



2012

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Recommended Citation

Skinner, M.A. (2012) "Wellness foods based on the health benefits of fruit: Gold kiwifruit for immune support and reducing symptoms of colds and influenza," *Journal of Food and Drug Analysis*: Vol. 20 : Iss. 1 , Article 29.

Available at: <https://doi.org/10.38212/2224-6614.2095>

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Wellness Foods Based on the Health Benefits of Fruit: Gold Kiwifruit for Immune Support and Reducing Symptoms of Colds and Influenza

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ABSTRACT

Fruits are excellent health foods and ingredients for functional foods. Plant & Food Research is developing new fresh and processed foods to promote gut health and immunity and protect from oxidative stress. The research involves testing using cell-based assays and animal models and validating health benefits in human trials. Using ZESPRI® GOLD Kiwifruit as an example, extracts stimulated human innate and adaptive immunity *in vitro* and a puree, but not a juice, enhanced a weak gut-associated immune response in mice. To validate an associated health benefit, a human trial determined the effects of gold kiwifruit consumption on symptoms of colds and influenza in older adults (≥ 65 years). Gold kiwifruit consumption significantly reduced head congestion and sore throat symptoms as well as plasma lipid peroxidation and significantly increased plasma vitamin C, α -tocopherol and lutein/zeaxanthin concentrations, and red cell folate concentrations. In a subsequent trial, regular consumption of gold kiwifruit by pre-school children (2 - 5 years) resulted in an overall reduction in the incidence of cold and influenza-like illnesses as well as a reduction in their symptoms. The results suggest that gold kiwifruit can modulate the immune system in a positive way and that consumption can improve health by reducing symptoms and incidences of colds and influenza, in groups whose immune status may result in a greater risk of contracting these types of infections.

Key words: fruit, wellness, kiwifruit, immune, colds and influenza

INTRODUCTION

Epidemiology studies have consistently provided evidence that diets rich in plant-based foods are associated with disease prevention. During the assessment of the chemical composition of these foods it became apparent that the health benefits did not correlate solely with nutrient content. Kiwifruit are some of the most nutrient-dense fruits and are good sources of vitamins, minerals, dietary fiber and phytochemicals, so they potentially provide health benefits beyond basic nutrition. The most commonly consumed kiwifruit are of the green (e.g. *Actinidia deliciosa* 'Hayward') or gold (e.g. *A. chinensis* 'Hort16A', ZESPRI® GOLD Kiwifruit) cultivars although a few others are starting to be produced in commercial volumes, for example the kiwiberry (*A. arguta*) along with other variants of green and yellow kiwifruit. Typically, green kiwifruit contain approximately 85 mg of ascorbate per 100 g fresh weight, and gold kiwifruit often contain 30 - 40% more⁽¹⁾. It has been estimated that an average kiwifruit can provide the daily vitamin C intake recommended for most of the population⁽¹⁾. Kiwifruit also contain vitamins E and K, folate, the minerals potassium,

magnesium and copper, as well as dietary fiber and phytochemicals, including carotenoids and polyphenols. Kiwifruit contain about 2 - 3% dietary fiber and so can contribute a significant proportion (10%) of the recommended daily dietary fiber requirement⁽¹⁾. As many of these compounds are associated with healthy immune function there was a natural link implied between the content of kiwifruit, immune health and protection from infections.

An initial screening of a selection of processed products from ZESPRI® GOLD Kiwifruit (henceforth referred to as gold kiwifruit) for their ability to support measures of human innate and adaptive immune responses *in vitro* led to the selection of two ingredients, a pasteurized puree and a 40° Brix juice, to test in a mouse model. A model system was used whereby mice were orally immunized with a protein, ovalbumin (OVA), given together with a sub-optimal dose of a mucosal adjuvant, cholera toxin, to generate a weak gut-associated immune response. When fed to mice at a dose of 200 μ L per day for 20 days, the kiwifruit puree enhanced the production of OVA-specific antibody (IgG) in the serum, and the ability of lymphoid cells from the mesenteric lymph node, but not the spleen, to proliferate on

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re-exposure *in vitro* to the immunizing antigen OVA⁽²⁾. In a further feeding trial it was established that OVA-specific antibody isotypes representative of both a T helper (h) 1 (IgG2b and 2c) and a Th2 (IgG1) immune response were produced. In addition, interleukin (IL) 5, a hallmark cytokine of Th2 responses, was produced by mesenteric lymph node cells from the mice when they were re-exposed to OVA *in vitro*⁽³⁾. These results suggested that the gold kiwifruit puree enhanced both Th1 and Th2 type immune responses. Interestingly a concentrated juice made from gold kiwifruit had no effect on this weak immune response when fed to the mice at a similar dose. The two processed kiwifruit products contained similar amounts of vitamins and minerals but the puree contained fiber and a higher concentration of carotenoids. These results led us to investigate the effects of gold kiwifruit on human immune responses, in particular the innate and adaptive immune responses of human blood cells *ex vivo* and to determine whether consumption of gold kiwifruit could reduce risk and/or symptoms of infections.

Upper respiratory tract infections typified by symptoms of colds and influenza (flu) are some of the most common illnesses, with adults having 1-3 per year and children 4-6 per year in a USA study⁽⁴⁾. There is no common anti-viral medication. Nutritional status is important for maintaining optimal immune function and recent evidence suggests that fruit, vegetables^(5,6) and tea consumption⁽⁷⁾ can reduce the symptoms and incidence of colds and flu. There are segments of the population that are particularly susceptible to these infections, for example pregnant women, athletes, students before exams, people with chronic health conditions and the old and the young. We therefore determined whether consumption of gold kiwifruit reduced symptoms of colds and flu and their severity in two 'at risk' populations', older adults aged 65 years or over and pre-school children aged 2 to 5 years.

MATERIALS AND METHODS

A measure of innate and adaptive immune cell function was determined by effects on natural killer cell activity, and a recall interferon γ (IFN γ) response to tetanus toxoid, respectively, using human blood cells. Blood samples or white blood cell fractions from tetanus toxoid-immunized human volunteers aged from 23 - 48 years (mean 33 years) were incubated with a sample prepared from the gold kiwifruit pasteurized puree, suitable for use in cell culture assays. The puree was tested for cytotoxicity on a human monocytic cell line (THP-1) and used to determine effects on blood cells at the highest non-toxic concentration and at a further three, 2-fold dilutions (1.6 - 13 mg/mL). Natural killer cell activity was measured by the ability of peripheral blood mononuclear cells to lyse K562 natural killer target cells⁽⁸⁾. IFN γ production after 24 h in the absence or presence of 5 μ g/mL tetanus toxoid was measured by ELISA. The study was approved by the

New Zealand Northern Y Human Ethics Committee (NTY/06/09/082).

For the intervention trials a cross-over design (Figure 1) was carried out using banana as the "placebo".

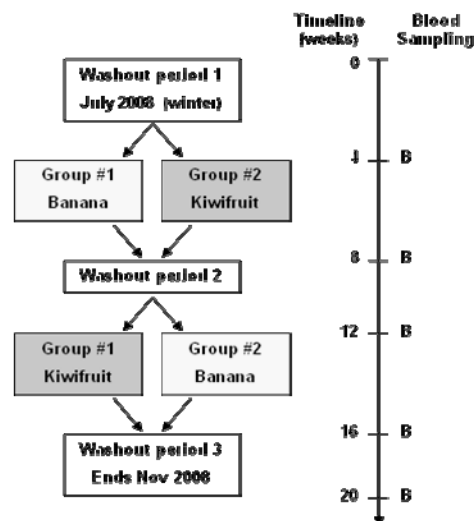


Figure 1. Design of intervention trial in older adults. B denotes that blood was taken from subjects at this time point.

For the study in older adults (approved by Northern A Human Ethics Committee 08/16), 32 healthy adults aged ≥ 65 years consumed either two gold kiwifruit plus two freeze dried kiwifruit or two freeze dried bananas per day for 4 weeks. The primary outcome variables were the duration and severity of symptoms of colds and flu recorded by subjects using the Wisconsin Upper Respiratory Symptom Survey (WURSS21). Plasma vitamin C, carotenoid content and lipid peroxidation and red cell folate were measured using standard laboratory procedures as described previously⁽⁸⁾. The rationale for providing banana as the "placebo" was to provide an alternative fruit that had relatively similar energy content, but lower nutritional value than gold kiwifruit with respect to most minerals, vitamins and carotenoids.

For the study in pre-school children (approved by Massey University Southern A Human Ethics Committee, 29/09), 66 children aged 2 - 5 years attending day care consumed two servings of either gold kiwifruit or banana for 5 days a week for 4 weeks. Symptoms and overall colds and flu-like illnesses were recorded by parents using the Canadian Acute Respiratory Illness and Flu scale (CARIFS) that has been validated for use with children. No blood samples were taken from in the study with children.

Significance analysis was carried out in SAS 9.1 using the MIXED procedure.

RESULTS AND DISCUSSION

I. Gold Kiwifruit and Immune Support

Natural killer cell activity was significantly increased with the addition of the kiwifruit sample in a concentration-dependent manner (Figure 2). The results suggest that gold kiwifruit may modulate cells associated with the innate immune response.

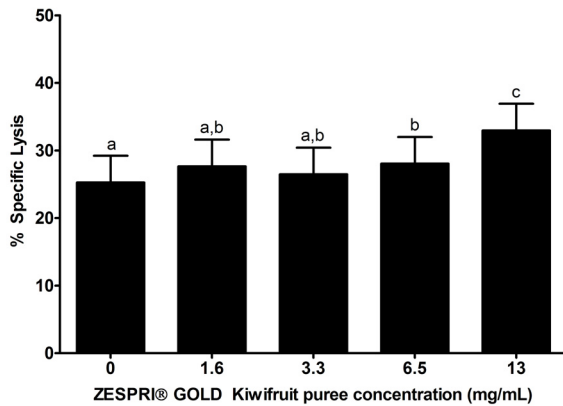


Figure 2. Effect of gold kiwifruit on human natural killer cell activity *in vitro*. Bars represent Least Squares Means + SEM of natural killer cells activity of 14 subjects at an effector: target ratio of 20:1. Values with the same letter are not significantly different from each other ($p < 0.05$).

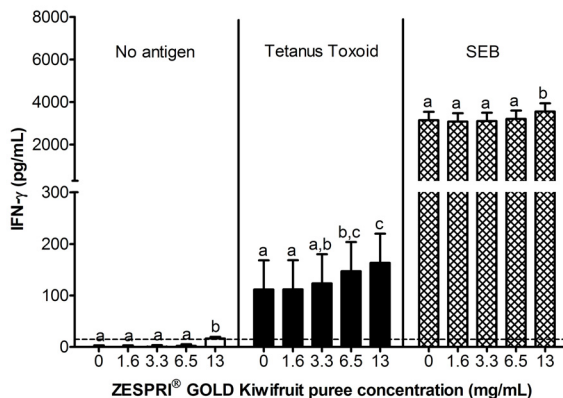


Figure 3. Effect of gold kiwifruit on interferon γ production *in vitro* in response to tetanus toxoid. Bars represent Least Squares Means + SEM of interferon γ production from blood of 14 subjects. *Staphylococcus aureus* enterotoxin B superantigen (SEB) was used as a positive control. Dashed line represents limits of interferon γ detection. Statistical analysis was carried out only between kiwifruit extract concentrations with the same antigenic stimulus. Values with the same letter are not significantly different from one another ($p < 0.05$).

In an *in vitro* recall response to tetanus vaccination (adaptive response) the concentrations of IFN γ were below the threshold of detection in the absence of antigenic stimulus, except when the kiwifruit sample was at a concentration of 13 mg/mL, when IFN γ was detectable. Addition of tetanus toxoid increased IFN γ secretion by an average of 100 pg/mL compared with the control. Co-incubation of tetanus toxoid with the kiwifruit sample resulted in an additional increase in IFN γ secretion compared with tetanus toxoid stimulation alone in a concentration-dependent manner, with significant increases at

the higher concentrations of kiwifruit sample tested (Figure 3).

II. Gold Kiwifruit Consumption and Symptoms of Colds and Influenza

In the first study with older adults, there were two symptoms, sore throat and head congestion that people had for significantly fewer days when they ate kiwifruit compared with banana. They experienced a sore throat for a mean of 5.4 days when they ate banana compared with only 2.0 days when they ate kiwifruit ($p = 0.02$). For the head congestion this was reduced from 4.7 days with banana to less than 1 day (0.9 days) with kiwifruit ($p = 0.03$). The severity of head congestion was also reduced so that the severity score out of 10 was reduced from 6.72 when people ate banana to 1.3 when they ate kiwifruit ($p = 0.01$). Consumption of gold kiwifruit compared with banana led to significantly higher concentrations of plasma vitamin C (72.9 μ M v. 53.7 μ M), α -tocopherol (35.6 μ M v. 32.5 μ M) and lutein/zeaxanthin (601 nM v. 498 nM) ($p < 0.05$), and enhanced the concentration of red cell folate (611 nmol/L v. 559 nmol/L, $p = 0.037$)⁽⁸⁾.

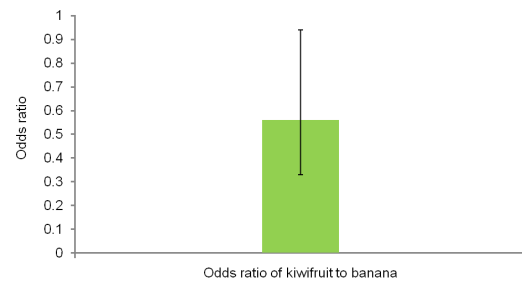


Figure 4. The odds of having a cold or flu-like illness when pre-school children consumed gold kiwifruit compared with banana. The odds ratio was calculated from the parent-reported incidence of colds and flu-like episodes of 66 children by comparing the gold kiwifruit intervention to the banana intervention outcome. Error represents 95% CI (0.32, 0.94) ($p = 0.03$).

In the second study with preschool children, the overall incidence of a cold or flu-like illness was considerably reduced when children ate kiwifruit compared with banana. The odds ratio of having a cold or flu-like illness was 0.55 when kiwifruit was consumed compared with banana, which means that the children had almost a 50% reduction in the chance of having a cold or flu when they ate kiwifruit (Figure 4). When individual symptoms were analysed there were significant improvements in a number of symptoms. Children had a better appetite ($p = 0.01$), did not feel so unwell ($p = 0.04$), had more energy ($p = 0.01$), cried less ($p = 0.04$) and their headaches ($p = 0.02$) and sore throats ($p = 0.04$) were not as bad⁽⁹⁾. The reduction in these last two symptoms is in agreement with the reduced head congestion and sore throat symptoms observed in the study with the older adults.

The results may be useful for giving consumers a better understanding of the health benefits of gold

kiwifruit and aiding in the development of health claims for processed functional foods containing kiwifruit ingredients⁽¹⁰⁾.

CONCLUSIONS

The *in vitro* results suggest that gold kiwifruit has the potential to influence immune responses, up-regulating key cells and cytokines for effective protective immunity. The possibility that gold kiwifruit can modulate the immune system in a positive way means that it may improve health and wellbeing by leading to lower incidences of common infections such as colds and flu or a reduction in the severity or length of symptoms. The results of intervention trials in two groups at higher risk of contracting colds and flu, older adults and young children, provide evidence that consumption of gold kiwifruit in these groups can reduce the symptoms of colds and flu and the overall incidence of infection. Whether these effects on infection are due to better protective immunity seems likely but has yet to be established.

ACKNOWLEDGMENTS

This work was financially supported by funding from the New Zealand Government *via* a FRST (now MSI) Wellness Foods Program (C06X0405) and by ZESPRI International Ltd. The following are thanked for their contribution to the work: Aselle Adaim, Denise Hunter, Mark Wohlers, Jingli Zhang (Plant and Food Research), Jacelyn Loh (University of Auckland), Lesley Stevenson (GSK), Marlana Kruger, Frances Wolber, Chris Booth, Rosanne Kruger, Welma Stonehouse (Massey University), Lynley Drummond (ZESPRI International Ltd).

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