of nutrients, using various methods to examine plant tissue at a cellular scale. One novel method will be the use of a recently established 'Dynamic Gastric Model', a computer-controlled simulation of digestion in the human stomach. We will also feed human volunteers with the same plant foods rich in fat and starch, to determine the effects of processing and mastication on nutrient release and digestion and the rate at which digested nutrients are transported into the blood stream. Finally, we will also produce a mathematical description of how fat and starch are released from edible plant tissues during digestion. It is envisaged that in the future, the use of mathematics will allow research scientists to predict the behaviour of similar foods in the gut without having to do so many laboratory experiments.</p> <p>This work will help the food industry to produce new food products or ingredients that have a controlled release of starch and fat in the gut, which could, for example, help to reduce the risk of heart disease. Indeed, Premier Foods, a large food manufacturer, has agreed to collaborate with us and provide scientific and technological expertise. Premier Foods has also agreed to provide cereals (e.g. wheat) and food products made with these cereals, all of which have been specially prepared to control starch release. These raw materials and food products will be used in our project to study how they behave in the gut and assess their potential benefits in reducing the risk of heart disease.</p>		

<p>Increased Propionate Production In The Colon Is Associated With Reduced Appetite, Body Weight And Improved Insulin Sensitivity</p>
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Obesity is the greatest public health challenge facing most developed and many developing countries. Obesity is directly related to increased mortality, causing 600 premature deaths in the UK per week. In the current obesity epidemic, using therapeutic foodstuff to tackle obesity is potentially a would be economically viable for industrial partners.

Recent epidemiological and experimental studies link the decline in consumption of non digestible carbohydrates (NDC) to the rise in obesity. NDCs are not broken down in the small intestine, but can be fermented by bacteria in the colon, part of the large intestine. Previous studies and our own pilot data shows that increasing the NDC in the diet of animals and humans reduces appetite and body weight and increase insulin sensitivity. Unfortunately, the high doses required to produce these effects are unpalatable and result in side effects, limiting the use of NDC supplements as a treatment for obesity or diabetes. Short chain fatty acids (SCFA) are molecules produced by the fermentation of NDC in the colon and are responsible for the biological effects of NDC. Recently, a receptor has been found that binds SCFAs, and in particular the SCFA propionate. This receptor is found on cells in the large bowel where it stimulates the release appetite-inhibiting hormones, and on fat cells where it acts to decrease the release of free fatty acids. Reducing free fatty acid levels within the body increases the sensitivity of the body to insulin and thus reduces the effect of insulin resistance which is present in type 2 diabetes. Until now, controlling the production of propionate in the colon has been impossible. Both the type of NDC ingested and the gut microbiota of an individual dictate the levels and types of SCFA produced in the colon. Recently, we have developed a novel molecule in which propionate is bound to a carrier molecule. The chemical bond that links propionate to its carrier molecule cannot be broken down in most parts of the gut. However, in the colon, this chemical bond is broken by the bacteria present there, delivering specific amounts of propionate to the colon. We have shown that supplementing the diet of rats with this propionate carrier molecule reduces their body weight compared to controls, and that in humans it reduces hunger and food intake.

This study will determine the effect of 24 weeks diet supplementation with this propionate carrier molecule on appetite, body weight and insulin sensitivity in obese volunteers. We will test the hypothesis that supplementing the diet with propionate carrier molecule will reduce appetite through gut hormone release and improve insulin sensitivity by reducing the concentration of free fatty acids in circulation. Industry will have an important role in developing products which produce propionate in the colon to reduce appetite and improve insulin sensitivity. In collaboration with Leatherhead Food International we will design foods which can be used to supplement the diet of the general population with propionate carrier.

Demonstrating the link between colonic propionate production and appetite regulation has significant implications for public health given current trends in obesity rates. However, if colonic production of propionate increases satiety, then simply adding any NDC may not be sufficient to increase propionate production to a significant level to impact on satiety. This study will determine if colonic propionate leads to a reduction in appetite and body weight and cause beneficial metabolic change, and will demonstrate proof of principle for using NDC esters to deliver SCFAs to the large intestine. These data will therefore provide valuable information for future studies investigating the effects of SCFAs on appetite regulation and insulin sensitivity.

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Influence of prebiotics on human gut microbiota, LPS and markers of metabolic syndrome.		
Glenn Gibson	University of Reading	£586,984.97
<p>Obesity is fast becoming the greatest health challenge of the 21st century. Central to our ability to intervene in this situation is a clear understanding of the link between diet and obesity and the ability of industry to deliver food products capable of reducing risk. This project will lay down the scientific rationale linking the gut microbiota with obesity, and provide a rationale for using functional foods in the obese for improved health, targeted towards modulating the gut bacteria.</p> <p>Traditional risk factors for obesity and associated metabolic disorders are dietary, genetic and exercise linked. However, there is the contention that these cannot fully explain the explosive increase seen in recent years. This was given added significance in 2006 when the first reports appeared suggested that gut bacterial profiles in obese and lean persons differed. It was hypothesised that the bacterial profiles variably affected calorie extraction rates from food and that some of their metabolites could influence satiety. This is still an area of some debate (and it may be that the traditional risk factors themselves affect microflora profiles), however what is not in question is that the gut microbiota has a major role to play in human metabolism. This is because of the huge numbers that are present and their constant supply of nutrients (principally diet).</p> <p>Should the gut microbiota differences be a factor in obese related conditions, then this opens up the possibility of altering the situation by using dietary ingredients that have a selective fermentation in situ. Prebiotics are functional food ingredients that exert major composition and metabolic changes in the human gut microbiota. Together with probiotics, they are attracting much attention for their ability to improve gastrointestinal health. New products are constantly being developed, with the main remit being improved digestion and wellbeing. Research on modulating the gut flora has largely targeted gut infections including links into chronic disorders like IBS and ulcerative colitis. Given the recent link between gut microflora and obesity, it makes sense to research whether prebiotics can exert a modulatory role. In this project, these have been chosen over probiotics as they are more efficacious in exerting change in bacterial populations within the gut.</p> <p>Our collaborative research in animal models has shown that LPS, a cell wall constituent of Gram -ve bacteria, can exert a metabolic endotoxaemia which is characteristic of diabetes in humans. Prebiotics target Gram+ve flora (principally bifidobacteria). Further studies in the murine showed that the toxic effects of LPS could be reversed by repressing Gram -ves at the expense of bifidobacteria.</p> <p>Here, we aim to replicate these experiments in humans at risk of metabolic syndrome. We will use a proven prebiotic (BiMuno, a type of galactooligosaccharide GOS), that was originally developed in our laboratories and is now commercially available, to target bifidobacteria in a human feeding study. Prior to this, food quality expertise will be applied towards assessing the most appropriate dietary delivery vehicle that maintains functionality of the ingredient and has optimal sensory qualities. A placebo will also be trialled. The outcomes will be an in vivo assessment of the capacity to alter gut microbiota and concomitant effects upon validated markers of metabolic syndrome.</p> <p>If the research is successful it will lead towards a dietary intervention product that positively affects the risks associated with obesity in a manner that changes the microflora influence. This would have much impact upon a major 21st century dietary problem and tally that with an extremely topical functional food approach. The outcome would also partly inform on the health attributes that can be expected from altering a person's gut microbiota and has relevance for both the probiotic and prebiotic industries.</p>		

Effects of Fruit Juice Processing and Human Metabolism on the Functionality of Anthocyanins for Cardiovascular Health		
Colin Kay	University of East Anglia	£290,952.70

Paul Kroon	Institute of Food Research	£74,849.19
Nigel Botting	University of St Andrews	£163,798.39

People who consume the highest quantities of fruits and vegetables appear to be more protected against heart disease than those who consume lower quantities. Evidence suggests that this protective effect is in part the result of substances in the fruits and vegetables called polyphenols. In recent years, berries and berry derived juices and wines have been promoted as especially healthy foods as they are high in a particular class of polyphenol called anthocyanins. These anthocyanins are reported to have activities that benefit the heart and blood vessels.

Cardiovascular diseases (CVD) and specifically stiffness of the arteries results from accumulated damage to blood vessel walls. There is a single layer of cells that lines the blood vessels which is sensitive to agents/compounds within the blood. When this layer is damaged as a result of injury or chronic disease, it loses its ability to maintain normal blood vessel function and becomes prone to processes that lead to heart disease. Anthocyanins and anthocyanin containing foods have been shown to have direct protective effects on this cell layer, thus restoring proper function to the blood vessels. However, the anthocyanins in the foods we eat often become altered during standard food processing and storage conditions, an effect that is believed to negatively alter their function relative to those in raw fruits or vegetables. As well, when we eat anthocyanins they become modified by our bodies, resulting in drastic changes to their original form. Previous experiments have used unaltered or original forms of anthocyanins to explore how these compounds affect the cells in our bodies and blood vessels. However, no studies have explored the true activity of anthocyanins as they exist within our bodies, as altered products in our circulatory system resulting from changes during processing and digestion. The effects of these alterations on the disease fighting properties of anthocyanins are currently unknown and could be greater, different or impartial to what we currently perceive.

The aim of the present program of research is to identify the actions of pure anthocyanins relative to their altered products of processing and digestion on CVD risk. In order to determine their functions, we must first identify their forms in the body after we eat them. We will identify changes that occur to anthocyanins (cyanidin-3-glucoside, the most abundant anthocyanin in nature) in common fruit juices on the UK market, during standard processing and storage conditions. We will also feed human participants a pure anthocyanin (cyanidin-3-glucoside) in order to trace its path and alteration through the body. We will then study the effects of the identified compounds on CVD risk by exploring their activities on the cells (cultured-cells) lining our blood vessels.

With this study we hope to prove the usefulness of anthocyanins as a dietary treatment for the prevention of cardiovascular disease, using the relevant compounds found in the body; thus providing informed advice on the health benefits of anthocyanins. The results of this study are also relevant to agricultural industries as levels of anthocyanins in food crops can be easily increased using breeding strategies and pre and post harvest manipulation. This project is particularly relevant to the processed food and beverage industry, as although the alteration of anthocyanins during food processing has generally been considered of negative consequence, the proposed research could establish this as a neutral or potentially beneficial outcome; providing valuable evidence to support the use of fruit juices for the delivery of beneficial components for health. This proposal will also generate findings that may be useful for future studies aimed at investigating the relative activity of other dietary polyphenols, such as those found in coffee, tea, wine or chocolate.

Impact of non-digestible carbohydrates on biomarkers of GI health: a human intervention study		
John Mathers	Newcastle University	£396,081.22
Ian Johnson	Institute of Food Research	£147,950.71

What we eat affects the health of all parts of the body including the gut. Symptoms, disorders and diseases of the large bowel are major causes of anxiety, visits to general practitioners and medical

treatment. In particular, the large bowel is one of the commonest sites for cancer development. Large scale observational studies of dietary practices and associated incidence of cancer provide very strong evidence that dietary choices and nutritional status (e.g. obesity) influence risk of cancer in the large bowel (colorectal cancer; CRC). Such evidence is very encouraging because it suggests that many cases of bowel cancer could be avoided by appropriate dietary choices and/or by the development of novel foods or dietary agents with anti-cancer properties.

Identification of beneficial dietary agents requires intervention studies i.e. carefully controlled experiments in which volunteers are given known amounts of the test agent. For both practical and ethical reasons, it is seldom appropriate to use the development of cancer as the endpoint in such experiments and there is a need to use surrogate outcome measures. This is analogous to using blood pressure or blood cholesterol concentration as surrogate outcome measures (or biomarkers) in studies of diet and heart disease risk. Unfortunately, in the area of diet and gut health, progress is hampered by the lack of robust biomarkers of CRC risk for use as surrogate endpoints. To address this gap, we have developed a number of novel biomarkers of diet-related CRC risk which can be measured in small samples (biopsies) taken during clinical examination of the large bowel. We have shown that these biomarkers can be detected BEFORE the development of CRC and so may be a useful tool to identify those at higher risk of the disease. In our on-going work, we are investigating relationships between what people eat (and other aspects of lifestyle) and these biomarkers in a cross-sectional study. The next logical step is to test how the most promising biomarkers respond to dietary intervention to determine how useful they will be as biomarkers of gut health.

We will do this by carrying out a carefully controlled experiment in which volunteers will be given food supplements of resistant starch (RS) and polydextrose (PD) - both are carbohydrates with special properties. They are widely used food ingredients for which there is already evidence that they may help reduce CRC risk. Both food agents show bioactivity in the large bowel where they appear to have beneficial effects on gut physiology and immune function including anti-inflammatory effects.

In our human intervention study, 70 healthy volunteers will be given RS and/or PD or another carbohydrate with no effects on the large bowel (a placebo) for 7 weeks. We will collect tiny pinch samples of the lining of the gut (mucosal biopsies) before and after the intervention for biomarker measurement. These biomarker studies will include measurement of genes which are known to be involved in the early stages of the development of cancer and which may be modifiable by changing diet. Dietary components such as RS and PD may influence how genes are switched on and off by affecting regulatory marks on DNA known as DNA methylation so we will quantify DNA methylation for a panel of key cancer-related genes. We will also measure the rates at which cells are being produced (cell proliferation) in the gut lining because faster cell proliferation appears to indicate higher CRC risk. In addition we will collect blood and stool for measurements of markers of inflammation. There is growing evidence that poor diet and obesity can lead to the development of a chronic inflammatory state and that this may predispose to CRC. Through their fermentation by bacteria in the large bowel, RS and PD may help reduce inflammation and so protect gut health.

Immunomodulatory effects of pre- and probiotics

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Richard Aspinall	Cranfield University	£51,325.39

The number of people aged 65 years and over is expected to rise by over 60% in the next 25 years, which presents an enormous challenge for the healthcare system. Elderly people suffer from more frequent and severe infections than younger people; influenza is particularly common in older individuals and is a major cause of death in the elderly population. Ageing dramatically affects immune function; as we age, the repertoire of cells potentially available to respond to a new pathogen shrinks and there is an accumulation of poorly functioning mature cells. This phenomenon is known as immunosenescence and partly explains the increased susceptibility for infection in older individual. Vaccination is recommended to protect the older people against influenza, but immunosenescence also reduces the efficacy of

vaccination. It has been estimated that 30-50% of older adults fail to mount protective antibody responses after influenza vaccination, representing a considerable waste of resource and a false sense of security for those receiving the vaccinations.

Probiotics are beneficial bacteria, which can be consumed and which have a long and safe record of use in humans. They have shown promise in the prevention or treatment of several disease states ranging from lactose intolerance, constipation and diarrhoea, alleviation of allergy and even to more chronic systemic diseases, such as cardiovascular disease and cancer. Often they are taken together with prebiotics, which are normally fermentable carbohydrates that provide a food source for the beneficial bacteria when they reach the lower gut. There is particular interest in the positive influences of pre- and probiotics in older people, who are subject to alteration in gut microflora composition as well as immunosenescence. Several studies have demonstrated beneficial effects of specific pre- and probiotics on immune function in older subjects. However, none of these studies have taken into account the age-related shift in immune cell populations. Furthermore, there is little understanding of the mechanisms underlying these effects. Despite this, probiotics have recently been proposed as prime candidates for 'anti-immunosenescence' therapy. We propose to investigate the impact of a pre- and probiotic on immune function in young and older subjects, taking into account the age-related shifts in immunity due to immunosenescence. We aim to establish biomarkers and methodology which could be exploited to establish the underpinning science behind the immunomodulatory effects of commercial pre- and probiotics.

The project will involve 60 young (18-35y) and 60 older (65-85y) subjects, who will take a pre- and probiotic mixture or a placebo for a total of 8 weeks. The probiotic is not currently commercially produced, but has been demonstrated to have particular ecological fitness and anti-pathogenic effects in the gastrointestinal tract in old age. A suitable prebiotic will be selected on the basis of ability to promote optimal growth and survival of this probiotic. After 4 weeks on the treatment, the subjects will receive an influenza vaccination. Blood samples will be taken before treatment, and at 4, 6 and 8 weeks after commencement. The samples taken at 6 and 8 weeks will be used to assess the immune response to the vaccination. A wide range of immune parameters will be assessed, taking into account the age-related shifts in immune cell populations. In conjunction with the human study, a series of in vitro studies will investigate the mechanistic basis for the impact of the probiotic on immune function and will determine whether there are differential effects of the probiotic in young and older subjects at the cellular and molecular level. The overall aim of both components of the project is to determine whether older subjects derive specific additional benefit from pre- and probiotics by modulation of the impact of immunological ageing.

Maximising satiety through manipulating expectations, sensory quality and nutrient content.

Martin Yeomans	University of Sussex	£539,704.80
<p>Understanding the processes which promote satiety and so decrease the risk of overeating and a consequent positive energy balance are critical to our future ability to counteract the worldwide rise in the incidence of obesity. The traditional view of satiety is that suppression of appetite after eating arises from physiological effects of the ingested nutrients. However, while it is clear that nutrients do generate satiety signals, there are many aspects of satiety that cannot be explained simply as an effect of nutrient ingestion. For example, nutrients ingested as beverages generate weak satiety, whereas nutrients in a different liquid context (soup) generate strong satiety. This implies that something about the context in which nutrients are consumed is critical to the subsequent experience of fullness. Taking a more cognitive view, this research considers the extent to which consumer expectations about how filling a food will be may modify satiety. Thus a beverage may be consumed on the expectation of reduced thirst but not satiety, thereby leading to a failure to attribute physiological satiety cues generated by the drink nutrients to the actual drink product. Conversely, if the expectation is that a food will lead to strong feelings of fullness, this may lead to greater suppression of appetite than that generated by the same nutrients in the absence of expectations. Preliminary data in our laboratory are consistent with this view:</p>		

protein was more effective in suppressing appetite when consumed in a context which was consistent with satiety than on its own. This research builds on this finding in a 3-phase programme designed to test the principle that expectations interact with physiological cues to modify satiety and so direct consumer behaviour. To achieve this, in Phase 1 we explore for the first time how expectations generated by label information and sensory quality modify the satiating effects of protein both in a snack and breakfast context. These experiments will first identify effective expectation manipulations and then examine their impact on satiety both when a product is first encountered and after repeat consumption. To ensure the outcome is both consistent and relevant to real-life behaviour, separate studies will use more controlled laboratory tests of eating and more naturalistic studies in a quasi-restaurant setting. The outcome of this 2-year Phase will be a clear test of the concept that expectations may interact with post-ingestive cues to generate satiety. How these expectations may modify actual physiological satiety responses will be tested in Phase 2, which examines how expectation-nutrient interactions modify the release of satiety hormones. As with Phase 1, these effects will be tested both acutely, and as a function of learning about the product as a consequence of repeated consumption. Finally, Phase 3 explores the utility of these findings for food product development by examining the effects of extended home consumption of products designed to generate maximum satiety through combinations of expectation and nutrient content. Separate home consumer trials will be run with the snack and breakfast products developed in Phase 1 and 2, and outcome measures will include effects of consuming high-satiety products on overall energy intake, and consumer evaluations of the products in terms of acceptability and future purchase intentions. The overall outcome of the programme will be both a detailed evaluation of the interaction between consumer expectations and physiological controls of satiety for the first time, and the use of such interactions to formulate new design rules for the development of future food products that generate consumer-perceivable satiety benefits.

Total £4,174,530.08