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## 3rd International Immunonutrition Workshop

### Session 1: Antioxidants and the immune system Gold kiwifruit (*Actinidia chinensis* 'Hort16A') for immune support

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Kiwifruit is a good source of several vitamins and minerals and dietary fibre, and contains a number of phytochemicals; so kiwifruit potentially provides health benefits beyond basic nutrition. Consumption of green kiwifruit can have positive effects on cardiovascular health through antioxidant activity, inhibition of platelet aggregation and lowered TAG levels, and gut health through improving laxation, aiding digestion and promoting a healthy gut microflora. The importance of nutrition on immune function is well recognised, with deficiencies in vitamins A, C, E, B<sub>6</sub> and B<sub>12</sub>, folic acid, Zn, Cu, Fe and Se being associated with impaired immune function and increased susceptibility to diseases. Evidence is growing that kiwifruit enhances immunity, with several small murine studies showing enhancement of innate and adaptive immune function. Few studies have examined the effect of kiwifruit on immune function in human subjects, but a recent study has revealed that kiwifruit up-regulates several 'immune' and 'DNA and repair'-related gene sets, and down-regulates one gene set related to Ig secretion. Taken together, the evidence from the literature provides supporting data for designing a human intervention trial to validate the ability of kiwifruit to support immune function in healthy and immunocompromised populations.

#### ZESPRI® GOLD kiwifruit: Health benefits of kiwifruit: Immune support

##### Health benefits of kiwifruit

A new global awareness of the role of healthy foods is emerging and consumers are responding by adapting their approach to health decisions, in particular placing greater emphasis on prevention rather than cure. Fruit has been identified as the future of functional foods as consumers are increasingly interested in health, sensory and convenience aspects of food in a form that is 'natural'<sup>(1)</sup>. Kiwifruit is a good source of several vitamins, minerals and fibre, and contains phytochemicals<sup>(2)</sup>, making it a suitable candidate as a fruit-based functional food or ingredient.

The most commonly consumed kiwifruit are of the green (*Actinidia deliciosa* 'Hayward') or ZESPRI® GOLD (*Actinidia chinensis* 'Hort16A', hereafter gold kiwifruit) varieties although a few others are now starting to be produced in commercial volumes, for example, the kiwiberry (*Actinidia arguta*) along with other variants of green and

yellow kiwifruit. Typically green kiwifruit contains approximately 85 mg ascorbate per 100 g fresh weight, and gold kiwifruit often contains 20% more<sup>(2)</sup>. It has been estimated that an average kiwifruit can provide the daily vitamin C intake recommended for most of the population<sup>(2)</sup>. Kiwifruit also contains vitamins E and K, folate, the minerals K, Mg and Cu, as well as dietary fibre and phytochemicals including carotenoids and polyphenols. For Cu, vitamin E and folate, it is estimated that a green kiwifruit gives 18, 11 and 10% of the US recommended daily intake, respectively, and gold kiwifruit gives 19, 11 and 8% recommended daily intake, respectively, of these important micronutrients<sup>(2)</sup>. Kiwifruit contains about 2–3% dietary fibre and so can contribute 10% of the recommended daily dietary fibre requirement<sup>(2)</sup>. While kiwifruit contributes a number of nutrients important for good health, in many instances the differences in content between green and gold kiwifruit are small and of uncertain biological value.

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Phytochemicals such as those present in kiwifruit, including carotenoids and polyphenols, may contribute to health and wellness by protecting from degenerative diseases and processes associated with ageing<sup>(3)</sup>. Gold and green kiwifruit contain approximately 620 and 590 µg carotenoids/100 g fresh weight, respectively, with the predominant carotenoids being lutein and β-carotene<sup>(4)</sup>. These beneficial phytochemicals often have antioxidant effects, assumed to be involved in scavenging excess free radicals that arise not only as a result of stress and disease but also as a part of normal metabolic processes. In comparison with other commonly consumed fruit, the antioxidant capacity of kiwifruit as measured by chemical antioxidant assays is average<sup>(5)</sup>. The reported antioxidant capacity of green kiwifruit consistently ranges from 6.0 to 9.2 µmol Trolox<sup>TM</sup> equivalent/g (fresh weight), when assayed by the oxygen radical absorbance capacity method<sup>(5–8)</sup>. The antioxidant activity of gold kiwifruit, as measured by oxygen radical absorbance capacity also, is reported to be 12.1 µmol Trolox<sup>TM</sup> equivalent/g<sup>(6)</sup>. Recently, the relevance of chemical-based antioxidant activity in predicting antioxidant activity in a biological setting has been criticised<sup>(9)</sup> and thus it is important to demonstrate the antioxidant efficacy of fruits in cell-based assays or *in vivo*. For example, in a cell-based assay, green kiwifruit juice concentrate protected human Jurkat T-cells from cell death induced by oxidative stress, as a result of exposure to hydrogen peroxide<sup>(10)</sup>. Similarly, chemical-induced oxidative damage was found to be reduced in rats fed a diet supplemented with 15 and 30% kiwifruit (species not specified)<sup>(11)</sup>. In addition, it has also been demonstrated that ingestion of kiwifruit juice improves scavenging of reactive oxygen species generated in human plasma<sup>(12)</sup>. These findings demonstrate that consumption of kiwifruit may protect the body from oxidative stress, although the effect of long-term supplementation on plasma antioxidant activity and the implications of this on specific health targets remain to be determined. There is increasing evidence to suggest that phytochemical antioxidant molecules may have indirect antioxidant effects in the body, by up-regulating endogenous protective enzymes and through regulation of a number of other cellular processes, including those involved in inflammation (reviewed by Stevenson and Hurst<sup>(13)</sup>).

The rate of DNA repair has been used as a marker for susceptibility to mutagenicity, and increased susceptibility is positively correlated with cancer<sup>(14)</sup>. An increase in the rate of DNA repair in lymphocytes has been demonstrated in healthy volunteers taking supplements of β-carotene or lycopene, although this may be due to a direct antioxidant effect rather than an anti-mutagenic effect<sup>(15)</sup>. Kiwifruit contains β-carotene<sup>(4)</sup>, and similar results have been achieved by feeding subjects with kiwifruit. For example, in a pilot trial two groups of six healthy subjects were given dietary and physical activity advice and one group was given one green kiwifruit daily (LR Ferguson, personal communication) for every 30 kg body weight<sup>(16)</sup>. Leucocytes from subjects eating kiwifruit daily were found to have an improved ability to repair DNA after a peroxide challenge *ex vivo*. However, it is not known whether this was due to carotenoid content, as the vitamin C, folate

and fibre content of kiwifruit may also have contributed to the positive results. In a randomised crossover trial, Collins *et al.*<sup>(17)</sup> fed fourteen subjects kiwifruit (species not specified) for 3 weeks, with a 2-week washout period between treatments. Feeding with between one and three kiwifruit was associated with a decrease in endogenous oxidation of pyrimidines and purines in DNA. These findings suggest that kiwifruit, in quantities that might be consumed as part of a normal diet, may provide protection against DNA damage associated with mutations and cancer<sup>(17)</sup>.

Adhesion and aggregation of platelets at the site of injury in atherosclerotic blood vessel walls are key factors in the pathogenesis of CVD. An intervention trial carried out with healthy volunteers, demonstrated that consumption of one or three green kiwifruit daily for 28 d inhibited platelet aggregation, raised plasma antioxidant activity and also lowered TAG levels<sup>(18)</sup>. This could potentially result in an alteration in the natural course of atherosclerosis and reduce risk of coronary arteriole disease, myocardial infarction and stroke.

The strongest evidence for a health benefit of green kiwifruit is in the area of digestive health. Kiwifruit has been used in traditional Chinese medicine as an aid to digestion and recent clinical studies have supported this traditional use. In a study of thirty-eight healthy elderly subjects, the consumption of one green kiwifruit (*A. deliciosa* 'Hayward') per 30 kg bodyweight daily for 3 weeks was associated with an improvement in several self-reported parameters of laxation compared with when the same subjects did not consume kiwifruit<sup>(19)</sup>. Although self-reporting is subjective and may be influenced by mood or misinterpretation by the respondent<sup>(20)</sup>, subjects of the trial were trained through discussion, example and feedback in the use of the diary used to record defecation (volume, ease and consistency)<sup>(19)</sup>. In another study, kiwifruit was shown to help with chronic constipation while having no adverse effects such as diarrhoea<sup>(21)</sup>. In both studies, it was suggested that dietary fibre in kiwifruit may contribute to the observed effects. This could be connected to unusual cell wall properties of kiwifruit, which appear to give kiwifruit fibre an exceptionally high-water-holding capacity<sup>(19,22)</sup>, an important feature for faecal bulking. Other components that may contribute to digestive health are actinidin, a proteolytic enzyme and non-digestible oligosaccharides. For example, a green kiwifruit extract containing actinidin enhanced gastric digestion of Na caseinate and beef muscle protein in an *in vitro* model, although digestion of whey protein isolate, soya protein isolate, collagen, gelatin and gluten was largely unaffected<sup>(23)</sup>. Extending this model, the kiwifruit extract enhanced digestion of whey protein isolate, zein, soya protein isolate, gluten and gliadin under conditions simulating small-intestine digestion<sup>(24)</sup>. However, these findings remain to be proven *in vivo*. The balance of microbe populations that make up the gut microflora are key to healthy gut mucosal immunity. The dietary fibre in kiwifruit can exert prebiotic effects with the potential to prevent adhesion of enteropathogens and enhance adhesion of probiotic bacteria as demonstrated *in vitro* with human Caco-2 colon epithelial-derived cells<sup>(25)</sup>. In addition to the prevention of

colonisation by potentially harmful bacteria, animal and human studies have shown that changes to gut microflora populations may modulate immune function, influencing Ig levels and cytokine production<sup>(26)</sup>. The effect of modulation of these immune parameters on clinical and health outcomes remain uncertain, but the prebiotic effect of dietary fibre present in kiwifruit may contribute to these benefits.

While the evidence regarding the beneficial effects of regular consumption of kiwifruit on human health remains promising, the limitations of the clinical studies cited above should be noted. In particular, the number of subjects is typically small, ranging from ten to thirty-eight subjects in those studies that were of cross-over trial design<sup>(12,17–19)</sup>, fifty-three subjects (thirty-three constipated subjects and twenty healthy control subjects) in a single intervention (kiwifruit) trial<sup>(21)</sup>, twelve subjects in a factorial-type design (six subjects randomised to consume kiwifruit)<sup>(16)</sup>.

### Fruit and immunity

It is well known that deficiencies in certain nutrients such as vitamins A, C, E, B<sub>6</sub> and B<sub>12</sub>, folic acid, Zn, Cu, Fe and Se can lead to significant impairment of immune function and an increased susceptibility to infection and chronic diseases<sup>(27)</sup>. In most cases, supplementation has been shown to restore immune function in deficient individuals. However, it is unclear to what extent supplementation may aid in maintaining an optimal balance within the immune system in adequately nourished individuals. Understanding the beneficial effects of non-essential/non-nutritive phytochemicals, such as carotenoids and flavonoids, on immune function has been the subject of recent studies<sup>(28,29)</sup>. For example, Bub *et al.*<sup>(30)</sup> showed that consumption of polyphenol-rich fruit juices led to increased lymphocyte responsiveness and natural killer cell function in healthy men. In addition, enhanced immune function and a reduction in the incidence of upper respiratory tract infections or symptoms in at-risk populations, including athletes<sup>(31)</sup> and the elderly<sup>(32)</sup>, has been demonstrated. These studies have included compounds present in fruits such as quercetin<sup>(31)</sup>, and vegetables high in carotenoids<sup>(33)</sup>. Both green and gold kiwifruit contain compounds that may promote a healthy immune system.

Several mouse feeding trials have demonstrated that kiwifruit can enhance immune cell function and modulate immune responses. For example, kiwifruit extract (species not defined) was shown to stimulate phagocytosis and enhance serum IgA, IgG and IgM<sup>(34)</sup>. In another study, a combined extract of green and gold kiwifruit was shown to enhance both innate and acquired immunity in response to immunisation with a vaccine<sup>(35)</sup>.

### Gold kiwifruit and immune support

The ability of gold kiwifruit to enhance an adaptive immune response in mice was demonstrated by feeding mice a pasteurised puree of this fruit. A model system was used whereby mice were orally immunised with a protein, ovalbumin, given together with a sub-optimal dose of an

oral adjuvant, to generate a weak immune response. When fed at a dose of 200 µl/d for 20 d the kiwifruit puree enhanced the production of ovalbumin-specific antibody (IgG) in the serum, and the ability of lymphoid cells from the mesenteric lymph node, but not the spleen, to proliferate on re-exposure *in vitro* to the immunising antigen ovalbumin<sup>(36)</sup>. In a further feeding trial, it was established that ovalbumin-specific antibody isotypes representative of both a T-helper 1 (IgG2b and 2c) and a T-helper 2 (IgG1) immune response were produced. In addition, IL-5, a hallmark cytokine of T-helper 2 responses, was produced by mesenteric lymph node cells from the mice when they were re-exposed to ovalbumin *in vitro*. These results suggest that the kiwifruit puree enhanced both T-helper 1 and T-helper 2 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 levels of vitamins and minerals but the puree contained fibre and a higher level of carotenoids<sup>(36)</sup>. These results led to an investigation of the effects of gold kiwifruit on human immune responses, in particular, the innate and adaptive immune responses of human blood cells *ex vivo*.

In a pilot study (*n* 14, healthy adults aged 23–48 years), the influence of a water-soluble extract prepared from gold kiwifruit-pasteurised puree on five measures of immune cell function (phagocytosis, oxidative burst, natural killer cell activity, T-cell activation and cytokine production) was investigated, following incubation with whole blood samples or leucocyte fractions<sup>(37)</sup>. Although variation between subjects was considerable, when this was accounted for using an appropriate statistical model (Proc Mixed in SAS 9.1, Type 3 tests and pair-wise comparisons using Tukey's honestly significant difference) the gold kiwifruit sample significantly enhanced all markers of innate immune function tested, stimulated T-cell activation, and modulated production of several cytokines<sup>(37)</sup>. Such a study provides supporting data for the design of an appropriate human intervention trial. A recent study has further revealed that daily consumption of three kiwifruit (species not defined) significantly up-regulated thirteen gene sets related to 'DNA and repair', and two immune-related gene sets, also down-regulating one gene set related to Ig secretion<sup>(38)</sup>. The influence this has on immunity and immune function remains to be determined. However, recently the influence of a vitamin E supplement rich in tocotrienols on immune response in healthy women was reported<sup>(39)</sup>. Healthy women aged 18–25 years consumed the supplement for 28 d prior to vaccination with tetanus toxoid, and continued the supplementation for a further 28 d. Supplementation resulted in greater production of interferon-γ and IL-4 by leucocytes in response to mitogen and tetanus toxoid stimulation, and higher plasma anti-tetanus toxoid IgG concentrations. Interestingly, a new vitamin E structure has been recently isolated from kiwifruit (*A. chinensis*)<sup>(40)</sup>, and our work demonstrates that consumption of gold kiwifruit enhances plasma vitamin E concentrations (DC Hunter, MA Skinner, FM Wolber, CL Booth, JMS Loh, M Wohlers, LM Stevenson and MC Kruger, unpublished results). This may point to positive



immunomodulation following regular consumption of kiwifruit in healthy and immunocompromised populations.

### Conclusions

Dietitians along with other health professionals have the responsibility of providing consumers with scientifically proven facts that help them make informed dietary decisions. It is clear that a diet rich in fruit and vegetables offers health and wellness benefits that go beyond basic nutrition<sup>(41)</sup>. There are not an extensive number of studies specifically reporting the health benefits of kiwifruit; perhaps this is because it is a relatively newly consumed fruit in western societies. Consumption of green kiwifruit contributes by having positive effects on cardiovascular and gut health. Research into the effects of consumption of whole foods rather than supplements on immune function is in its infancy. The scientific evidence that kiwifruit may support immune health is growing and preliminary data supports progression to human intervention trials. The possibility that gold kiwifruit can modulate our immune system in a positive way means we may, in the future, look to kiwifruit consumption to improve health and well-being through leading to lower incidences of common infections such as colds and influenza or a reduction in the severity or length of symptoms. Two intervention trials with this focus have been completed, targeting different age groups, the younger and older members of our population, as they have a greater susceptibility to such infections, and manuscripts reporting these results are in preparation.

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