New Project Summary Report for FV 420: Carrot & Parsnip - consumption on biomarkers of human health

Project Number	31304200
Title	Carrot and Parsnip: Intervention study to assess the effect of consumption on biomarkers of human health
Short Title	FV 420
Lead Contractor	Newcastle University
Other Contractors	
Start & End Dates	01 September 2013 - 31 August 2016
Industry Representative	Mr Martin Evans, Fresh Growers Ltd
Project Budget	£74,415 plus In kind contribution of vegetables from the vegetable industry.
AHDB Contribution	£74,415

The Problem

In the project a human intervention study will be carried out to measure the effect of consumption of selected root vegetables on biomarkers for cancer and inflammatory diseases.
Within the Field Vegetables Sector, the project addresses the British Carrot Growers Association's strategy Objective 5, 'To provide information which can be used to promote consumption of high quality home grown vegetables', specifically the target to 'Encourage consumption of vegetables and exploit health benefits' which includes recognised gaps in areas with high priority.
Observational studies have found a negative correlation between consumption of carrots and the risk of cancer, which originally were believed to be caused by the antioxidants α- and β-carotene, however intervention studies showed that the carotenes gave little or no benefit for health, high intakes even increased the risk of cancer. Laboratory studies then showed that polyacetylenes, phytochemicals found mainly in carrots, parsnips and celeriac, have beneficial effects on cell culture models of inflammation and cancer, and also reduced cancer growth in a rat model. Due to the unexpected harmful effects experienced when volunteers were given supplements with pure β-carotene, it is no longer allowed to carry out human trials with high doses of isolated compounds. However it is ethically acceptable to ask volunteers to consume a diet with a pre-defined amount of specific vegetables such as carrots or parsnips, also when this provides them with equivalent amounts of the phytochemicals.
Inflammation is involved in the development of both cancer and cardiovascular disease, as well as specific inflammatory diseases such as arthritis.
Biomarkers of human health can be used to measure changes in a person's risk of a disease, and are therefore used to test how certain foods can improve our health by reducing our risk of disease, even among people who are already healthy. For cancer a well-known biomarker is measurement of damage to DNA in lymphocytes from blood samples (the comet assay). Different aspects of inflammation can be

assessed by measurement of specific proteins, C-reactive protein (CRP) and α1-acid glycoprotein (AGP), (7) as well as the signalling molecules called cytokines.
The new EU legislation on health claims (Regulation (EC) No 1924/2006) allows for authorisation of health claims for foods with beneficial effects on human health, but at the same time bans any advertisements referring to health benefits that are not in the form of an approved health claim. This means that compared with today where any producer can tout their product as a superfood, after December 2012 those few foods that have had their health claims approved will stand out against all those that have not. One of the key requirements for EFSA approval is human intervention studies that document a benefit, and which does this in a way where it can reasonably be assured that the food with the claim will also work the next time around, that it was not just the one batch that was tested. EFSA requires that the food is sufficiently characterised, that the effect of it is beneficial, and that a cause and effect relationship can be documented between the food and the benefit.
Changes in validated biomarkers such as the comet assay is presently the only type of evidence that the European Food Safety Authority (EFSA) has approved as appropriate evidence for health claims for reductions in disease risk.
While and yellow carrots contain polyacetylenes, but not carotenes. Celeriac and parsnips also contain polyacetylenes, but not the same spectrum of specific compounds as carrots. Parsnips contain another type of phytochemicals, furanocoumarins, which also may have health benefits in their own right. This means that appropriate combinations of the different vegetables will make it possible to test if an effect of vegetable consumption is caused by the polyacetylenes or maybe furanocoumarins, and define how much of these compounds must be provided in the diet to obtain the health benefit.

Aims and Objectives

The project will carry out a human intervention study to measure the effect of consumption of selected root vegetables on biomarkers for cancer and inflammatory diseases. Laboratory studies have shown that polyacetylenes, phytochemicals found mainly in carrots, parsnips and celeriac, have beneficial effects on cell culture models of inflammation and cancer, and observational studies have found a negative correlation between consumption of carrots and the risk of cancer. Inflammation is involved in the development of cancer, cardiovascular disease, and specific inflammatory diseases such as arthritis. Biomarkers of human health can be used to measure changes in a person's risk of a disease. For cancer a well-known biomarker is measurement of damage to DNA in lymphocytes from blood samples (the comet assay), while other health aspects such as inflammation can be assessed by measurement of other biomarkers in the blood.

During the first year the student will learn how to carry out intervention studies and measure DNA damage and other biomarkers, analyse polyacetylene content in samples of relevant plant material, plan the full-scale trial in detail and apply for ethical approval. In the second year a full-scale intervention trial will be carried out with 20 or more middle-aged and older participants, who will consume an intervention diet and a placebo diet, each for 6-10 weeks. Blood samples will be collected before and after each of the intervention periods, together with measurements of other health indicators such as blood pressure and grip strength.
During the third year the samples will be analysed and the results published as scientific papers and the PhD thesis

Approach

During the first year, in addition to attending the University's generic training courses, the student will learn how to carry out intervention studies and how to measure DNA damage, as well as how to prepare samples for analysis of other relevant biomarkers such as blood lipids, cholesterols, proteins and cytokines. S(he) will produce a literature review of the literature on health benefits of apiaceous vegetables, analyse contents of polyacetylenes and furanocoumarins from carrots and parsnips from a range of varieties and growing conditions and do short-term intervention tests (2-3 weeks) with the

analysed samples to select the best options regarding the detailed design of the full-length study, including the precise formulation of the intervention diet(s) and the placebo treatment. The full-length trial will be planned in detail and ethical approval applied for.
In the second year a full-length intervention trial will be carried out, with 20 or more middle-aged and older participants, who will consume an intervention diet and a placebo diet, each for 6-10 weeks. Blood samples will be collected before and after each of the intervention periods, together with measurements of other health indicators such as blood pressure and grip strength. DNA damage will be assessed on live cells from the fresh blood samples using the comet assay.
After the second year the project will be reviewed by the HDC and the project plan revised if relevant.
During the third year the samples will be analysed for biomarkers based on the results until then and developments of state of the art, and the results will be written up as scientific papers and the thesis.
The budget applied for will cover the cost of analyses of carrot and parsnip samples and for carrying out the trial with 20 volunteers including collection and storage of blood samples and the measurements of DNA damage markers, blood pressure and grip strength. If more funding is obtained, the number of volunteers can be increased, improving the probability to detect health benefits in the study. During the project, assuming it develops as planned, additional funds will be applied for, to pay for additional analyses of the stored blood samples. This can be done during the third year, or even after the project is finished. These additional analyses are not a prerequisite for a successful PhD thesis, however they will be a very cost-effective way of increasing the value of the project, at a stage where it is already known if the intervention itself has been successful (in the terms of recruitment of volunteers, preparation and distribution of diets, that the volunteers have consumed the diets and attended the testing sessions etc.).
If the results of the study indicate benefits of consuming the vegetables, but the effects are too small to be significant with this number of volunteers, then the data can be used as ‘pilot data’ to calculate how many volunteers are needed in order to obtain documentation for a benefit (a power calculation). In this case it will be relevant (and relatively easy) to apply for funds from e.g. the Research Councils to repeat the intervention with more volunteers and obtain the full documentation. This strategy (a pilot trial with a small number of volunteers, followed by a full-scale trial based on the pilot trial data) is considered best practice for human intervention studies, since it minimises the burden on the volunteers.