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ScienceDirect
Nutrition Research 28 (2008) 251–257
Nutrition Research
www.elsevier.com/locate/nutres

Feeding ZESPRI™ GOLD Kiwifruit puree to mice enhances serum immunoglobulins specific for ovalbumin and stimulates ovalbumin-specific mesenteric lymph node cell proliferation in response to orally administered ovalbumin

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Received 31 October 2007; revised 4 January 2008; accepted 17 January 2008

Abstract

The health benefits of fruits have been recognized for some time, but only recently have their effects on the immune system been investigated. Kiwifruit contains vitamins, minerals, and phytochemicals that are known to be important for normal functioning of the immune system. In this work, the influence of feeding 2 ZESPRI GOLD Kiwifruit processed products (Tauranga, New Zealand) on immune function in mice was investigated. Using a model to demonstrate adaptive immune responses in the gut, mice were fed either ZESPRI GOLD Kiwifruit puree or ZESPRI GOLD Kiwifruit 40° Brix Juice concentrate for 20 days, during which time they were immunized via the oral route with ovalbumin and subsequently given a suboptimal dose of the mucosal adjuvant cholera toxin. ZESPRI GOLD Kiwifruit puree enhanced the response to ovalbumin by significantly increasing the levels of total immunoglobulins and immunoglobulin G specific for ovalbumin and enhanced the antigen-specific proliferation of cells from the draining mesenteric lymph nodes compared with mice fed a 20% sugar control. These results indicate that ZESPRI GOLD Kiwifruit can modulate an antigen-specific immune response and suggest that ZESPRI GOLD Kiwifruit may represent a new type of functional food ingredient.

Keywords: *Actinidia*; Carotenoids; Food processing; Kiwifruit; Mice; Mucosal immune response
Abbreviations: ANOVA, analysis of variance; CT, cholera toxin; Ig, immunoglobulin; IL, interleukin; MLN, mesenteric lymph node; OVA, ovalbumin; PBS, phosphate-buffered saline; SI, stimulation index; Th, T-helper cells.

1. Introduction

The major function of the immune system is protection of the host from infectious diseases, and a properly functioning immune system is crucial for good health. Immune system function can be divided into nonspecific innate immunity, the first line of defense, and adaptive immunity, which includes a component of memory. Adaptive immunity operates at the systemic level as well as at the local level, which includes mucosal tissue such as that found in the upper airways and the gut [1].

Variation in nutrient status may influence immune function; and human trials have established that supplementation with micronutrients and qualitative changes in macronutrients can influence immune function, although the effect on well-

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doi:10.1016/j.nutres.2008.01.009

nourished people is unclear [1]. Micronutrients that have been shown to influence the immune system include minerals such as zinc, selenium, copper, and iron; vitamins A, C, and E; essential amino acids [2]; and phytochemicals [3] present in fruits and vegetables as well as in wine and tea. Although supplementation with vitamins and nutrients may restore immune function in the presence of a specific micronutrient deficiency, evidence suggests that intake of a cocktail of nutrients, particularly in the form of fruit and vegetables, has a beneficial effect on the maintenance of the immune system and in the prevention of disease [4].

Kiwifruit (*Actinidia* sp) is one of the most nutrient-dense fruits [5] and is a good source of vitamins C, E, and K; potassium; magnesium; and dietary fiber [6]. In addition, kiwifruit also contain phytochemicals, predominantly carotenoids. ZESPRI GOLD Kiwifruit (*Actinidia chinensis* 'Hort16A'; Tauranga, New Zealand) has been reported to contain a range of carotenoids, namely, β-carotene, lutein, violaxanthin, and 9′-*cis*-neoxanthin [7]. The health benefits of phytochemicals are being increasingly recognized, and there is evidence that phytochemicals can modulate immune function. For example, the consumption of a high-carotenoid vegetable product (tomato juice) by healthy men significantly enhanced the secretion of interleukin (IL)-2 and IL-4 from T-helper cell (Th) 1 and Th2 lymphocytes, respectively, after a period of plasma carotenoid depletion [8]. This finding demonstrates that depletion of carotenoids in plasma reduces T-lymphocyte function, but optimal function can be restored with the intake of a high-carotenoid tomato juice.

Research suggests that kiwifruit can provide several specific benefits for maintaining health and well-being. For example, kiwifruit has been described as a useful agent for cancer therapy in traditional Chinese medicine [9], possibly reducing some risk factors associated with cancer [10, 11], and may also be beneficial in cardiovascular disease [12]. A link has also been made between kiwifruit and digestive health, as kiwifruit has been shown to enhance measures of laxation significantly in the elderly [13]. Evidence suggests that kiwifruit also modulates immune function in a number of ways. Preliminary results have demonstrated that supplementation with kiwifruit enhances phagocytosis and the levels of immunoglobulins (Igs) in mice [14]. An imbalance of Th1/Th2 cell activity in an ovalbumin (OVA)-sensitized mouse model can be partly corrected with extracts from *Actinidia arguta*, leading to a reduction in the overproduction of IgE, which is involved in allergic response [15]. A similar effect was found in an OVA-sensitized mouse model of asthma, with *Actinidia polygama* inhibiting the accumulation of eosinophils into airways by reducing IL-4, IL-5, IL-13, and IgE, and modulating the Th1/Th2 cytokine imbalance, thus demonstrating an antiasthmatic effect [16].

There is an increasing demand for the development of evidence-based functional fruit products, and the gold standard by which products should eventually be measured is randomized clinical trials. During the early developmental stage of fruit-based functional products, it is not feasible to carry out such trials; therefore, cell-based and animal models are used. The aim of this study is to provide supporting evidence toward developing functional products that promote immune function. Such products may be useful in maintaining general immune function but may be particularly helpful in elderly populations, as aging, frailty, and chronic diseases are associated with impaired immune function; and this is often compounded by immune dysregulation from malnutrition [17]. The hypothesis of this study is that ZESPRI GOLD Kiwifruit products will enhance a gut-associated adaptive immune response (Ig levels and lymphocyte proliferation) in mice fed either of 2 ZESPRI GOLD Kiwifruit processed products for 20 days, during which time they are immunized with OVA by the oral route, with a suboptimal dose of cholera toxin (CT) adjuvant. A positive result will indicate that ZESPRI GOLD Kiwifruit products modulate immune responses in the gut and may promote health.

2. Methods and materials

2.1. Mice

All procedures on the mice were approved by the Wallaceville Research Centre Animal Ethics Committee (Upper Hutt, New Zealand). Female C57B1/6 mice aged 8 to 10 weeks were obtained from the Hercus Taieri Resource Unit, Dunedin, New Zealand. They were allowed free access to mouse feed pellets (Sharpes Stockfeeds Ltd, Carterton, New Zealand) and tap water throughout. The mouse feed pellets consisted of diet 86, containing wheat, barley, meat and bone meal, grass meal, lime yeast, plus added vitamins, minerals, and salt.

2.2. ZESPRI GOLD Kiwifruit samples

The ZESPRI GOLD Kiwifruit puree and 40° Brix Juice concentrate were supplied by ZESPRI Group Ltd. To prepare the puree, fruit was pureed and passed through a screen to remove seeds and skin; and the puree was heated to 68°C, held at temperature for 2 minutes, and then immediately cooled. To prepare the 40° Brix Juice concentrate, fruit was pulped, enzyme treated, and pressed to squeeze out the juice; pasteurized to 98°C; held at temperature for 30 seconds; and then concentrated to 40° Brix. Samples were stored at −80°C until use. Analyses for minerals and vitamins were conducted by a commercial food analysis laboratory (AgriQuality Limited, Auckland, New Zealand). Additional nutritional composition measurements were made including folate (Pathwest Laboratory Medicine, Perth, Australia); vitamins A [7], E [18], and C; and total ascorbic acid [19], as well as carotenoid content including *trans*-lutein, zeaxanthin, *trans*-α-carotene, *trans*-β-carotene, and total carotenoid content [7].

2.3. Experimental design

In a single trial, 3 groups of 10 randomly assigned female C57B1/6 mice were fed daily, by oral gavage, with a control