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## Abstract

Evidence suggests that diets rich in fruits and vegetables boost the body's natural defences against diseases caused by infection. Gold kiwifruit is rich in vitamin C and contains several phytochemicals that may influence immune function. The effect of consumption of ZESPRI® GOLD kiwifruit on the incidence, and symptoms of upper respiratory tract infection (URTI) have been investigated in children attending local crèches and play-centres. In a randomised, crossover trial, 66 children (aged two to five years) were randomised into one of two groups following a 2-week washout period and consumed an equivalent of 2 servings of gold kiwifruit (group A) or 2 servings of banana (group B) daily for 4 weeks. This was followed by a 2-week washout period and a cross-over of the treatments i.e. group A consumed 2 servings of banana and group B consumed 2 servings of gold kiwifruit for a further 4 weeks, followed by a final 2-week washout period. Parents completed a daily questionnaire of URTI symptoms, the validated Canadian Acute Respiratory Illness and Flu Scale (CARIFS), which assessed the incidence of cold-and flu-like illnesses and the severity of those symptoms. The fruit and vegetable consumption of the children along with their food liking was also investigated at baseline and at the end of the intervention. Additionally, children's fruit and vegetable eating habits along with their parent's motivation to provide them with fruits and vegetables were also investigated. The odds ratio of having a cold- or flu-like illness was 0.55 (95% (0.32, 0.94),  $P=0.03$ ) for the kiwifruit intervention compared to the banana intervention. The sum of total URTI symptoms scores over the treatment periods were significantly lower for the kiwifruit treatment compared to the banana treatment ( $P<0.05$ ), along with the incidence of certain URTI symptoms (including cough, headache, and feeling unwell). Children's fruit consumption did not differ from baseline to end but their kiwifruit liking scores improved. There was a strong positive correlation between children's fruit and vegetable consumption and parents' motivation to provide them with fruits and vegetables ( $r$  values ranged from 0.22-0.61). In conclusion; Children had a lower incidence of cold- and flu-like illnesses during the kiwifruit intervention phase of the trial compared to banana phase. Some of the URTI symptoms were significantly less during the kiwifruit intervention phase compared to the banana phase. Regular consumption of Zespri® Gold Kiwifruit during the cold & flu season may reduce the incidence or symptoms of URTI in children.

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# Chapter 1

## 1.1 Introduction

The “common cold” seems to be appropriately named because it is in fact the most “common” cause of respiratory illness and the most “common” reason people seek primary care (Proud and Chow, 2006, Eccles, 2005, Purssell, 2009, Wald *et al.*, 1991). It does not denote a precisely defined disease, but it is a familiar illness to practically everybody (Proud and Chow, 2006, Eccles, 2005). The common cold is an upper respiratory tract infection (URTI) illness caused by more than 200 viruses, the most common of which is the rhinovirus (responsible for 35% of common cold and flu episodes) (Eccles, 2005).

The common cold presents a major disrupt to daily living activities and it is associated with direct and indirect costs related with health care and loss of productivity through missed work and school days (Wald *et al.*, 1991). It is also one of the most common causes of morbidity around the world (Wald *et al.*, 1991). Therefore a cure for the common cold would be of a great therapeutic and economic value to everyone. However, in spite of the current vast advances in medical treatment, the cure for the common cold has remained elusive (Eccles, 2005).

## 1.2 Justification of the study

Eating “5+ a day” servings of fruit and vegetables is commonly recommended worldwide and in New Zealand (<http://www.5aday.co.nz> (accessed December 2010)) to achieve general health and wellbeing. It is generally assumed that fruit and vegetable consumption will

contribute to reduction in the susceptibility to URTI including cold-and flu-like illnesses (Meneghetti, 2007, Brown, 2010, Li and Werler, 2009) since fruits and vegetables are rich dietary sources of a variety of vitamins, nutrients, and bioactive compounds (Ministry of Health, 2010).

Numerous trials have investigated the effects of vitamins and minerals as a treatment or as a preventive measure for URTI (Douglas *et al.*, 2007, Hemila *et al.*, 2006, Hemila *et al.*, 2002). The most common and comprehensive of these studies are in regards to the role of vitamin C in the treatment or prevention of the common cold. A recent Cochrane (Douglas *et al.*, 2007) review has found that regular supplementation with vitamin C compared to placebo had no effect on the incidence of the common cold with the exception of participants exposed to severe physical stress or extreme physical environments (Hemila, 1999). However, the authors of the review found that regular supplementation with vitamin C alleviated the symptoms of the common cold (Douglas *et al.*, 2007). These findings indicated that there was a dose-response effect and that higher doses of vitamin C (e.g. 600 mg or more) were more beneficial than lower doses (200mg or less) (Douglas *et al.*, 2007). All of these studies used vitamin supplementation rather than food sources of vitamin C and most of these studies included adult subjects with the exception of a handful of studies dating back to the late seventies on children (<14 years old) (Karlowski *et al.*, 1975, Ludvigsson *et al.*, 1977, Martin *et al.*, 1982). The data indicate that children benefited more than adults from vitamin C supplementation (Hemila, 1999).

Vitamin E was the second most commonly studied vitamin in relation to the common cold (Graat *et al.*, 2002, Hemila and Kaprio, 2004, Hemila and Kaprio, 2008, Hemila and Kaprio, 2008). This is possibly due to the proposal that vitamin E supplementation may prevent or

may be beneficial against infection (Beck *et al.*, 2004, Chavance *et al.*, 1989, Ravaglia *et al.*, 2000, Vogel *et al.*, 1997, Wu *et al.*, 2006), which is largely extrapolated based on findings from *in vitro* immunological studies and animal studies (Beck *et al.*, 2004, Field *et al.*, 2002, Gay *et al.*, 2004, Hayek *et al.*, 1997, Lee and Wan, 2000, Meydani *et al.*, 2005). The small number of human studies, investigating the effects of vitamin E on URTI, have been mostly limited to elderly subjects over sixty years of age and the findings from them have been inconclusive (Chavance *et al.*, 1989, Graat *et al.*, 2002, Harman and White-Miller, 1986, Hemila and Kaprio, 2004, Hemila *et al.*, 2004, Hemila *et al.*, 2006). Similarly, studies investigating the relationship between  $\beta$ -carotene supplementation and the common cold have been inconclusive (Hemila *et al.*, 2002, Hughes, 1999, van der Horst-Graat *et al.*, 2004, Vogel *et al.*, 1997).

It is sometimes assumed that antioxidants including vitamins such as C, E, and beta-carotene might have a reliable unidirectional wide-ranging benefit for the human system by protecting it from free radicals (Ames *et al.*, 1993, Halliwell, 1996). The discrepancy of results in the literature that supplementation of certain vitamins and antioxidants may significantly increase, decrease, or have no effect on the risk of the common cold depending on a number of modifiers, is inconsistent with the concept of uniform benefits from antioxidant supplementation. Whole fruits and vegetables provide a natural balance of multiple nutrients and bioactive compounds which may support and improve immune function against exogenous invaders such as viruses in a complementary and synergistic way (Lampe, 1999, Jacobs *et al.*, 2009). Therefore using a nutritional intervention with a fruit that is rich in vitamins and minerals and bioactive compounds, may produce a better outcome than using single nutrients or vitamins.

Zespri® Gold kiwifruit (*Chinesis Actinidia* ‘Hort 16A’) is an excellent source of vitamin C (105.4mg/100g fruit), potassium (316mg/100g), vitamin E (1.49mg/100gm), folate (34mg/100gm), and carotenoids (43mg/100g) (USDA National Nutrient Database for Standard Reference, Release 22 (2009)). Additionally, kiwifruit has been shown to promote immune support in *in vitro* studies and *in vivo* animal models (Chang, 2009, Collins *et al.*, 2001, Farr *et al.*, 2008, MA *et al.*, 2006, Molan *et al.*, 2008, Shu *et al.*, 2008). Furthermore, the lower oxalate and actindin content and the mild taste of gold kiwifruit varieties make it more acceptable to some consumers than other varieties (Ferguson, 2003, Rush *et al.*, 2002). This, along with the reduced associated side effects such as laxative effects make gold kiwifruit an ideal choice for a nutrition intervention particularly in children (Ferguson, 2003, Rush *et al.*, 2002).

Children under the age of five make an excellent target population for an URTI study. The incidence of URTI is at its highest in this age group (six to eight colds per year) (Proud and Chow, 2006). One study estimated that preschoolers suffer “cold-like” symptoms 23.4% of the time (Tarafder *et al.*, 2009). This high prevalence of this illness is largely due to the developing nature of children’s immune system (Bramley *et al.*, 2009).

This research aimed to investigate the effects of consuming two servings of Zespri® Gold kiwifruit on the incidence and symptoms of URTI in preschoolers attending crèches or play centres. Banana was used as the control fruit in the study because it has relatively similar energy content, but lower nutritional value than gold kiwifruit with respect to most minerals, vitamins and carotenoids (<http://www.nal.usda.gov/fnic/foodcomp/search/>) (accessed 11/08/2010). The rationale for using another fruit as the control (placebo), was to determine

whether changes to symptoms of URTI or incidence was fruit specific or due to improved general wellbeing.

Children's fruit and vegetable consumptions were also investigated at baseline and upon completion of the intervention to assess if the study impacted on their normal fruit and vegetable consumption. Additionally, a secondary purpose of this study was to investigate the participating children's fruits and vegetables consumption, liking, and habits and their relation to the parents' motivation to providing their children with fruits and vegetables.

### **1.3 Purpose of the Study**

#### 1.3.1 Aim

To investigate the effect of consuming gold kiwifruit on the incidence and symptoms of URTI in preschool children.

#### 1.3.2 Objectives

##### 1.3.2.1 Primary Objective

To compare the effect of consuming either two servings of gold kiwifruit or banana per day for five days a week over a four week period on the incidence and symptoms of URTI in preschool children aged two to five years attending crèches or play centres in Auckland.

### 1.3.2.2 Secondary Objectives

To investigate the fruit and vegetable consumption habits of the children in the study, along with their fruit and vegetable liking and their parents' motivation towards providing fruits and vegetables.

### 1.3.3 Hypothesis

H<sub>1</sub>: It is hypothesised that the consumption of two servings of gold kiwifruit daily compared to two daily servings of banana will reduce the incidence and symptoms of URTI in two to five year old children.

## 1.4 Structure of the thesis

The literature will be reviewed in chapter 2 regarding the effects of fruit and vegetables or fruit and vegetable components and the common cold. Chapter 3 will describe the materials and methods used in our investigation. This will be followed by chapter 4 where the results and outcome of the intervention will be reported. The findings will be discussed in chapter 5. Finally Chapter 6 will summarise the study, its strengths, limitations, and a conclusion and recommendation for future studies will be made.

# Chapter 2

## 2. Literature Review

When health professionals consider the serious health problems facing our community and country, diseases such as obesity, heart disease, and cancer come to mind as a result of their direct association with mortality. Therefore it might seem rather bold to include the common cold in this list of serious health problems. Upon consideration of the nature of cold and influenza(flu)- like illnesses, the magnitude of their associated costs, the frequency of occurrence (adults suffering from two to four colds per year, and children suffering from six to eight colds per year) (Proud and Chow, 2006) and the associated risks of further complications, the true enormity of cold and flu-like illnesses becomes apparent (Eccles, 2005, Purssell, 2009, Wald *et al.*, 1991). This is a serious health problem facing every community in New Zealand and worldwide (Wald *et al.*, 1991). Moreover, who would not exult to have a relief from those exasperating symptoms like coughing, sore throat, runny nose and headache?

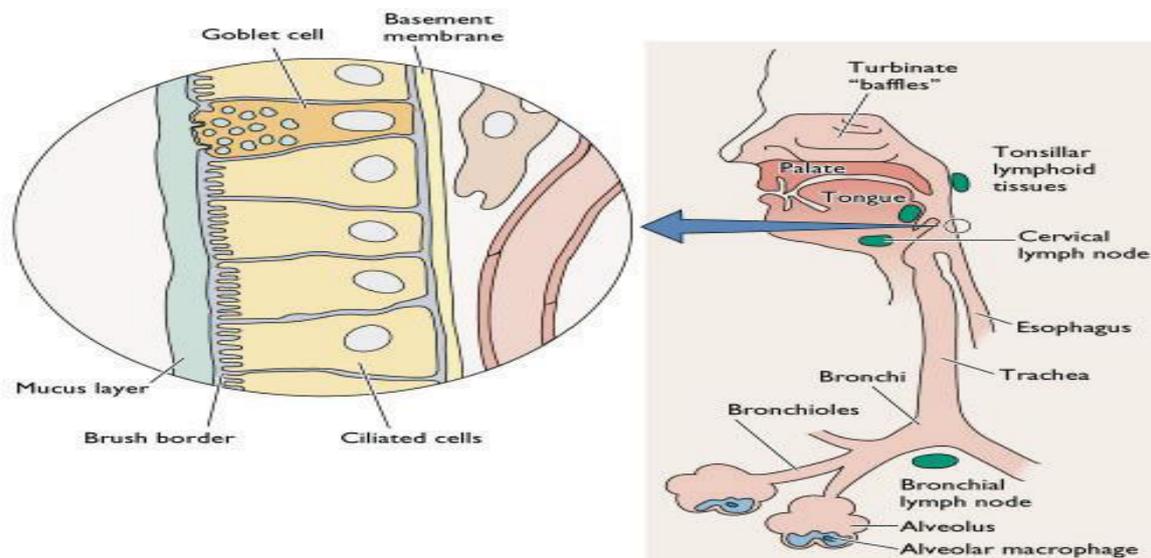
Currently there is no known cure for the common cold and the treatments focus on symptomatic relief (Turner, 2001). Treatments include rest, drinking extra fluids, and using over-the-counter cold remedies, as well as alternative treatments including supplements like vitamin C, Echinacea, or Zinc- containing products. The efficacy of these treatments have not been proven in human clinical trials yet they remain very popular nonetheless (Bukutu *et al.*, 2008). Another alternative therapy that has always been associated with treatment of the

common cold is the use of a dietary intervention such as eating more than 5 servings of fruit and vegetables per day. This is commonly recommended to reduce the susceptibility to common respiratory infections such as cold and flu-like illnesses (Meneghetti, 2007, Brown, 2010). The consumption of a diet rich in fruits and vegetables has long been associated with health benefits (Lampe, 1999). High intakes of fruits and vegetables have been found to be associated with a lower incidence of flu-like illnesses in Japanese children (Hirota *et al.*, 1992) and with fewer episodes of acute respiratory tract infections in the general Australian population (Douglas and Muirhead, 1983). Another yearlong study in the United States found that students who were given an apple a day had fewer cold episodes (Averill and Averill, 1968), while another similar study in adolescents found that orange juice reduced the incidence of respiratory symptoms from nasally inoculated rubella virus infection and the incidence of experimental rhinovirus infection (Ganguly and Waldman, 1977). There is considerable epidemiological evidence linking fruit and in particular citrus fruit with respiratory health (Farchi *et al.*, 2003, Fortes *et al.*, 2000, Forastiere *et al.*, 2000). Whole fruits provide a natural balance of numerous nutrients, vitamins, and bioactive compounds, which may improve host immune function against exogenous bacterial or viral attacks in a complementary synergistic manner (Lampe, 1999, Jacobs *et al.*, 2009). This approach may yet prove to be more effective than the single nutrient supplementation approach widely described in the scientific literature (Jacobs *et al.*, 2009). The current chapter includes a discussion on the feasibility of consuming gold kiwifruit as an affordable and accessible alternative therapy to combat the common cold. Evidence from the literature will be examined in regards to the health benefits of gold kiwifruit or its components, and in relation to combating upper respiratory tract infection.

To introduce the subject a short review of the respiratory tract, the immune system and upper respiratory tract infections in children is presented.

## 2.1 The respiratory tract

The respiratory tract can be divided into two parts, the upper respiratory tract and lower respiratory tract (Campbell and Reece, 2004). The upper tract comprises the ear, nose, throat tonsils, pharynx and sinuses, while the lower tract comprises the trachea, the two bronchial tubes, the bronchioles, and the lungs (Campbell and Reece, 2004) (refer to figure 2.1). Upper respiratory tract infection (URTI) is a viral infection the upper respiratory tract, and it is one of the most common infections in the world, while lower respiratory tract infections are generally more serious than their upper respiratory tract infection counterparts (Campbell and Reece, 2004).



*The respiratory tract can be divided into two parts, the upper respiratory tract and lower respiratory tract. The upper tract comprises the ear, nose, throat tonsils, pharynx and sinuses, while the lower tract comprises the trachea, the two bronchial tubes, the bronchioles, and the lungs*

Figure 2.1 The upper respiratory tract and its components (Campbell and Reece, 2004)

The upper respiratory tract has a multitude of defence systems because of its constant contact with the exterior environment including air that potentially contains pathogenic organisms and other disease causing substances (Janeway *et al.*, 2005). These defense systems include physical barriers such as hair in the nostrils, which serves to filter the air and trap particles together with the mucus layer so that they can then be removed through coughing or sneezing, secondary lymphoid organs such as tonsils and mucosa associated lymphoid tissue (MALT), and the extensive blood supply, which allows for utilization of the innate and adaptive immune responses (Albers *et al.*, 2005, Tregoning and Schwarze, 2010). Hence, the respiratory tract has a significant degree of protective mechanisms and defense systems to prevent and respond to the continuous invasion of harmful factors (Wilson, 2004, Rouse and Sehrawat, 2010).

### 2.1.1 The immune system

The immune system is made up of a collection of complex and highly interactive networks of cells and plasma proteins, with its primary function being to protect against external disease-promoting factors (Fahey *et al.*, 1998, Rouse and Sehrawat, 2010). The system has two components; innate (natural and non-specific) and adaptive (acquired and specific) immunity (Abreu and Arditi, 2004, Medzhitov and Janeway, 2000, Medzhitov and Janeway, 1999, Janeway and Medzhitov, 2002, Medzhitov, 2001). There is a redundancy in the mechanisms to ensure a robustness to fight off infection (Medzhitov and Janeway, 2000).

The innate (first line of defence) component of the system is represented by a sub-set of immune cells that recognise and respond to foreign intruders and any infectious agents

(Chaplin, 2010). The adaptive (second line of defence) component is capable of mounting a vigorous and effective response on re-exposure to a specific foreign intruder (Schnare *et al.*, 2001). Hence the adaptive response has an element of memory to it, but may take three to five days to respond if the pathogen is new, whereas the innate immune response occurs immediately. There are several cell types that are involved in mounting an immune response: including phagocytes, dendritic cells, lymphocytes, and neutrophils. Phagocytes and dendritic cells are mainly involved in the innate immune response and are able to recognise pathogens based on the conserved molecular structures of microbes. Phagocytes then engulf, kill and dismantle the microbes (Medzhitov and Janeway, 2000). Dendritic cells play a role in the identification of microbes and the expression of microbe specific antigens on their cell surface, allowing recognition by lymphocytes, and induce further innate and adaptive immune responses (Medzhitov and Janeway, 1999).

Lymphocytes are for the most part, while not exclusively, involved in the adaptive immune response. They are comprised of two major types; T cells and B cells (Wan, 2010, Janeway *et al.*, 2005). T-cells primarily differentiate in the thymus and are able to recognise ‘foreign’ antigens, presented by dendritic cells, and play a central role in the control and development of immune responses. B cells, which develop in bone marrow, recognize antigens and produce antibodies in response to those antigens (Medzhitov and Janeway, 2000). In both cases the outcome is an amplification of the immune response resulting from the recognition of the foreign antigen (Medzhitov and Janeway, 2000).

T and B cells have a vast and diverse collection of antigen-specific surface receptors (a result of random re-arrangement of a limited number of receptor genes during lymphocyte differentiation) and are thought to be able to recognise 10<sup>11</sup> different antigens (Janeway *et al.*, 2005). Mature lymphocytes are released from the thymus and bone marrow and migrate to different secondary lymphoid tissue such as the spleen, lymph nodes and MALT, including the tonsils (Janeway *et al.*, 2005). Antigens are carried into lymphoid tissues where they may then be recognised by specific lymphocytes.

However, if an associated antigen is not detected by the lymphocytes, it will then be re-released back into the blood for further recirculation to a new site (Rouse and Sehrawat, 2010, Chaplin, 2010, Wan, 2010). This process allows for continuous surveying of the body for new infections. On the other hand, if a 'foreign' antigen is detected, this will lead to the activation of the lymphocyte. Once the lymphocyte is activated it will cease migration, enlarge and proliferate rapidly so that within 3-5 days there are sufficient daughter cells with the required antigen receptors to mount an effective immune response (Rouse and Sehrawat, 2010, Chaplin, 2010, Wan, 2010).

B cells form the humoral immune response (i.e. circulating antibodies or immunoglobulins specific for the antigen in question), which assists with the recognition of pathogens. T cells are responsible for the cell-mediated response, which involves the production of cytokines by T-helper cells to induce an appropriate defensive response and the generation of effector-cells such as cytotoxic lymphocytes (Schnare *et al.*, 2001).

T-helper cell responses can be categorised as Th-1 or Th-2 (Adkins *et al.*, 2004, Schnare *et al.*, 2001) type responses according to their cytokine profile. Th1-type responses tend to induce cell-mediated immunity (i.e. cytotoxicity, phagocytosis, etc), whereas Th2-type responses are typically associated with a humoral (production of antibodies) and allergy responses (Adkins *et al.*, 2004, Schnare *et al.*, 2001).

Another type of lymphocyte that is involved in the innate response is the natural killer (NK) cell, which represents approximately 10-15% of lymphocytes (Abreu and Arditi, 2004). NK cells primarily identify and destroy virus-infected and specific tumour cells by releasing their toxic granule content (Schnare *et al.*, 2001). They are also involved in the initial cytokine cascade which leads to the activation of appropriate effector cells (Abreu and Arditi, 2004).

### 2.1.2 The immune system of children

The unique nature of a child's immune system critically impacts on their susceptibility to respiratory viral infections (Adkins *et al.*, 2004). This nature is characterised by what can be construed as a general naivety of the immune system in the early years of life. Part of this naivety stems from a lack of immunological memory, due to limited or lack of exposure to pathogens, while the rest stems from a general reduction in innate and adaptive immune responses associated with an immature immune system (Adkins *et al.*, 2004). This tendency towards hypo-immune responses is a paramount adaptation to survive early onset exposure to first encounters with non-pathogenic antigens of both 'self' and 'foreign' origins (Bjorksten, 1999).

Before birth and in neonates the developing immune system is associated with a strong predisposition in favour of Th2 cytokines (humoral) rather than Th1 cytokines (Adkins *et al.*, 2004, Levy, 2007). Studies suggest that Th1 cytokines are harmful in pregnancy and can lead to damage of the placenta either directly or through activation of cytotoxic pathways (Bjorksten, 1999, Adkins *et al.*, 2004). This Th2 skewing extends into early childhood and can predispose the child to infection (Levy, 2007). In addition, there are several mechanisms that are still underdeveloped in the child's immune system such as underdeveloped cell-mediated immunity, inability to produce certain iso-types of immunoglobulins, inflammatory cytokine responses and most importantly, defences against intracellular pathogens (Levy, 2007). Table 2.1 below summarises the differences between the infantile immune system and the adult immune system (Bjorksten, 1999).

Table 2.1      Function of the neonatal immune system in relation to that of adults  
(Bjorksten, 1999)

The difference of function between the neonatal immune system and the adult immune system	
T cells	<ul style="list-style-type: none"> <li>● Increased proportion of naive cells</li> <li>● Decreased cytotoxic capacity</li> <li>● Decreased helper function</li> <li>● Impaired cytokine production</li> <li>● More easily Th2 skewed</li> </ul>
B cells	<ul style="list-style-type: none"> <li>● Defective isotype switching</li> <li>● Defective affinity maturation</li> <li>● Decreased antibody secretion</li> </ul>
Antigen-presenting cells	<ul style="list-style-type: none"> <li>● Defective Th1-inducing capacity</li> <li>● Decreased cytokine production</li> </ul>
Granulocytes	<ul style="list-style-type: none"> <li>● Defective neutrophil function</li> <li>● Eosinophilia common</li> </ul>

Infants receive some protection from URTI, such as the common cold and flu, at birth from maternal antibodies, which are transferred *in utero* from mother to foetus (Bjorksten, 1999). As a result a gradual loss occurs over time, however this protection can be extended through breast feeding (Duchén, 1999).

By one year of age virtually all maternal antibodies will have been depleted, resulting in a window of susceptibility after 6 months of age between the time when there is a loss of maternal antibodies and when the infant can produce their own antibodies and their immune system is sufficiently mature to effectively protect them from infections (Wold and Adlerberth, 1998). Young children appear to be more susceptible to URTI than adults (Nordlie and Andersen, 2002). This may be associated with various factors e.g. their close contact with adults and other children, their inability to practise good personal hygiene such as hand washing, and covering coughs and sneezes. They are certainly the primary reservoir for cold viruses (Gwaltney and Ruckert, 1997, Jackson and Dowling, 1959).

## **2.2 Upper Respiratory Tract Infections**

### **2.2.1 Causes**

The clinical condition of URTI is defined as a heterogenous group of diseases caused by more than 200 diverse viruses (Tregoning and Schwarze, 2010). These viruses include adenoviruses (AV), coronavirus (CoV) (causing 10-15% of URTI), enterovirus (EV), human metapneumovirus (hMPV), influenza virus (IV) (causing 5-15% of URTI), parainfluenza virus (PIV), rhinovirus (RV) (most common, causing 30-50% of all colds), respiratory

syncytical virus (RSV) and recently identified “new” viruses such as bocavirus (BoV), and polyomaviruses (Tregoning and Schwarze, 2010).

### 2.2.2 Clinical presentation and symptoms

The clinical presentation of URTI such as the common cold is subject to the type of infecting virus, however, other factors such as age and immunological experience, nutrition and physiological status, and perhaps even genetic factors, may also influence the presentation of symptoms at onset (Eccles, 2005). This results in a wide variation of symptoms and/or severity, and in turn, makes it difficult to have an exact definition for the syndrome of the common cold and flu (Tyrrell *et al.*, 1993).

In general, URTI tends to be a short mild illness, including common early symptoms such as sneezing, sore throat, headache and general lethargy, and usually resolves itself within 24-48 hours (Tyrrell *et al.*, 1993). This is followed by other more intense symptoms such as nasal stiffness, discharge, cough, hoarseness and low grade fever (Eccles, 2005, Tyrrell *et al.*, 1993, McChlery *et al.*, 2009). The severity of the latter symptoms tend to peak around day 3 or 4 and are resolved by day 7-10 (McChlery *et al.*, 2009). The nasal discharge, at the peak of illness, can become thick and postulant and often is incorrectly diagnosed as a bacterial sinus infection (McChlery *et al.*, 2009). Common cold and flu symptoms are illustrated in table 2.2.

Table 2.2 Typical symptoms associated with the common cold (adapted from (Eccles, 2005))

Typical symptoms	Description
Sore throat	A scratchy sensation of throat irritation. Often the first symptom of a URTI
Sneezing	A prominent early symptom associated with URTIs (like sore throat)
Nasal congestion	A later symptom of URTIs It increases in severity over the first week of symptoms
Sinus pain	The origin of sinus pain may be related to several factors e.g. pressure changes in the sinus air space or in the blood vessels draining the sinus
Watery eyes (Epiphora)	A common symptom (especially in children) associated with allergic and infectious rhinitis
Cough	A common symptom associated with URTIs It may persist for 3 weeks or more
Headache	A common early symptom associated with URTIs.
Chilliness and fever	A sensation of chilliness is an early symptom of common cold, and is sometimes explained as an initial stage of fever, since vasoconstriction of skin blood vessels may cause a fall in skin temperature that is perceived as chilliness It is a symptom that is frequently identified in children Adult cases are rarely accompanied by fever

*\*URTIs; Upper Respiratory Tract infection*

### 2.2.3 Diagnosis

The diagnosis is typically made clinically, however, four different techniques can be used to confirm clinical diagnoses; namely: virus culture, serology, antigen detection, and PCR-based tests (Tregoning and Schwarze, 2010). The diagnostic methods are beyond this review.

Nevertheless, it is worthwhile mentioning that these methods are rarely used to validate the clinical diagnosis due to factors such as cost, labour intensiveness, and time required with some methods taking up to 10-14 days to produce results, by which time the infection may be resolved (Tregoning and Schwarze, 2010). In addition, knowledge of the infectious agent rarely alters the course of treatment (Tregoning and Schwarze, 2010) perhaps with the exception of saving unnecessary antibiotics prescription. The common cold has been documented as the second most common reason for antibiotic prescription in general practice (Jacobs *et al.*, 2000, Arroll and Kenealy, 2002). It is more appropriate to focus on ways to reduce symptoms unless a secondary bacterial infection becomes established such as a streptococcal throat infection or sinusitis.

### 2.2.4 Pathology

The symptoms of URTI are manifestations of a viral infection (Tregoning and Schwarze, 2010). These viruses are transmitted to an individual either by direct contact or as large particle aerosols (Tregoning and Schwarze, 2010). After a 48-72 hour incubation period, the virus infects the nasal epithelial cells. It is thought that the host's immune response to the virus plays the main role in its pathogenesis with the immune response to infection being the main factor causing the symptoms (Conti *et al.*, 2004, Proud *et al.*, 1988). The body's immune cells are attracted to the site of infection and release a complex mixture of pro-inflammatory cytokines and mediators, which leads to the symptoms of the common cold.

The patho-physiological mechanisms underlying the common cold are beyond this review, however, these are described in great detail in a review by (Eccles, 2005).

### **2.3 Epidemiology of cold and flu and burden of disease**

The statistics on the epidemiology of URTI are probably not accurate because a great number of these infections are treated at home and likely are unreported (Purssell, 2009).

Nonetheless, the common cold is a very prevalent infection in humans (Eccles, 2005). In New Zealand, the highest prevalence of this illness occurs during the winter months of May to October, with the incidence being at its highest for children and in particular those between one and four years of age (Ardagh, 2006, Blackmore, 2005, Cross *et al.*, 2009, Curry *et al.*, 2006). In an American study by Tarafder *et al.*, of 273 toddlers attending 52 day-care centres, it was found that cold and flu-like illness were by far the most prevalent illness in this age group and 10 times more prevalent than the next frequent illness, namely diarrhoea (Tarafder *et al.*, 2009).

While most URTI are mild, self-limiting, and have a low mortality rate, the overall disease burden is still considerable because of its high prevalence ensuing an associated economic cost for the general population or an increased risk of complications for certain subgroups of the population (Proud and Chow, 2006, Proud *et al.*, 1988, Proud, 2008). The centre of disease control and prevention in the United States (US) estimates that 22 million school days are lost annually in the US due to cold and flu-like illnesses (Proud, 2008). In addition, the cost of such illness has been estimated around the world. In the US it is estimated to be between US\$140 (Ehlken *et al.*, 2005) to US\$240 (Lambert *et al.*, 2008) per case, with an annual cost of US\$40 billion (Lambert *et al.*, 2008). A study in Germany estimated the

annual cost of cold and flu-like illness in children to be around US\$213 million (Ehlken *et al.*, 2005). These estimates include the financial impact of medical cost, days of work, and the possibility of severe complications for at-risk groups.

As stated earlier, the incidence of cold and flu-like illness is at its highest in children. They suffer far more cold and flu illnesses than any other subgroup of the population. A manipulation of diet may provide a good natural alternative therapy if it reduces the rates of infections.

## **2.4 The role of nutrients in the prevention of cold and flu**

### **2.4.1 Antioxidants**

Over the past decade or so, consumers have become aware of the term “antioxidants” and it is often associated with human health (Edeas, 2009, Verhagen *et al.*, 2010, Watanabe *et al.*, 2009). A dietary antioxidant, as was defined by the Food and Nutrition Board at Institute of Medicine (IOM) of the National Academies USA, is “a substance in the food that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both, on normal physiological function in humans”(Institute of Medicine, 1998). Antioxidants have been associated with positive effects against cancer (Halliwell, 1996, Young and Woodside, 2001), degenerative diseases (Ames *et al.*, 1993), cardiovascular illness (Harris and Kris-Etherton, 2010, Obrenovich *et al.*, 2008, Pandey and Rizvi, 2009), and modulation of the immune system (Webb and Villamor, 2007). Vitamin C, vitamin E, and carotenoids are among those antioxidants that have received attention regarding their health-promoting roles in combating illnesses and/or supporting an optimal immune function (Webb and Villamor, 2007), in particular their role in supporting the immune system and

warding off infections like cold and flu-like illness (Wintergerst *et al.*, 2006, Rao and Rao, 2007, Hemila and Kaprio, 2004).

#### 2.4.1.1 Vitamin C

Vitamin C, also known as ascorbic acid, is a water soluble vitamin found in many fruit and vegetables at varying concentrations. Vitamin C deficiency results in the neurological disease of scurvy, as a consequence of its association with production of neurotransmitters and glucose metabolism. It is also necessary for iron absorption, wound healing and collagen formation (Hemila and Douglas, 1999). However, the main interest of this review is the association between vitamin C and any beneficial effects it may have against cold and flu-like illnesses.

Prior to 1970 a small number of physicians had observed that vitamin C administration might be beneficial against the common cold (Hemila, 1992). In fact, even the authors of the renowned Sheffield trial (Arthur *et al.*, 1967) that investigated the effects of vitamin C deprivation in human subjects noted that “common cold episodes lasted longer in the deprived group”. However, these observations did not reach the wide mainstream popularity until Linus Pauling (Mead C and Hagar T, 2008), one of the greatest chemists of the last century and a popular politician wrote a best-selling book titled *Vitamin C and the common cold* (Pauling, 1970). Not only did the book receive the Phi Beta Kappa award for the best scientific book of the year, it also received wide readership amongst the lay community sparking the interest of millions of Americans and people globally. This world-wide interest resulted in increased demands for vitamin C. The resulting increased consumption of vitamin C was said to be associated with a 39 % increase in productivity by 1971 and an annual 18% growth rate (CMR, 1972a). Consuming vitamin C to combat the common cold and /or cope

better with the symptoms remains a popular therapeutic strategy (Bukutu *et al.*, 2008) and a popular topic of scientific investigation to this day.

#### 2.4.1.1.1 The effect of vitamin C on the incidence of the common cold

The prophylactic effect of vitamin C on the common cold has been reviewed extensively. The most notable of these reviews were three Cochrane systematic reviews conducted by Douglas *et al.*, in 2000, 2004 and 2007 (Douglas *et al.*, 2004, Douglas *et al.*, 2000, Douglas *et al.*, 2007). The earliest one of these reviews found that daily supplementation of vitamin C during the cold and flu season did not alter the incidence of the common cold. The initial review examined studies that used dosages as high as 100 mg/day (Douglas *et al.*, 2000). This finding remained consistent across the second and third reviews. The second updated Cochrane meta-analysis examined the effect of a daily dose of vitamin C of 200 mg or more on the incidence of the common cold. It involved 11,077 mostly adult subjects across 29 placebo controlled trials, ascertaining that there was no difference in the incidence of the common cold among those who regularly supplemented with vitamin C compared to those who consumed placebo (Douglas *et al.*, 2004).

Furthermore, the third and most recent meta-analysis review covered 30 placebo-controlled trials involving 11,350 subjects, and the findings supported those of the previous reviews (Douglas *et al.*, 2007). Overall, it appears that almost all of the comprehensive reviews into vitamin C and the common cold showed no marked effect of vitamin C on the incidence of the illness in “normal healthy adults”. The only exception to this were studies conducted with subjects who had less than optimal immune status including athletes, subjects training under extreme conditions, or young children with immature immune systems. Notably, six of the placebo-controlled trials reviewed in the second review of Douglas *et al.* (2004) involved 642

athletes (skiers and marathon runners) and soldiers that were training in sub-arctic conditions. They found that these subjects who supplemented with 200 mg or more per day of vitamin C had a 0.5 reduced risk (95% CI 0.38-0.66) of developing the common cold compared to the placebo group. Furthermore, Hemila in 2004 (Hemila, 2004) also systematically reviewed the prophylaxis effects of vitamin C against the common cold in seven studies of military personnel, in three studies of students living in crowded student accommodation, and in two studies of marathon running athletes. Five of these studies showed a 45%-91% reduction in the incidence of the common cold in those supplementing with vitamin C compared to those in the placebo group, while the remaining seven studies found no statistically significant difference between the vitamin C group and placebo group in relation to the incidence of the common cold. The variation of the effect of vitamin C supplementation on the incidence of the common cold between 'normal healthy subjects' and those 'other' groups could either be as a result of the status of the 'other' subjects' immune system (if the immune system of these 'other' subjects is operating at a sub-optimal level or if it was exposed to severe conditions that require higher than usual immune response) they are more likely to benefit from an additional aid compared to subjects whose immune system is functioning at an optimal level) or as a result of the more controlled environment in which the 'other' subjects studies were conducted. To elaborate further; subjects living in student hostels or military academies are more likely to live under comparable conditions consuming a similar diet, and any differences arising from the treatment compared to the placebo subjects will be observed more easily. The background noise or variation might not allow for a similar observation in studies conducted in the general population or as 'free living' subjects. It is probable that a combination of the two explanations mentioned above, i.e. the sub-optimal status of the immune system of subjects and the more controlled environment of the study, together promote a better insight into any conferred benefits from the vitamin C intervention.

#### 2.4.1.1.2 The effect of vitamin C on the duration and severity of the symptoms of the common cold

Overall, the most promising evidence regarding the beneficial effects of vitamin C in combating cold like illness are in regard to the duration of the episodes of a common cold illness. The strongest evidence from placebo-controlled trials using doses of 200 mg or more per day indicate a reduction in the duration of cold episodes and, moreover those who are immune-limited (either due to their physiological status or due to extraneous environmental factors) such as children and athletes benefited more than healthy adults. The findings also suggest that there is a dose-response effect and that higher doses of vitamin C (e.g. 600 mg or more) were more beneficial than lower doses (200mg or less) (Douglas *et al.*, 2004, Karlowski *et al.*, 1975, Hemila, 1996, Hemila, 1999).

The systematic review conducted by Douglas et al in 2004 (Douglas *et al.*, 2004) covered 12 placebo-controlled trials with children (under 14 years of age) who “regularly” supplemented with vitamin C. Four of these trials used a dose of 200 -750 mg of vitamin C/day and found a 7% reduction on average in the duration of the common cold episodes (95% CI, -19-5). Six of these trials used a dose of 1000 mg of vitamin C/day and found an 18% reduction on average in the duration of the common cold episodes (95% CI, -32--3), and two trials used a dose of 2000 mg of vitamin C/day and found a 25% reduction on average in the duration of common cold episodes (95% CI, -5- 0.1).

Thus, based on these findings the authors calculated a 13.6% reduction in the duration of an episode of the common cold due to supplementation with vitamin C of 200 mg or above/day. While this equates to a reduction of approximately one day in an average 10 day episode of

the common cold for children, it must be noted that this calculation may grossly underestimate the effect of high doses of vitamin C supplementation because stratification of the “regular” supplementation trials also shows a tendency for a vitamin C dose dependency effect on common cold episodes. The authors also calculated a similar estimated reduction for an adult, which is about an 8% total reduction on average in the duration of an episode of the common cold, with trials using doses ranging from 200mg-1000 mg vitamin C/day, and a total reduction ranging from -0.07%-39% in the average duration of an episode of the common cold. Again these calculations in trials using adults are likely to be subjected to the same underestimations as discussed above in trials with children (Douglas *et al.*, 2007).

Supplementations with vitamin C soon after the onset of a cold or flu episode, referred to as “therapeutic” vitamin C supplementation, have also been proposed. Nonetheless, to date there is only one trial that examined “regular” vitamin C supplementation compared to “therapeutic” vitamin C supplementation. Healthy adult volunteers (N=190) working at the National Institute of Health in the United States of America (USA) completed a trial where they were either randomised into a placebo group or a 3000 mg/day vitamin C treatment group (1000 mg of vitamin C taken 3 times daily).

At the onset of an episode of a cold illness, the subjects were given an additional 3000 mg daily dose of either vitamin C or placebo. The investigators concluded that there was no difference between regular or therapeutic supplementation; however they did observe that the 6000 mg/day dose was associated with as much as twice the benefit of the 3000 mg/day vitamin C group on the duration and severity of the common cold. The number of trials examining therapeutic supplementations with vitamin C is limited and the results are inconsistent, most likely due to the wide variation in methodologies used (Douglas *et al.*,

2007). Moreover, these trials were all conducted with adult subjects and to date, there are no trials in children investigating effects of therapeutic supplementation with vitamin C and the common cold.

Treatments of the symptoms of the common cold remain the most popular therapy (Kogan *et al.*, 1994, Kemper, 1998) in spite of the lack of evidence regarding the efficacy of such treatment (Smith and Feldman, 1993, Bukutu *et al.*, 2008). In the most up-to-date review by Douglas *et al.* (Douglas *et al.*, 2007) 15 trials involving 7045 cases of the common cold reported a decrease in the severity scores of the symptoms in subjects regularly consuming 200 mg per day of vitamin C or more compared to the placebo groups, although, the decrease was not statistically significant.

However, a statistically significant decrease in the number of missed work days among those in the vitamin C treatment group were observed ( $p=0.02$ ) compared to placebo treatment, albeit, the actual number of days was not reported. It must also be noted that in studies that supplemented with vitamin C therapeutically, there was no difference in symptom severity or the number of missed days from work or school.

The vitamin C trials reviewed above are not without limitations, which may explain the variations in some of the outcomes. One of the biggest limitations of some of the studies discussed above is not taking into account the subjects' dietary intake of vitamin C. Several dietary studies in the United Kingdom with male subjects suggest that low dietary intake of vitamin C may increase the incidence of cold episodes (Ludvigsson *et al.*, 1977). However, these findings are not always consistent.

In a recent cohort study of 21,795 male smokers drawn from the ATBC study, (Hemila and Kaprio, 2008, Hemila *et al.*, 2004, Hemila *et al.*, 2006), no association was found between low dietary vitamin C and the incidence of the common cold. Another study in the United Kingdom suggested that low dietary vitamin C intake may increase the incidence of cold-like illnesses. In the United Kingdom trials, the subjects were either school students or children, while in Hemila's study the subjects were mostly 50 years of age or older. Therefore, low vitamin C intake may increase the risk of colds in younger subjects only.

Other limitations of some of the vitamin C studies included; the unavoidable subjective nature of the data regarding duration and severity of common cold symptoms, the limited number of subjects in some of the trials, and the self-administration of the treatment in an uncontrolled setting hence restricting an exact measure of compliance.

In summary, the placebo controlled vitamin C trials that were reviewed used at least 200 mg of vitamin C per day. In most of these trials vitamin C was administered as a regular daily pill supplementation. Overall, the trials showed strong evidence that vitamin C shortens the duration of cold-like illnesses and alleviates its symptoms, although this might translate to moderate results in real-life situations.

Evidence discussed above indicates that children are likely to demonstrate a better benefit from vitamin C supplementation than healthy adults because children have immature immune systems and are likely to also benefit from therapeutic vitamin C and for this reason such therapeutic supplementation trials in children are warranted.

#### 2.4.1.2 Vitamin E

Vitamin E also known as  $\alpha$ -tocopherol is a fat soluble vitamin that is rarely found in fruits. Surprisingly, a large amount of vitamin E is found in kiwifruit (about 1.49 mg/100g of fresh weight), albeit there have been some reports suggesting that vitamin E from kiwifruit may not be bioavailable (Ferguson, 2003). Conversely, a recent study by Chang (Chang, 2009) it was found that regular consumption of kiwifruit has been associated with significant increases in plasma vitamin E concentrations. Hunter et al have found (unpublished observations) consumption of Gold kiwifruit “Hort16A” for 4 weeks can provide 2.2-2.6 mg of vitamin E per 100g serving of flesh.

The suggestions that vitamin E supplementation might have an effect on the immune system, and susceptibility to infection, largely dates back to animal-based studies conducted in the 1970s. A report by Sabin and Duffy that was published as far back as 1940 suggests that vitamin E might beneficially affect the severity of viral infection (Stone, 1941). The proposal that vitamin E supplementation may prevent or may be beneficial against infection is largely extrapolated from *in vitro* immunological studies and animal studies. Immune markers measured *in vitro* and in animal studies are only proxies of susceptibility to infection and it is not clear whether these observed immune effects will in fact translate or have any bearing in a clinical setting with human subjects (Gay *et al.*, 2004, Graat *et al.*, 2002, Harman and White-Miller, 1986, Hayek *et al.*, 1997, Hemila and Kaprio, 2004, Hemila *et al.*, 2006, Lee and Wan, 2000, Meydani *et al.*, 2004, Vogel *et al.*, 1997, Wu *et al.*, 2006).

Furthermore, most of the vitamin E trials with human subjects have been limited either by the small number of subjects in the study or by the shortness of their duration (Gay *et al.*, 2004, Graat *et al.*, 2002, Harman and White-Miller, 1986, Hayek *et al.*, 1997, Hemila and Kaprio,

2004, Hemila *et al.*, 2006, Lee and Wan, 2000, Meydani *et al.*, 2004, Vogel *et al.*, 1997, Wu *et al.*, 2006).

On the whole, findings from human studies in regards to the effects of vitamin E supplementation on respiratory infection have been inconclusive (Gay *et al.*, 2004, Graat *et al.*, 2002, Harman and White-Miller, 1986, Hayek *et al.*, 1997, Hemila and Kaprio, 2004, Hemila *et al.*, 2006, Lee and Wan, 2000, Meydani *et al.*, 2004, Vogel *et al.*, 1997, Wu *et al.*, 2006). Although some authors have reported apparent benefits regarding the risk and incidence of common cold infections (Meydani *et al.*, 2004, Liu *et al.*, 2007) other studies found none (Harman and White-Miller, 1986, Chavance *et al.*, 1989), yet others obtained negative outcomes (Graat *et al.*, 2002, Hemila *et al.*, 2006). Overall, the discrepancy of the outcomes from human studies may be explained by the variation in methodology used, the supplementation dose, and the differences in subjects' vitamin E status at baseline, since the differences in baseline prevalence of nutrient deficiency is an important predictor of response (Chavance *et al.*, 1989).

Results from animal studies indicate that while vitamin E can contribute to protection against viral infection it does not appear to do the same for bacterial infections (Hayek *et al.*, 1997, Gay *et al.*, 2004). Cold and flu-like illnesses are usually caused by viruses although on occasions a bacterial infection can also be the underlying cause of the illness. The definition criteria for respiratory infections in the medical literature do not differentiate between viral or bacterial aetiology and most of the diagnostic criteria used in the human vitamin E studies did not include microbiological evaluation due to the associated cost and time limitations.

Therefore the divergence in outcome could be a result of differences in underlying infectious

agents i.e. whether this was of viral or bacterial origin if vitamin E is more effective against viruses but not bacteria in humans as it has found to be in studies on animals.

Meydani et al (Meydani *et al.*, 1997) reported a 30% non- statistically significant reduction in subjects supplemented with 60-800mg/day vitamin E compared to placebo in a small trial with 88 subjects  $\geq 65$  years. The authors then followed this small trial with another larger randomised, double-blind trial, with 617 people aged 65 years and older, receiving either a 200mg/day vitamin E supplement or placebo for one year (Meydani *et al.*, 2004). This was a very rigorous study using published criteria for infection surveillance that trained nurses performed weekly. The findings from this latter trial indicated that subjects in the vitamin E group had a lower incidence of the common cold compared to placebo (0.66 vs. 0.83 per subject per year, RR=0.8, CI=0.64-1.00,  $p=0.046$ ), and fewer subjects in the vitamin E group acquired one or more common cold episodes compared to the placebo group (46% vs. 57%, RR=0.79, CI=0.63-0.96,  $p=0.016$ ).

The vitamin E group also had fewer days with common cold per person per year compared to the placebo group, although the difference was not statistically significant (22% less,  $p=0.11$ ). Yet another trial by Chavance et al (Chavance *et al.*, 1993) reported that a yearlong 30mg/day supplementation with vitamin E in subjects  $\geq 65$  years had no effect on non-specific infection. Moreover, Graat et al (Graat *et al.*, 2002) reported in their 15 month long trial with 652 non-institutionalised subjects  $\geq 60$  years of age that 200 mg/day of vitamin E supplementation had no effect on the incidence of respiratory infection, but vitamin E supplementation unexpectedly increased the number of symptoms ( $p=0.03$ ), the duration of illness ( $p=0.02$ ) and the percentage of participants with fever ( $p=0.09$ ) compared to the

placebo treatment. Therefore, vitamin E supplementation may in fact be harmful in some subjects.

The conflicting results in the literature may reflect differences in the populations studied. For instance, the study by Graat et al 2002 (Graat *et al.*, 2002), included older community dwelling subjects, whereas Meydani's 1997 study focused on institutionalised subjects. This heterogeneity of results in older subjects was also observed in the Alpha-Tocopherol Beta-Carotene (ATBC) study cohort (Hemila *et al.*, 2006). The overall results of the ATBC study showed no effect of 50 mg/day vitamin E supplementation (intervention lasted 5-8 years, 6.1 years median) on the incidence of the common cold (RR=0.99; CI=0.98-1.01) compared to placebo. Although subgroup analysis of the ATBC study showed that vitamin E supplementation was associated with significant reduction in common cold incidence in subjects aged  $\geq 65$  years (RR=0.95, CI= 0.9-1.00), there were no effects in participants younger than 65 years of age. Further investigative analysis of the older subjects  $\geq 65$  years showed that age, smoking and residential neighbourhood were all modifying factors of the effect of vitamin E supplementation on the common cold.

Taking these variables into consideration, the analysis revealed that vitamin E supplementation, in city dwellers aged  $\geq 72$  years who smoked  $< 15$  cigarettes/day, reduced the risk of the common cold (46% less, CI=0.37-0.8), while increasing the risk of getting the common cold (58% more, CI=1.23=2.01) in those who smoked  $> 15$  cigarettes/day and lived outside of the city. The modification of the risk of common cold by age, smoking, and residential environment may be associated with the physiological effects of antioxidants as there is evidence indicating that free radical generation may play an important role in the

pathogenesis of certain viral and bacterial illnesses (Hemila, 1992, Goode and Webster, 1993, Akaike *et al.*, 1998).

#### 2.4.1.3 Carotenoids

Carotenoids are a group of phytochemicals that are responsible for the different orange, yellow and red colours of some plants (Namitha and Negi, 2010). They are one of the most widespread groups of pigments in nature and more than 600 of these have been identified (Namitha and Negi, 2010) and are recognized as playing an important role in the prevention of human diseases and maintaining good health (Rao and Rao, 2007). The most common carotenoids found in western diets are alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and lycopene (Rao and Rao, 2007, Thurnham, 1994). In addition to being potent antioxidants some carotenoids also contribute to dietary vitamin A (Vogel *et al.*, 1997).

$\beta$ -carotene has been studied extensively in regards to its effect on immune response and function; whereas there is little information in regards to the effects of other carotenoids on the immune system (Jyonouchi *et al.*, 1996, Hughes, 1999, O'Neill *et al.*, 2001). The findings from  $\beta$ -carotene studies have been controversial, with some showing beneficial immune effects from  $\beta$ -carotene supplementation or enhanced immune responses at high plasma  $\beta$ -carotene levels (Watson, 1991, Fuller *et al.*, 1992, van Poppel *et al.*, 1993, Murata *et al.*, 1994, Santos *et al.*, 1998, Hughes, 1999) while others have not (Ringer *et al.*, 1991, Daudu *et al.*, 1994).

Although clinical endpoints such as infectious illnesses have much greater public health relevance than simply measuring markers of immune function, studies investigating the

effects of carotenoids intake and supplementation on illnesses are scarce. In one study the relationship between  $\beta$ -carotene supplementation and the common cold was investigated in a cohort of 21,796 male smokers that were drawn from the ATBC Cancer prevention study.

The relationship between dietary  $\beta$ -carotene and 20 mg long term  $\beta$ -carotene supplementation on the incidence of the common cold was investigated (Hemila *et al.*, 2002). Neither dietary  $\beta$ -carotene nor long term  $\beta$ -carotene supplementation had an association with the incidence of the common cold (Hemila *et al.*, 2002). In contrast to this, Graate et al. (van der Horst-Graat *et al.*, 2004) found a beneficial association between plasma  $\beta$ -carotene levels and the incidence of the common cold in elderly subjects. Non- institutionalised subjects (n=652) aged  $\geq 60$  years participated in a retrospective study. Illness incidence and severity of infection over the previous year were self reported by means of a questionnaire and answers were investigated in relation to six major carotenoids ( $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein, and zeaxanthin). No association was observed between  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein, and zeaxanthin plasma levels and incidence or severity of infection. Plasma  $\beta$ -carotene concentration was not associated with severity of illness, however, the incidence rate ratio of acute respiratory infection at high plasma  $\beta$ -carotene concentrations ( $0.83 \pm 0.33 \mu\text{mol/L}$ ) was 0.71 (95% CI 0.54-0.92) as compared with the low  $\beta$ -carotene ( $0.18 \pm 0.05 \mu\text{mol/L}$ ) concentration group (van der Horst-Graat *et al.*, 2004).

The authors of this study state that while their findings suggest that high plasma  $\beta$ -carotene concentrations are associated with a lower incidence of respiratory infection, they do recommend further investigation especially regarding the effect of intake of carotenoids, rich fruits or vegetables on respiratory infection illnesses in a human trial. Boosting plasma levels

with carotenoids through the consumption of fruits, vegetables, or both, provide a safer option, since mega-dose supplementation of  $\beta$ -carotene have been associated with increased mortality in certain subgroups of the population (Hemila *et al.*, 2004).

It is sometimes assumed that antioxidants including vitamin C, vitamin E, and beta-carotene might have a reliable unidirectional wide-ranging benefit for the human system by protecting it from free radicals (Ames *et al.*, 1993, Halliwell, 1996). The discrepancy of results in the literature that certain vitamin and antioxidants supplementation may significantly increase, decrease, or have no effect on the risk of the common cold depending on a number of modifiers is inconsistent with the concept of uniform benefits from antioxidant supplementation.

#### 2.4.2 Other nutrients

Micronutrient status is an important contributing factor to efficient immune function (Lampe, 1999, Larralde and Martinez, 1989, Hughes, 2005, Mazari and Lesourd, 1998, Lesourd, 1997, Samartin and Chandra, 2001, Chandra and Kumari, 1994). Vitamins and minerals like folate, vitamin K, potassium, and copper are all necessary nutrients for proficient immune function (Lampe, 1999, Larralde and Martinez, 1989, Hughes, 1999, Bistran, 2004, Hughes, 2005, Mazari and Lesourd, 1998, Lesourd, 1997, Samartin and Chandra, 2001, Chandra and Kumari, 1994, Gleeson *et al.*, 2004, Hoyles and Vulevic, 2008, Field *et al.*, 2002) but currently there is no evidence in regard to the effects of these nutrients on cold and flu-like illnesses.

## **2.5 The potential role of gold kiwifruit in preventing or relieving symptoms of cold and flu-like illnesses**

### 2.5.1 Introduction

Gold kiwifruit, commercially known as the fruit *Chinesis Actinidia*, is a common and popular fruit in New Zealand. Kiwifruit is a nutrient-dense fruit, on average and per fresh weight basis (wt/wt), kiwifruit has more vitamin C than an orange (approximately 50% more), considerably more than banana, and ten times as much as an apple (Ferguson, 2003). In fact, only a few readily available fruits such as blackcurrants are a richer source of vitamin C than kiwifruit (Ferguson, 2003). . kiwifruit has a similar potassium content to banana (wt/wt), and is a source of carotenoids such as lutein and zeaxanthin (McGhie, 2009, Taylor *et al.*, 2002). Table 2.3 below shows the concentrations of antioxidants and vitamin compounds in kiwifruit and other fruit commonly found in western diets.

Table 2.3 Concentrations of a selection of the antioxidant compounds present in some of the major fruits consumed globally (McGhie, 2009)

Antioxidants component	Kiwifruit	Banana	Orange	Apple	Peaches	Strawberries	Raspberries	Blackberries	Grape
Antioxidant capacity <sup>A</sup> ( $\mu\text{mol TE}/100\text{g}$ )	882	879	1819	3082	1814	3577	4882	5347	1260
Vitamin C <sup>B</sup>	75	8.7	45	4.6	6.6	58.8	26.2	21	4
Vitamin A <sup>B</sup>	175	64	225	54	326	12	33	214	100
Vitamin E <sup>B</sup>	1.46	0.10	0.18	0.18	0.73	0.29	0.87	1.17	0.19
Carotenoids									
Lutein	138	22	92	51	60	17	n.d.	n.d.	49
$\beta$ -carotene	30	38	25	22	84	8	20	78	23
$\beta$ -cryptoxanthin	n.d.	n.d.	266	10	70	n.d.	n.d.	n.d.	n.d.
Flavonoids <sup>C</sup>									
Quercetin	n.d.	n.d.	0.58	4.272	0.68	1.14	1.23	1.76	138
Kaempferol	n.d.	n.d.	0.01	0.02	n.d.	0.46	0.09	0.06	0.01
Myricetin	n.d.	n.d.	0.02	n.d.	n.d.	n.d.	n.d.	0.67	n.d.
Hesperetin	n.d.	n.d.	27.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
Naringerin	n.d.	n.d.	15.32	n.d.	n.d.	0.26	n.d.	n.d.	n.a.
Total ACNs <sup>C</sup>	n.d.	7.39	n.d.	2.44	1.61	33.63	38.38	90.46	44.86
Total PP <sup>D</sup>	28.1	51.5	31.0	179.1	59.3	263.8	51.7	49.5	195.5
Total PA <sup>E</sup>	3.2	3.4	—	127.8	71.8	141.7	25.1	23.3	61.6

<sup>A</sup>- USDA Oxygen Radical Absorbance Capacity (ORAC of selected foods-2007 (<http://fnic.ral.usda.gov/>))

<sup>B</sup>- USDA National Database for Standard Reference Release 18 (<http://fnic.nal.usda.gov/>)

<sup>C</sup>- USDA Database for the Flavonoid Content of Selected Food Release 2.1 (<http://fnic.nal.usda.gov/>) CAN= anthocyanidins

<sup>D</sup>- PP=polyphenols, GAE=gallic acid equivalent

<sup>E</sup>- USDA Database for the Proanthocyanidin of Selected Foods (<http://fnic.nal.usda.gov/>); PA=pranthocyanidins

TE= Trolox® Equivalents; n.d.=no data

Units are  $\mu\text{g}/100\text{g}$  where not specified

Gold kiwifruit “Hort16A” is one of the mildest tasting varieties of readily available kiwifruit in the market and this milder taste is probably due to the fact it contains less oxalate (Ferguson, 2003) . In green kiwifruit, the oxalates are bound in insoluble needle-like crystals of calcium oxalate and are commonly associated with the “catch” in throat experienced when green “Hayward” kiwifruit is eaten (Perera *et al.*, 1990). Some varieties of kiwifruit contain large amounts of a protein called actindin (Ferguson, 2003, Bublin *et al.*, Lucas *et al.*, 2007).

It has been suggested that actindin has laxative effects in some consumers (Chan *et al.*, 2007, Rush *et al.*, 2002) and it may also be associated with the allergic response caused by kiwifruit in 2-3% of the total population, though extreme allergic responses to kiwifruit are uncommon (Bublin *et al.*, Lucas *et al.*, 2007). Nonetheless, actindin is barely detected in cultivars of *Actinidia chinensis* including gold “Hort16A” kiwifruit resulting in hardly any laxative effects (Bublin *et al.*, 2004).

### 2.5.2 Nutrient content

According to Food Standards Australia New Zealand (FSANZ) guidelines ([www.foodstandards.gov.au/](http://www.foodstandards.gov.au/) accessed March 2010) a product can be claimed as a high source of a nutrient if it provides at least 20% of the RDI of the nutrient, while a product may be claimed to be a good source of a particular nutrient if it provides at least 10% or more of that nutrient.

Kiwifruit can be considered as an excellent source of vitamin C, with two medium size kiwifruit (150g) providing an equivalent of 270% RDI of vitamin C. One hundred and fifty grams of gold kiwifruit also contains 19% of the copper RDI, 15% vitamin E and 11% of the folate RDI. It also contains as much potassium as bananas, (13% of RDI, for half the

calories). As mentioned earlier optimal nutrient status is necessary for optimal immune function (Lampe, 1999, Larralde and Martinez, 1989, Hughes, 2005, Mazari and Lesourd, 1998, Lesourd, 1997, Samartin and Chandra, 2001, Chandra and Kumari, 1994). An immune system functioning at optimal levels is, likely, more capable of fighting off or warding off infections and illnesses. This along with new emerging evidence indicating that host nutritional status not only influences host response to pathogen, but can also influence the genetic make-up of the viral genome (Beck *et al.*, 2004), it seems the old nutritional adage “You are what you eat!” is still valid and relevant today.

### 2.5.3 Research on immunological effects

The nutrients provided by kiwifruit as mentioned above, vitamin C, vitamin E, folate, copper, and potassium, are all essential nutrients for efficient immune function (Lampe, 1999, Larralde and Martinez, 1989, Hughes, 1999, Bistrrian, 2004, Hughes, 2005, Mazari and Lesourd, 1998, Lesourd, 1997, Samartin and Chandra, 2001, Chandra and Kumari, 1994, Gleeson *et al.*, 2004, Hoyles and Vulevic, 2008, Field *et al.*, 2002), therefore it is not surprising that the health benefits of kiwifruit have been investigated. There have been a number of human intervention trials with kiwifruit targeting health areas such as oxidative stress, immune support and natural protection, gut health and many other health areas (Beck *et al.*, 2010, Chang, 2009, Rush *et al.*, 2009, Sun-Waterhouse *et al.*, 2009, Rush *et al.*, 2002, Collins *et al.*, 2001, Hunter *et al.*, 2008, Skinner *et al.*, 2008, Chan *et al.*, 2007).

Regular consumption of kiwifruit or kiwifruit juices could be important in protecting against oxidative stress during stressful or normal conditions. Ko et al (Ko *et al.*, 2005) demonstrated that kiwifruit juice consumption enhanced antioxidant capacity of plasma. Ten subjects consumed 150 mL kiwifruit juice and blood was collected at various intervals after digestion.

The antioxidant capacity of plasma was assessed by the rate of inhibition of reactive oxygen species (ROS) generation. This was measured by the level of prevention of oxidation of a non-fluorescent dye 2',7'-dichlorodihydrofluorescein (DCFH) to its fluorescent product dichlorofluorescein (DCF). Kiwifruit juice consumption enhanced antioxidant capacity of plasma within 30 min, and this enhancement was sustained for up to 90 min (Ko *et al.*, 2005). Furthermore, in a similar study consumption of 300g of green 'Hayward' kiwifruit also significantly increased total plasma antioxidant capacity, measured using the Oxygen Radical Absorbance Capacity (ORAC) method, up to five hours post consumption, during which time the authors also observed an accompanied increase in plasma vitamin C (Prior *et al.*, 2007). Nevertheless, while kiwifruit may enhance plasma antioxidant capacity after consumption, the effect of long term supplementation on plasma antioxidant activity and the implication this has on specific health targets is yet to be determined.

Kiwifruit may also play a role in immune support through immune modulation. In an *ex vivo* study using human blood samples from 20 healthy women and men, aged 32-48 years, a 'Hort 16A' water extract from a pasteurised puree, was shown to significantly enhance phagocytosis, oxidative burst response, and NK cell activity (Skinner *et al.*, 2009). The effect of kiwifruit on markers of immune function and modulation has also been investigated in a number of animal studies. Ma *et al.* (Ma *et al.*, 2006) demonstrated using Kunming mice that a high dose of kiwifruit extract supplementation comprising up to 30% of diet improved the lymphocyte transformation and the phagocytosis of phagocytes as well as enhancing serum immunoglobulin levels (IgA, IgG, and IgM).

Furthermore, Shu *et al.* (Shu *et al.*, 2008) investigated the effect of kiwifruit extracts on markers of innate and acquired immunity in a murine mouse model. Using mice (BALB/c), it

was demonstrated that aqueous and supercritical fluid extracts of 'Hort 16A' and 'Hayward' kiwifruit enhanced markers of both innate and acquired immunity in these animals. Overall, enhancing non-specific NK cell activity and cytokine production of interferon- $\gamma$  and TNF (Shu *et al.*, 2008). The influence of feeding two ZESPRI® Gold kiwifruit processed products on gut associated immune function in mice has also been investigated (Hunter *et al.*, 2008). Three groups of ten randomly assigned female mice were fed daily for twenty days, either gold kiwifruit puree or gold kiwifruit 40% brix juice concentrate or a control sample (20% sugar, 1:1 ratio of glucose and fructose). During this time, they were also immunised orally with ovalbumin in conjunction with a sub-optimal dose of the mucosal adjuvant cholera toxin. The oral immunisation with ovalbumin plus sub-optimal cholera toxin was used to create a model to provide a weak adaptive immune response in the gut. The gold kiwifruit puree was shown to significantly enhance this weak adaptive immune response increasing antigen-specific proliferation of cells from the draining mesenteric lymph nodes compared to the gold kiwifruit 40% brix juice concentrate and the control sample. The Kiwifruit fibre, present in the puree but not in 40% brix juice concentrate, was suggested to contribute to the modulation of the gut adaptive immune response by the puree (Hunter *et al.*, 2008). Carotenoids could also be contributing bioactives to these observed immunomodulatory effects (Hunter *et al.*, 2008) because the puree had a much higher level of carotenoids including lutein compared to the 40% brix juice concentrate.

Immune function is strongly influenced by gut microflora, and changes to the microbial populations colonising the gastrointestinal tract may modulate immune function through influencing cytokine production and immunoglobulin levels (Cummings *et al.*, 2004). Kiwifruit is a reasonable source of dietary fibre, because the cell walls of kiwifruit are unusual during ripening and they swell much more than those of other fruit, comprising

approximately 2-3% of kiwifruit fresh weight (Hallett *et al.*, 1992). Various kiwifruit extracts from the edible flesh have stimulated the growth of beneficial gut bacteria and reduced the colonisation of harmful gut bacteria *in vitro* (Molan *et al.*, 2008). The contribution of these effects to health targets, such as supporting the immune system to more effectively combat infection, is yet to be determined in a human intervention setting. Nevertheless, polysaccharides such as glucuronoxylans and xyloglucans have been shown to have effects on the immune system and the dietary fibre in kiwifruit may be a contributing factor to the immune-modularity effects observed *in vitro* and in animal studies (Molan *et al.*, 2008, Lim *et al.*, 2005).

To summarise, kiwifruit is a nutrient dense fruit, and the scientific information supporting the health benefits of kiwifruit is growing rapidly. The antioxidant compounds found in kiwifruit could contribute to the mechanism involved in its health promoting effects. Results from human intervention trials do provide evidence for improved oxidation status of blood after consumption of kiwifruit but it is unclear how such benefit may influence the health benefits of individuals.

Kiwifruit may also play a role in immune support and this has been demonstrated in animal and very limited human trials. It remains to be proven whether regular consumption of kiwifruit may have a beneficial effect on disease outcome. It is likely that the antioxidants and the various bioactives, vitamins and minerals present in kiwifruit work synergistically to support optimal immune function. These unique synergistic combinations in the whole kiwifruit consumed as a food are likely to be more beneficial in supporting optimal health than if compounds, vitamins, or bioactives were consumed individually as supplements.

## **2.6 Methods to investigate the effect of kiwifruit on cold and flu in children**

### **2.6.1 Study design**

Most human based kiwifruit research has focused on cardiac health (Chang, 2009, Rush *et al.*, 2009), or intestinal well-being (Rush *et al.*, 2002, Molan *et al.*, 2008), or supporting the immune system (Skinner *et al.*, 2008, Hunter *et al.*, 2008). There is no available human data on specific disease outcome such as the prevention of cold and flu or their symptoms. These illnesses are the most common afflictions in humans. Further recent studies that call into question the inefficacy of zinc based products (Eby and Halcomb, 2006), and Echinacea (Melchart *et al.*, 2000) make research into cold and flu therapy more important.

Conventional medicine relies on repeated, large-scale randomised controlled trials of standardised design to support the efficacy of various therapies in URTI treatment or prevention. In a recent review by Ryan *et al.* (Ryan *et al.*, 2010), the author thoroughly reviewed standard design elements present in the controlled-trial design of conventional antiviral influenza therapies and provided a recommendation to follow those conventional guidelines for any new URTI treatment. The author argues that in order for a new URTI treatment to be accepted by health professionals as valid and effective it must be subjected to those same rigorous testing standards applied to mainstream therapy such as antiviral flu drugs.

However, when using a dietary intervention such as using a fruit (e.g. kiwifruit) as a therapeutic agent to combat cold and flu-like illnesses there are considerable challenges facing the investigator in terms of intervention trial design and the consistent elements commonly accepted as a standard in trial design may not be applicable in this situation.

Double blind randomised controlled trials (RCT) are the golden standard when it comes to human intervention trials (Fang *et al.*, 2010). While randomisation across subject-blinded study arms is fundamental to account for any accuracy or bias that may arise from such subjective-perception regarding what classifies as a common cold episode. Blinding will always be an issue when using a fruit rather than a pill as the treatment. One way to overcome this obstacle is by giving the subjects another type of fruit and comparing the outcome of the treatment from the two fruits while at the same time not informing the subjects which fruit is of interest to the investigator (partial blinding). A cross over design is also essential when using subjective outcome measures as this allows for subjective confounders to be randomised across both treatments.

Another point raised by Ryan *et al.* (Ryan *et al.*, 2010) in their review is that standardised clinical trials often use laboratory tests to verify the presence of the cold or flu virus. In nutrition intervention trials however, where subjects are often free-living, it is not possible or practical to conduct laboratory tests to verify a cold episode. Instead the definition of a cold episode can be based on self-diagnosis which is usually reliable (Gwaltney and Ruckert, 1997). Although subjective perception of what is classified as a cold episode may vary between participants, such inaccuracy in outcome assessment does not lead to consistent differences between study arms but rather, the inaccuracy renders the difference smaller than they may actually be (Hemila *et al.*, 2006). A randomised cross-over design trial will ensure that the subjective perceptions of participants are spread over both treatments (i.e. each subject acting as its own control or reference point).

In summary, a study investigating the effect of kiwifruit consumption on cold and flu-like illness should therefore be a randomised controlled trial. Every effort must be taken in order

to ensure subject-blinding in regards to the fruit of interest. If subjective outcome measures are used such as questionnaires or self-reported cold and flu episodes then a cross-over design is also necessary to account for any confounders that might arise from the self-reported subjectivity of participants. The study must be long enough and statistically powered to detect differences between treatments and a consultation with a statistician to ensure this is necessary. In addition, as symptoms of cold and flu-like illnesses are the diseases of interest here, the study needs to be conducted over the winter months or during the cold and flu season to obtain best outcomes (Cross *et al.*, 2009).

### 2.6.2 Survey instruments

The most common childhood illnesses are URTI such as cold and flu-like illnesses. Small or young children suffer from URTI considerably more than adults due to their immature immune systems (Bramley *et al.*, 2009). There are a number of treatments that are commonly used by parents and health practitioners in the treatment of childhood URTI, yet, few of these treatments have been validated due to the lack of validated outcome measures.

In a systematic review by Fahey et al (Fahey *et al.*, 1998), 12 studies that investigated treatments of URTI in children were reviewed and it was found that each of the studies used a different method of assessing the effects of treatment on URTI and none used a validated paediatric outcome measure of disease severity. Five studies used outcomes recorded by medical practitioners, although the subjects were all treated as outpatients, and therefore the medical practitioners were likely to have a limited insight into their illness and symptoms (Fahey *et al.*, 1998). While the remaining seven studies used symptom diaries recorded by parents, none of them gave data on the measurement properties of the diaries (Fahey *et al.*, 1998).

There are several challenges involved in the development of paediatric disease assessment tools. Limited language of young children will limit their communication ability (depending on their age) to articulate their complaints hence precluding self-reporting measures and necessitate the use of proxies such as parents, caregivers, or medical practitioners. The illness may also manifest itself through functional problems alone that will have to be reported by these observing proxies (Stein *et al.*, 1987). Children's emotional peculiarities and issues such as growth, development, and relationships with the family all have to be considered when assessing a child's quality of life and their functional ability/disability (Stein and Jessop, 1990). The measures also need to be suitable for assessing severity of outpatient acute illness.

Stein *et al.* (Stein *et al.*, 1987) classifies disease severity measures based on the scope of disease effects, according to four scale levels; biological, clinical, functional, and burden of disease (financial and social). An appropriate disease severity measure of URTI, such as cold and flu illnesses, for this age group therefore has to account or be able to assess all the points mentioned above with a particular emphasis on impact of disease as this is the most relevant point to outpatients. The Canadian Acute Respiratory Illness and Flu Scale (CARIFS) fulfil these needs (Jacobs *et al.*, 2000). The CARIFS is one of very few available instruments for measuring the severity of URTI illness, and to our knowledge it is the only one that has been empirically constructed and formally validated in children  $\leq 5$  years of age (Jacobs *et al.*, 2000).

Consequently it has been used as the primary outcome measure in a number of recent clinical trials with children around the world including the United Kingdom and Australia (Butler *et*

*al.*, 2002, Shepperd *et al.*, 2004, Vohra *et al.*, 2008). CARIFS is based on a conceptual framework that defines illness severity as having three domains; physiological (e.g. cough), functional (e.g. play), and burden of illness (e.g. impact on parents), and it is designed to reflect these domains by measuring three dimensions of childhood illness; symptomless, function, and parental impact (Jacobs *et al.*, 2000). The survey instruments require the caregiver to rate up to 18 aspects of their child's symptoms and behaviour using four response categories; major, moderate, minor, or no problem.

Table 2.5 Symptoms assessed by CARIFS along with their measures of internal consistency (Shepperd *et al.*, 2004).

Scale dimensions and items	Correlation coefficient	Cronbach's $\alpha$ statistics
<b>Symptoms</b>		<b>0.54</b>
Fever	0.39	
Cough	0.21	
Nasal congestion, runny nose	0.27	
Vomiting	0.24	
Feels unwell	0.69	
<b>Function</b>		<b>0.77</b>
Not interested in what's going on	0.55	
Poor appetite	0.46	
Irritable	0.44	
Low energy, tired	0.62	
Not playing well	0.64	
<b>Parental impact</b>		<b>0.7</b>
Needing extra care	0.75	
Clinginess	0.64	
Crying more than usual	0.66	
Unable to get out of bed	0.28	
Not sleeping well	0.23	

Shepperd *et al.* (Shepperd *et al.*, 2004) evaluated the performance of the CARIFS in a European primary care setting (table 2.5). The author concluded that the CARIFS is a good

measure of functional severity (Cronbach  $\alpha$ = 0.77 and correlation coefficient (CC) > 0.5 for functional symptoms) and burden of illness on parents (Cronbach  $\alpha$ = 0.7, and CC > 0.6 for three out of 5 symptoms). It is perhaps a less robust measure of physiological severity (Cronbach  $\alpha$ = 0.5, and all their physiological symptoms had a less than 0.5 CC with the exception of one (feels unwell)). The author concluded that their findings can probably be generalised to other community settings, where the socioeconomic status of the parents and standard of living are similar to the UK and Canada (Shepperd *et al.*, 2004). Overall, the CARIFS appears to be a satisfactory measure across a wide range of illness domains (functional, physiological, and parental impact). The CARIFS has been well validated as a disease severity measure for children's respiratory infections. This measure can be suitable for many research applications including studies of cold and flu-like illnesses and their determinants, treatment studies, and studies investigating the impact of these illnesses on children and their families. As this instrument has been trialled in countries similar culturally and economically to New Zealand (NZ) (Butler *et al.*, 2002, Shepperd *et al.*, 2004), this instrument is likely to be suitable to be used in NZ.

### 2.6.3 Dietary assessments

Dietary assessment is an important aspect of human intervention studies. Diet can be an important confounder of outcome and therefore it has to be monitored in some way throughout the study. The accurate assessment of dietary intakes is a task of immense difficulty due to the absence of an absolute gold standard method for dietary assessment (Willett, 1998). Any evaluation method of dietary intake is limited by the validity and reproducibility of the dietary assessment tool used for measuring the intake (Willett, 1998). This challenge is amplified in the paediatric population, particularly those at pre-school age (under five), due to the rapid changes that characterise the diet of this particular age group

including the transition from consuming frequent quantities of milk to weaning foods and finally to table or family foods over a relatively short space of time, coupled with social factors such as attendance at day care or kindergarten (Birch and Ventura, 2009, Bryant-Waugh *et al.*, 2010, Hesketh and Campbell, 2010, Kerzner, 2009, Nicklaus, 2009). Together, these factors limit the parents ability to report accurately what their child might have consumed (Gibson, 2005).

Techniques commonly used to assess pre-schooler's dietary intakes can be divided into either prospective methods such as; diet/ food records, or retrospective methods such as; 24 hour recalls, or food frequency questionnaires (FFQ) (Gibson, 2005). Nevertheless, whatever the assessment method used, it is crucial that they are valid, reliable, age appropriate, and essentially practical and suited to the needs of the researcher (Gibson, 2005). A careful consideration must be given when selecting a dietary assessment method and the selection of the method is largely dependent on the objectives of the study. For instance, a content specific food frequency questionnaire or food survey may be more suitable to use if the aim was to explore eating habits of a specific food (Gibson, 2005). On the other hand if the aim was to assess macro and micro nutrient intakes and possible deficiencies then a detailed diet record may be a more suitable method to use (Gibson, 2005).

### 2.6.3.1 Retrospective research methods

#### 2.6.3.1.1 Food Recalls

Food recalls are usually administered by trained interviewers to collect information on everything the subject consumed in the previous 24 hours (Gibson, 2005, Thompson, 1994). For pre-schoolers, caregivers or guardians are usually asked to provide detailed information

about the child's dietary intake such as ingredients of dishes, food preparation methods and estimated amounts consumed, often during a one on one interview with researchers.

Visual prompts for quantifying portion sizes such as food models or pictures are often used (Gibson, 2005, Gibson, 1990). In a review by Serdula et al (Serdula *et al.*, 2001) 12 food recall studies in preschool-aged children were reviewed. The author concluded that the validation standards varied widely, making conclusions difficult (Serdula *et al.*, 2001). In general the author observed that with dietary recalls, respondents were more likely to omit than add food items and there was no consistent pattern when it came to estimation of portion size (some studies reported over-estimation while others reported under-estimation).

Obviously mothers who were with their children most of the day were more able to report intake compared to mothers who had their children at pre-school for more than 4.5 hours per day (Serdula *et al.*, 2001). Overall, using food recalls, respondents are more likely to recall main meals of their children rather than snack foods or food they consumed in between meals (Serdula *et al.*, 2001). The 24-hour food recall can be used to both rank and quantify food intake, however due to the day to day variability in food intake multiple recalls are required to obtain a more accurate verification of the subject's food intake. Limitations of this method includes inaccuracy in reporting due to failure to remember certain foods consumed, which may lead to under-estimation, and the significant effort required by both the researcher / the respondent / the proxy to complete multiple interviews.

#### 2.6.3.1.2 Food Frequency Questionnaire

A Food Frequency Questionnaire (FFQ), typically includes a long list of foods and beverages, and respondents (or their parents) are asked to report frequency of consumption, and some may require the respondents to report portion size as well (Gibson, 1990,

Thompson, 1994). For pre-schoolers, the usual referent period ranges from 1 month-1 year. Quantitative FFQ portion sizes are collected for all food, while for semi-quantitative FFQs, portion sizes are collected only for foods that are consumed in typical or usual portion sizes (e.g. slices of bread, glasses of juice). On the other hand, in a non-quantitative FFQ, portion sizes are not collected, and the FFQ mainly aims to investigate the frequency and types of foods consumed in the diet (Gibson, 1990, Thompson, 1994).

In the review by Serdula et al (Serdula *et al.*, 2001) on the dietary assessments of pre-schoolers, the author examined the validity of this method in 9 studies. Most of the studies reviewed used the FFQ primarily to assess general dietary intake, and the period assessed ranged from 1 week-1 year (Serdula *et al.*, 2001). Generally, the author found that FFQs tended to over-estimate mean energy intake when compared to other methods. In one study the FFQ used over-estimated mean energy intakes by 73% when compared to the 24-hour dietary recall method, while another study found that there was a 59% energy difference between the FFQ used and the doubly-labelled water method (Serdula *et al.*, 2001).

In a study with 224 children aged 44-60 months, Stein et al. (Stein *et al.*, 1992) investigated the utility of the Willett semi-quantitative food frequency questionnaire in assessing the habitual diets of preschool children. The author found that the FFQ used consistently over-estimated the frequency of intake of food servings such as meat, dairy products, and fruit and vegetables when compared to a 24 hour recall (Stein *et al.*, 1992).

In another study by Linneman et al (Linneman *et al.*, 2004) the accuracy of parents as reporters of their 2 to 5-year-old children's fruit and vegetable intake was assessed using an FFQ. This was a community-based observational study (n= 61 participants), with an

independent observer assessing one-meal intakes, followed by a telephone survey to determine the previous day's consumption using a 29-item fruit, juice, and vegetable food frequency questionnaire.

The investigators found that parents accurately reported their children's intake on most fruits and vegetables ( $\kappa=0.59-0.61$ ) and parents were the least accurate in recalling the consumption of raisins from oatmeal cookies ( $\kappa=0.05$ ) and 100% juice ( $\kappa=0.17$ ) (Linneman *et al.*, 2004). While the results from this study show that parents can be accurate proxies to report fruit and vegetable consumption using an FFQ, it must be noted that this was an unusual setting and the recall period required was the previous 24 hours only. The FFQ method is a relatively easy one to administer and is unlikely to affect dietary intake. In general, FFQs seem to overestimate total energy intake or the quantities of food consumed and some investigators believe that they are better at ranking rather than quantifying usual intake (Serdula *et al.*, 2001). Therefore FFQs are a good method to use to investigate the dietary eating habits and eating patterns of subjects. They are particularly if the objective is to focus on a specific food in the diet such as fruits and vegetables.

Self-administered FFQ while it may be a low cost method for the investigator and a low burden method on the subject (can be filled out at a convenience time), they are limited by the possibility of over-estimation. Therefore if a self-administered FFQ is used in a study, it might be sensible to use another method that assesses the same food but does not have the same limitation. In a study by Kennedy *et al.* it was demonstrated that a food-liking survey of fruits and vegetables was a feasible predictor of fruit and vegetable consumption in preschoolers (Kennedy *et al.*, 2008).

## 2.6.3.2 Prospective research methods

### 2.6.3.2.1 Food Records

This method requires the caregiver of the child to record detailed information about all the food and beverages (including preparation methods) consumed during a specified time period. The amount consumed is usually quantified by either weighing the food item or visual estimation of portion size compared to a standard reference (Thompson, 1994, Gibson, 1990). Ideally, the information needs to be recorded at consumption to improve accuracy and eliminate the problem of forgetting (Gibson, 1990, Thompson, 1994). This method is by far the most labour intensive one (compared to FFQ and 24-hour dietary recalls) and carries a high respondent burden. Studies evaluating the validity of food records in children under the age of 5 are scarce in the literature (Davies *et al.*, 1994, Harbottle and Duggan, 1993).

In a yearlong cross-sectional study by Harbottle *et al.* (Harbottle and Duggan, 1993) the validity of food records were evaluated compared to diet history on 117 Indo-Asian children aged 4-40 months and living in the UK. The weighted 4-5 days food records (dietary inventories) of the children were compared to their diet histories of that same period. The diet history method gave higher estimates of the dietary intake of most nutrients than the weighed inventory method. The differences of the mean intakes of energy (7% more), protein (9% more), fat (3% more), iron (9% more) and vitamin C (6% more), and were significant for energy, protein and iron when compared to food records (Harbottle and Duggan, 1993).

In another study, that also examined the validity of (4 day) weighed food records, this time compared to the doubly labelled water method (Davies *et al.*, 1994). In a cohort of 81 children aged 1.4-4.5 years of age, the energy intake was calculated from the weighed food records and compared to the energy expenditure calculated from the doubly labelled water

method (Davies *et al.*, 1994). The authors also reached a conclusion that while outcome of both methods correlated well (0.41 correlation) with the mean relative bias between children 154kJ/day (and the mean relative bias between the older children aged 3.5-4.5 by as little as 37 KJ/day), mean energy intake from food records tended to under-estimate total energy expenditure from the doubly labelled water (Davies *et al.*, 1994).

Quantified food records provide a suitable and valid method of dietary assessment for children under the age of 5 albeit, under-estimation of foods consumed is likely with this method. In addition, this is a high burden method for participants especially with parents of children under the age of 5 who already require significant attention. This may explain why studies utilising this method in this age group are not often reported in the literature.

To investigate the link between diet and health, it is essential to be able to monitor the nutritional status, eating habits, and dietary consumption of the population of interest. All dietary assessment methods are limited by the validity of the tool used to measure intake. Finally, when selecting a dietary assessment method for a nutritional study the researcher must consider the purpose of the assessment, the reliability of it, the constraints of the setting along with the age appropriateness of the method.

## **2.7 Summary**

Upper respiratory tract infections (URTI) including cold and flu-like illnesses are one of the most prevalent illnesses in the world resulting in misery, loss of productivity, and absence from work and school (Bukutu *et al.*, 2008). There is no known cure for the common cold (Bukutu *et al.*, 2008). Therefore, alternative prophylactic or treatment of symptoms options for cold and flu-like illnesses are of importance to public health (Bukutu *et al.*, 2008).

Nutrients that are required for the immune system to function efficiently include essential amino acids, the essential fatty acid  $\alpha$ -linolenic acid, folic acid, vitamins A, B6, B12, C and E, zinc, copper, iron and selenium (Chandra and Kumari, 1994). Therefore it is not surprising that some of these nutrients and others have been investigated in various URTI prevention and treatment studies (Hemila *et al.*, 2002, High, 2001, Bukutu *et al.*, 2008, Meydani *et al.*, 2004). The effects of vitamin C, vitamin E, and carotenoids on the incidence of URTI illnesses and symptoms have been investigated, and the findings have been contradictory or inconclusive (Hemila *et al.*, 2002, High, 2001, Bukutu *et al.*, 2008, Meydani *et al.*, 2004). These studies used a supplementation approach and were mostly in adults or elderly subjects (Hemila *et al.*, 2002, High, 2001, Bukutu *et al.*, 2008, Meydani *et al.*, 2004). There was a lack of studies in children subjects in spite of the high incidence of cold and flu-like illnesses in this age group.

The essential nutrients required for optimal immune function can also be obtained through food (Lampe, 1999). In recent studies it was demonstrated that URTI illnesses were negatively associated with fruit and vegetable consumption in pregnant women (Li and Werler, 2009), suggesting that a nutritional intervention may be a valuable tool for preventing URTI illnesses and/or reducing the symptoms. The diet of subjects is a large confounder in human studies and must also be accounted for through suitable dietary methods (Gibson, 1990, Gibson *et al.*, 1998).

# Chapter 3

## 3. Materials and methods

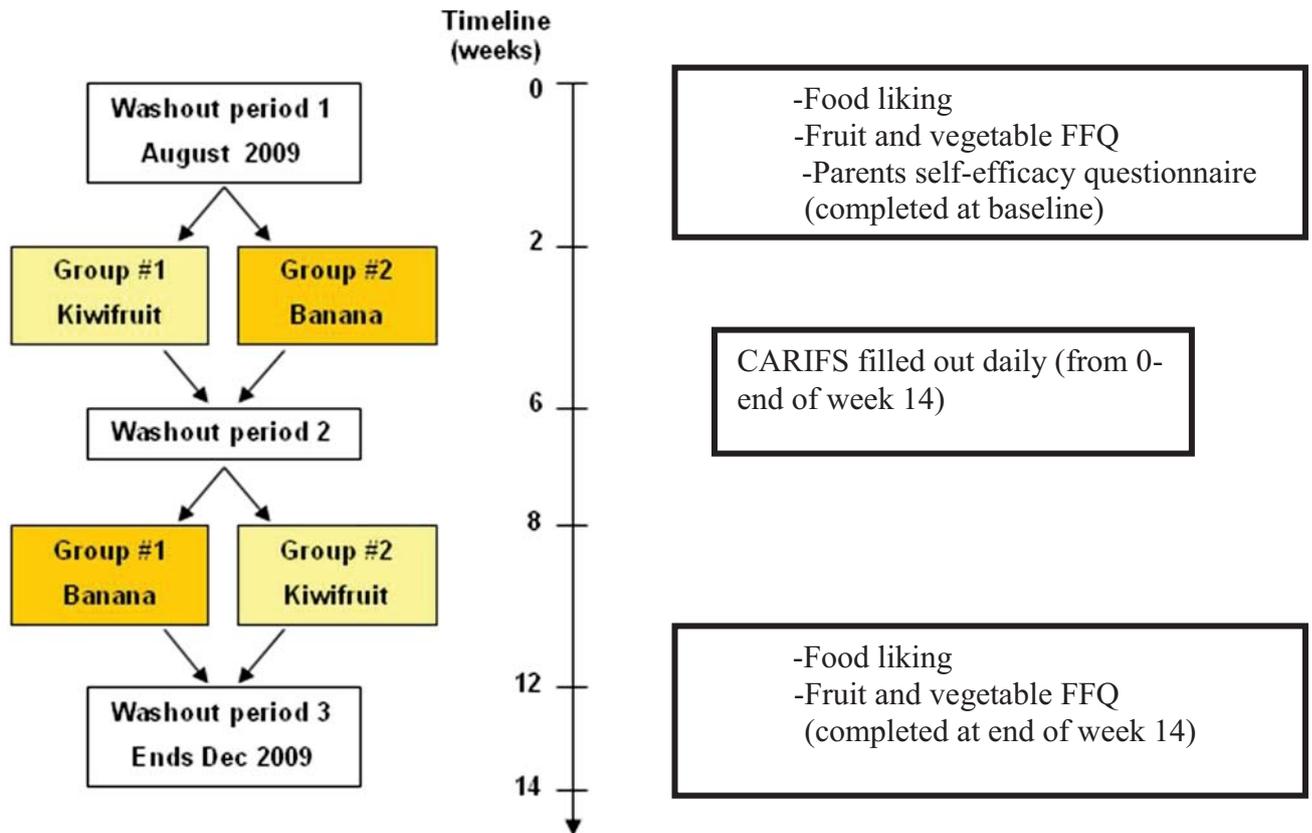
### 3.1 Introduction

The study investigated the effect of gold kiwifruit consumption on the incidence and severity of respiratory infections, such as the common cold, in children aged 2-5 years old. The objective of this chapter is to present the study design, methods, settings and procedures used to carry out the investigation.

### 3.2 Study design

Children were recruited from local crèches, randomised into one of two groups, either receiving two servings of gold kiwifruit per day or two servings of banana per day for five days a week, for four weeks, then, they were crossed over to the alternative treatment, i.e. children who received kiwifruit then received banana and vice versa. Parents were required to fill out a fruit and vegetable intake survey, a food liking questionnaire, and monitor their child for the duration of the study to see whether they experienced any respiratory symptoms by daily completion of the Canadian Acute Respiratory Illness and Flu Scale (CARIFS), a valid respiratory symptom survey in preschoolers. The study was designed as a randomised double cross-over trial, including three wash-out periods (figure 3.1)

N=100 (2-5 year old pre-schoolers)



*N*=total number intended to be recruited (100)  
Group 1 *n*=34 children  
Group 2 *n*=32 children  
FFQ= Food Frequency Questionnaire

Figure 3.1 Research Design

### 3.3 Funding and Ethics Approval

The investigator was awarded an industry fellowship by ZESPRI® Group Ltd which covered the cost of the trial. Additional running costs were covered by the Institute of Food, Nutrition and Human Health, Massey University. All extra costs including the investigators time were covered by The New Zealand Institute for Plant & Food Research.

Ethical approval for the study was acquired from the Massey University Human Ethics Committee: Southern A (application 29/09), and in accordance with this the subjects' legal guardians gave informed consent for their children to participate in the study. Additional permissions were sought from crèches and play-centres to recruit participants.

### **3.4 Subjects**

The study population was pre-school children, aged two to five years old, attending either crèches or play-centres in the Mount Albert and central Auckland area.

#### **3.4.1 Inclusion/Exclusion Criteria**

The inclusion criteria for this study:

- Children between 2-5 years of age and attending a crèche or play-centre
- No allergy to gold kiwifruit
- No allergy to banana

#### **3.4.2 Participants Recruitment**

More than 15 local crèches and play-centres in Auckland were approached by the investigator and they were invited to take part in the study (see appendix A). Once a crèche/play-centre consented to taking part in the study (see appendix A), recruitment of children began from the consenting crèche/play-centre. Parents/caregivers were made aware of the study via advertisements in the form of flyers and posters (see appendix B) that were distributed at the consenting crèches/play-centres. Advertisements were focused on the Mount Albert and central Auckland geographical area. In total four crèches and four private play centres took

part in the study. In addition, parents who heard about the study (even if they were not approached by the investigator) and wished to take part in it were included as long as their child met the inclusion criteria. Prior to the commencement of the study the investigator visited the participating crèches and play centres, and the study procedure was explained in detail to the teachers. Once parents registered interest in the study they were sent further information in the form of an information sheet about the study (see appendix C). This gave a description of the outline of the study, an explanation of the procedure involved, confidentiality measures and the rights of the participants. If the parents wished their children to participate they were given a screening questionnaire to complete and return along with a consent form (see appendix D). The questionnaire was designed to ensure that the children met the inclusion criteria of the study. The study was explained to the consenting parents through a one on one interview with the principal researcher prior to commencement of the study. The interview was conducted either over the telephone or face to face (depending on the parents preference), during which the principal investigator explained in detail how the food questionnaires should be completed and how the CARIFS diary should be completed. Demographic information, including medical history and supplementation intake were also collected in the screening questionnaire. Children who met the inclusion criteria were randomly assigned to one of two groups. The flow chart (Figure 3.2) outlines the recruitment process.

### **Identification of Crèches**

- Crèches/play-centres identified and advertisements focused on the Mount Albert and geographical area;
- However children who attended crèches or play-centres from various areas throughout the greater central Auckland area were also enrolled in the study upon request of parents.



### **Visit to Crèches**

- The principal researcher approached the crèches/ play-centres
- Informed the management staff about the study and delivered the study information sheet and consent form
- Once consent was obtained a follow up visit was made to the crèche/play-centre and the study was explained to the rest of the staff to be involved



### **Participant Identification and Recruitment**

- Children's recruitment commenced (upon the crèche/ play-centre consenting)
  - Posters were posted around each crèche



### **Recruitment of Parents**

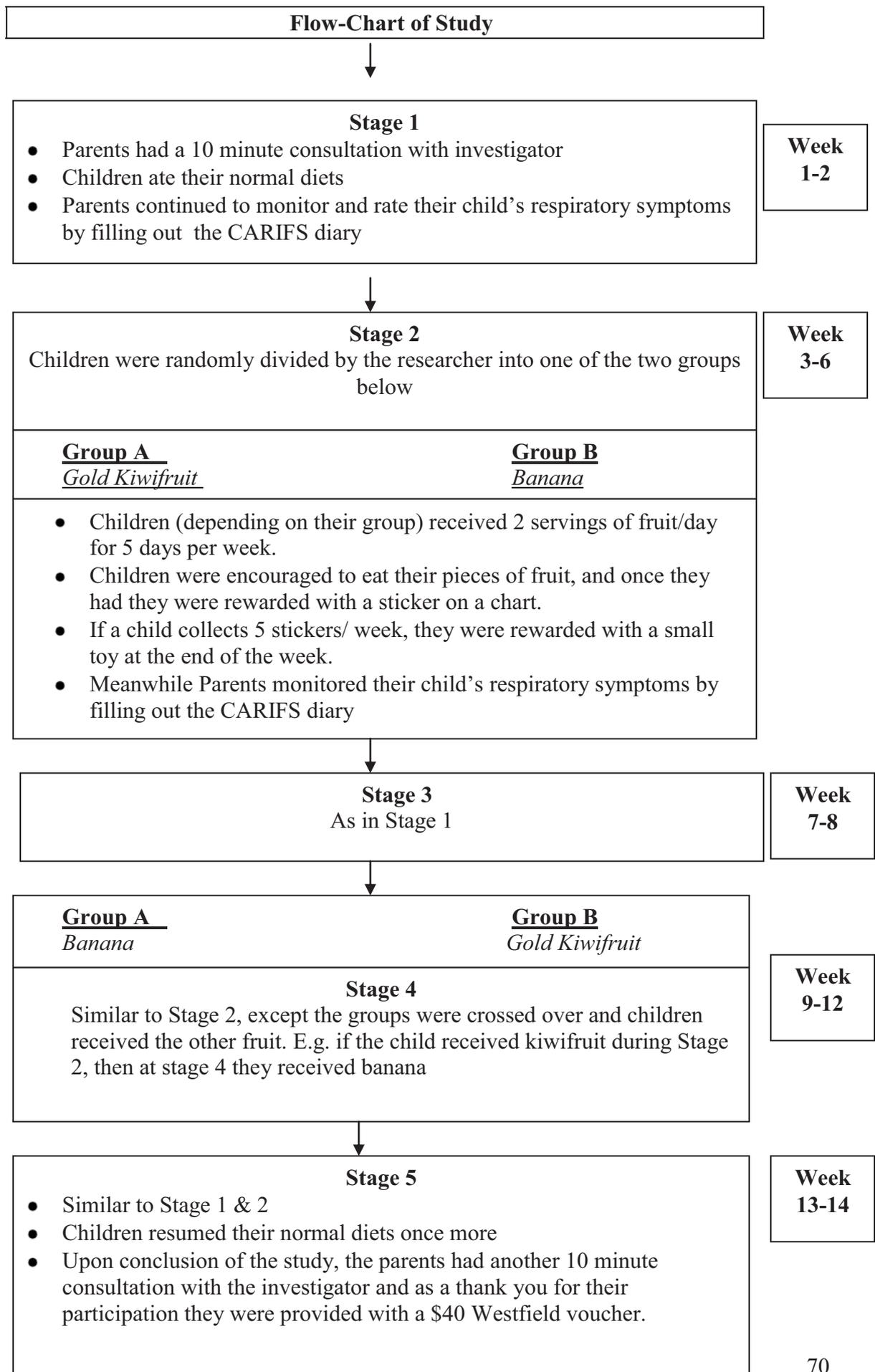
- Once parents registered interest in the study they were sent an information sheet explaining the study



### **Recruitment of children**

- If parents wished their children to participate they were given screening questionnaires to complete
- If they were eligible, then they were invited to take part in the study and required to sign the consent form

Figure 3.2 Flowchart detailing the study recruitment process



3.3 Flowchart detailing the study process

### 3.5 Study process

This study was carried out under free-living conditions. The study process is outlined in a flowchart presented in Figure 3.2. The children were required to consume two servings of their allocated fruit per day (kiwifruit or banana, depending on what group they were randomised into). This was either done at the crèche, or at home, if they weren't attending crèche for that day. Each child received their allocated fruit every Monday. It was usually delivered to the crèche, however some children did not attend their crèche on Mondays and in that case the fruit was delivered to their household or alternative arrangements were made with the parents to collect the fruit from Plant & Food Research. When the child consumed their two allocated servings of fruit, their caregiver (parent or teacher) rewarded them with a sticker on their personalised chart. When all the stickers were collected for one week (five stickers in total) they were rewarded with a small toy at the end of that week. In this way it was possible to track and foster compliance among the participating children. The parents/guardians of the children were given cold and flu diaries and asked to track their children's symptoms with it. These diaries were a collection of CARIFS surveys (see appendix E), with one page per day to fill out. The parents were requested to commence filling in the CARIFS diary for two weeks prior to the start of the treatment to obtain baseline measurements for the children and the diary was filled out for the duration of the trial (3months). At the end of the trial the parents were given the food-liking and intake questionnaire to complete again. The parents or the guardians of the children including teachers at the crèche were provided with gold kiwifruit or banana. As most children did not attend crèches or play centres full time, both the cooperation of the teachers and the parents were needed for the success of the study.

## 3.6 Intervention

### 3.6.1 Gold kiwifruit intervention

Children received 2 servings of gold kiwifruit/day during the kiwifruit intervention phase. Two servings of gold kiwifruit are equivalent to one average size kiwifruit (150g)/ or two small gold kiwifruits (about 75 g each). Serving size of fruit was determined in reference to the New Zealand Ministry of Health guidelines for children between 2-12 years of age (Ministry of Health, 2010).

The ZESPRI® Group Limited provided the gold kiwifruit used in this intervention. The fruit was delivered weekly (from ZESPRI® International Limited, Mount Maunganui South, New Zealand) to Plant & Food Research in Mount Albert, Auckland.

The investigator received the fruit every Friday, and the fruit was sorted into paper bags so the children in the kiwifruit group received at least 5 servings more than their minimum requirement to allow for extra in case of spoilage, the bags were labelled with the participants name and delivered to the crèche every Monday, unless prior arrangements were made with the investigator. Additional fruit was also provided for siblings of participants or children who were attending the crèche but were not taking part in the study.

### 3.6.2 Banana

Children received 2 servings of banana/ day during the banana intervention phase. Two servings of banana is equivalent to one medium size banana (110g). Serving size of fruit was determined in reference to the New Zealand Ministry of Health guidelines for children between 2-12 years of age (Ministry of Health, 2010).

Medium sized bananas were purchased weekly by the investigator and collected from MG Marketing (801 Great South Road, Westfield 1060, New Zealand) on Saturday mornings.

The bananas purchased were at different levels of ripeness (ranging from ripe and ready to eat to semi-ripe (green at the tips)) to ensure they remained palatable and acceptable to the children until the end of the week. Similar to the kiwifruit, the bananas were sorted into paper bags and the children received at least 5 servings more than their minimum requirement to allow for extra fruit in case of spoilage. The bags were labelled with the participants name and delivered to the crèche or parents on the Monday. Additional fruit was also provided for the siblings of participants or children who were attending the crèche but were not taking part in the study.

### **3.7 Fostering and tracking compliance**

Each child had their own personalised chart (see appendix F). The stickers and rewards given to children for consuming their allocated servings of fruit was a way of fostering and encouraging compliance yet at the same time monitoring it. The charts were collected at the end of the study by the principal investigator.

Children who did not comply with the study, still received their allocated toys, but they were not given the toys until the end of the study and they were not made aware of that during the study. However the parents and crèche staff were made aware that the children will be receiving the toys at the end of the study even if they failed to comply with the study fully.

This was a measure to encourage accurate reporting of children's compliance by parents and crèche staff.

### 3.8 Instruments/ measurements

#### 3.8.1 The Canadian Acute Respiratory Illness and Flu Scale (CARIFS) survey instrument

The CARIFS survey instrument is a validated cold and flu symptoms measure for this age group (Jacobs *et al.*, 2000, Shepperd *et al.*, 2004). It is based on a conceptual framework that defines illness severity as having three domains; physiological, functional, and burden of illness (Stein *et al.*, 1987). It has been designed to reflect these domains by measuring the three dimensions of childhood illness namely; symptoms, function, and parental impact. The survey instrument requires the child’s caregiver (most likely to be one of parents) to rate 18 aspects of their child’s symptoms and behaviour using four response categories namely; major, moderate, minor, or no problem (figure 3.4) (Jacobs *et al.*, 2000).

	No Problem	Minor problem	Moderate problem	Major problem	Don't Know or Not Applicable
1. Poor appetite					
2. Not sleeping well					
3. Irritable, cranky, fussy					
4. Feels unwell					
5. Low energy, tired					
6. Not playing well					
7. Crying more than usual					
8. Needing extra care					
9. Clinginess					
10. Headache					
11. Sore throat					
12. Muscle aches or pains					
13. Fever					
14. Cough					
15. Nasal congestion, runny nose					
16. Vomiting					
17. Not interested in what's going on					
18. Unable to get out of bed					

Figure 3.4 Canadian Acute Respiratory Illness and Flu Scale (CARIFS) survey instrument (Jacobs *et al.*, 2000)

## 3.8.2 Fruit and vegetable consumption questionnaires

### 3.8.2.1 Food liking questionnaire

The food liking questionnaire was developed and validated at the University of Connecticut in the USA (Kennedy *et al.*, 2008). The questionnaire contained 23 items including all food groups but with an emphasis on fruits and vegetables (see appendix G). Parents completed the food liking questionnaire at baseline and at the end of the study.

### 3.8.2.2 Fruit and vegetable consumption and food frequency questionnaire

Parents were required to complete the fruit and vegetable FFQ at baseline and at the end of the study (see appendix H). The fruit and vegetable FFQ was designed by the principal investigator to examine the children's fruit and vegetable consumption over a four week period. Parents were required to think back over the previous four week period and estimate how many servings of 16 different fruits and 17 different vegetables they provided their child with. The fruits and vegetables list provided in the questionnaire were based on the types of fruits and vegetables that are listed in the most recent NZ CNS (Ministry of Health, 2002). The FFQ was non-interviewer based.

### 3.8.2.3 Children's fruit and vegetable eating habits

At baseline, parents were asked general questions regarding their children's fruit and vegetable consumption habits (see appendix H). This second part was used as an internal measure to control for the reliability of data reported in the FFQ.

### 3.8.2.4 Parents' motivation to providing their children with fruits and vegetables

Parents were asked to rate (on a 6 point scale) how certain they are that they will provide their child with fruits and vegetables in six particular situations based (see appendix H). The self-efficacy questions were added as a moderator of fruit and vegetable consumption. These sets of questions capture the parents' beliefs of whether they are confident that they can undertake the desired behaviour when faced with specific barriers. These barriers comprise of more superficial factors such as when your child is sick, when you are in a hurry or during weekends as opposed to broader social, cultural, and financial barriers. These questions were based on a fruit and vegetable self-efficacy model developed by Brug et al. (Brug *et al.*, 1995) and are detailed in table 3.1.

Table 3.1 Fruit and vegetable questions used to determine parent's motivation to providing their children with fruit and vegetables based on Brug's self-efficacy model (Brug *et al.*, 1995).

<b>Questions based on Brug's self-efficacy model</b>	
<b>Parents' fruit self-efficacy</b>	<p>On a scale from 1-6 how certain are you that you will give your child fruit in the following situations (not counting fruit juice)</p> <ul style="list-style-type: none"> <li>-In the evening, after your child is home from crèche.</li> <li>-During the weekends.</li> <li>-When your child is sick.</li> <li>-When the fruit is messy or requires preparation (e.g. peeling or need a spoon).</li> <li>-During winter when there is less choice.</li> <li>-When you are really busy, in hurry or having a hectic day.</li> </ul>
<b>Parents' vegetable self-efficacy</b>	<p>On a scale from 1-6 how certain are you that you will give your child at least two servings of vegetables in the following situations (not counting potatoes or kumara)</p> <ul style="list-style-type: none"> <li>-In the evening, after your child is home from crèche.</li> <li>-During the weekends.</li> <li>-When your child is sick.</li> <li>-When the fruit is messy or requires preparation (e.g. peeling or need a spoon).</li> <li>-During winter when there is less choice.</li> <li>-When you are really busy, in hurry or having a hectic day.</li> </ul>

### 3.9 Data handling and analysis

A simulation was used to estimate the power required to detect a treatment effect at the 5% level for the probability of developing a cold during the month. The simulated data did not include any time, subject or sequence effects. It also assumed that each observation was independent which may not be the case with the actual clustered data. The probability of getting a cold for the control treatment in a given month was taken to be 0.6 while the assumed probability while taking the kiwi fruit treatment was set at 0.3. Based on 10,000 simulated datasets the power to detect this treatment effect was calculated to be 87% when a sample size of 50 was used. We decided to recruit 100 people to allow for drop-out as this was a high burden study, and no previous data was available to indicate the likelihood of children achieving more than 80% compliance.

Symptoms measured by CARIFS were analysed using Statistical Analysis software (SAS) 9.2 (SAS Inc., Carry, NC, USA). The symptom scores for each symptom were summed for each child, per study day. The averages of these sums were calculated separately for the kiwifruit and banana intervention periods per child. A mixed effects model was then fitted using SAS proc mixed with these averages as the dependant variable and children treated as a random effect partial eta squares (Cohen, 1988). The intervention, intervention groups, and intervention period were all fixed effects. Evidence of a treatment effect was assessed at the 5% level using type-3 sums of squares. The probabilities of individual symptoms were analysed using Proc Genmod to fit a Generalised Estimating Equation (GEE). The ordinal scores are likely to be correlated due to the repeated measures over successive days. The GEE model allows for unknown correlation structures and produces robust standard errors. The model fitted used a cumulative logit link function and assumed a multinomial probability

distribution, which was appropriate given that the response variable is ordinal. Significance tests were carried out at the 5% level, and odds ratios comparing the two treatments were also produced. A statistically significant group effect in the mixed effect model used to analyse the data could be due to either a treatment by phase interaction or differences between the two study interventions (kiwifruit or banana). However for the symptoms grouping analysis there were no instance of a significant grouping effect, indicating that there was no treatment phase interaction.

Children's dietary analysis of fruit and vegetable consumption, liking, and eating habits employed standard statistical software, Statistical Package for the Social Sciences (SPSS) v.16 (SPSS Inc., Chicago, Il, USA).

The variables were tested to see if they were normally distributed using Kolmogorov-Smirnov and Shapiro-Will test together with examining Normal Q-Q, box and steam and leaf plots. Differences within groups between baseline and end values were analysed using the repeated measures ANOVA test for parametric variables and the Wilcoxon ranked-sum test for non-parametric variables. Spearman's Correlations Coefficients were used to determine any significant mono-tonic relationships. Normally distributed variables are presented as mean  $\pm$  standard deviation and non-normally distributed variables as medium (25<sup>th</sup>, 75<sup>th</sup> percentile). A  $p$  value  $< 0.05$  was considered to be statistically significant.

# Chapter 4

## 4. Results

### 4.1 Characteristics of study children

One hundred children were recruited for this study. In total, 66 children completed the study and had more than 80% compliance (i.e. they consumed at least eight out of their ten servings of fruit per week). Only 22 children did not complete the study (dropped out) and 12 children had less than 80% compliance. Children's characteristics are summarised in table 4.1.

Table 4.1 Characteristics of children

Characteristics	Values
Gender ( <i>boys/girls</i> )	30/36
Age ( <i>year</i> )	$3.4 \pm 0.46$
Children recruited from crèches/ day-care (N)	49
Children recruited from play-centres (N)	19

*Results expressed as mean  $\pm$  SD and frequencies*

The majority of children were recruited from crèches (49 children) with the rest being recruited from play-centres (19 children). The mean age of the children was  $3.4 \pm 0.46$  years. The majority of participants were of European ethnicity (50 children), however all the major ethnicities found in New Zealand were represented.

Detailed medical histories were also collected from children (summarised in table 4.2 below). No children were excluded from the study, as this was intended to be a normal free-living situation study. Three children had asthma and three children had wheezing/breathing problems, however, they did not respond differently to the intervention and therefore were included in the final analysis. Six children were consuming a multivitamin supplement prior to the commencement of the study, and they were not excluded from the study as long as they continued to consume them as normal through-out the study. The only food allergies reported were allergy to peanuts (1 child), and allergy to green kiwifruit (2 children). All participants were up to date with their vaccines. None of the children had received the flu vaccine, possibly due to recent reports of febrile convulsion among children under the age of five within 24 hours of receiving the flu vaccine (Ministry of Health, 30 April 2010). The current Ministry of Health's advice for New Zealanders is that people, including children under five, at risk of increased complications from flu should be vaccinated against it (Ministry of Health, 30 April 2010).

Table 4.2 Medical history/status and ethnicity of children by gender

	Boys	Girls	Total (N) (%)
Participating children	30	36	66 (100%)
<b>Ethnicities:</b>			
European	22	28	50 (75.8%)
Maori	1	0	1 (1.5%)
Pacific Islander	2	0	2 (3%)
Asian	2	3	5 (7.5%)
Indian	3	3	6 (9%)
Other	0	2	2 (3%)
<b>Health issues:</b>			
Asthma	1	2	3 (4.5%)
Wheezing/ breathing problems	2	1	3 (4.5%)
Supplements (multivitamins)	2	4	6 (9%)
Food allergies (green kiwifruit)	1	1	2 (3%)
Food allergies (peanuts)	1	0	1 (1.5%)
Up to date with their vaccines	30 (100%)	36 (100%)	66 (100%)

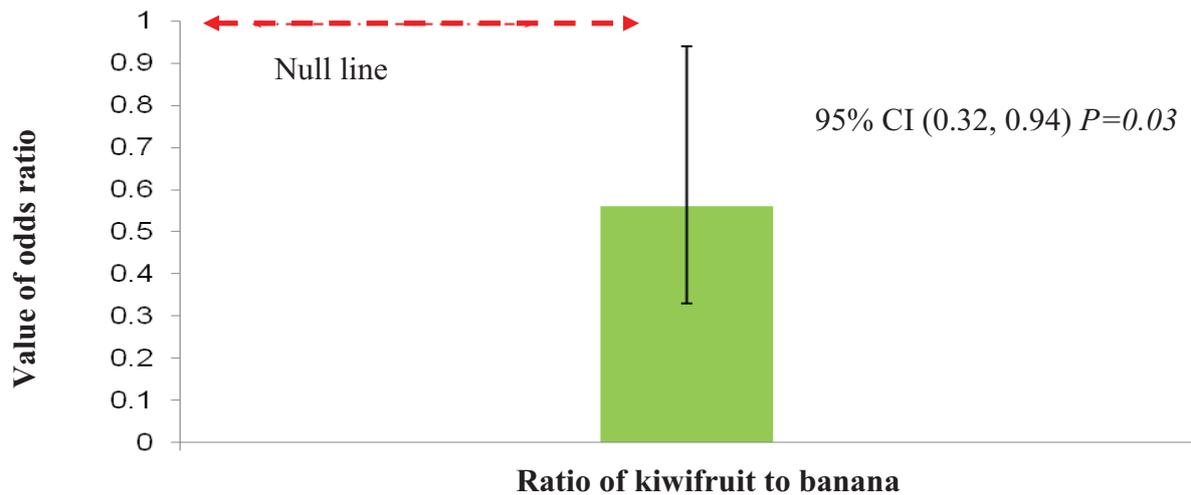
*Results expressed as frequencies and percentages*

## **4.2 The effects of the intervention on upper respiratory tract infection**

### **4.2.1 Incidence of cold-and flu-like illness**

The average yearly incidence of cold- and flu-like illnesses in children in this study was extrapolated from the reported cold and flu episodes. Children had an average of three cold- and flu-like episodes per year. The odds of having a cold-and-flu-like illness was almost half

as much (odds ratio (OR) = 0.55 (95% confidence interval (CI) 0.32, 0.94),  $P=0.03$ ) during the kiwifruit part of the intervention compared to the banana part of the intervention. This indicated clearly that during the kiwifruit part of the intervention, the incidence of cold-and-flu-like illness was greatly reduced.



*Odds ratio calculated from the parents reported incidence of cold- and flu episodes of 66 children by comparing the kiwifruit intervention outcome to the banana intervention outcome. Error represents 95% confidence interval (CI)*

Figure 4.1 The odds ratio of having a cold-and-flu-like illness when consuming kiwifruit compared to banana.

#### 4.2.2 Upper respiratory tract symptoms score (CARIFS scores)

The CARIFS instrument has a total of 18 symptoms; each symptom was scored with one of four values depending on the experienced severity (not applicable=0, no problem=1, minor problem=2, and major problem=3). When the total CARIFS scores were calculated (see table 4.3), the average score over the kiwifruit intervention period ( $21.6 \pm 0.15$ ) was lower than that of the banana intervention period ( $22.1 \pm 0.15$ ), and the difference was significant ( $P=0.015$ ).

The various symptoms measure by CARIFS can be clustered into three different domains; namely physiological (i.e. symptoms that measure physiological effects of disease), functional (i.e. symptoms that measure the impact of disease on the child's day to day activity), and parental impact (symptoms that measure the impact of disease on parents/caregivers) (see table 4.3). The total score for both the physiological symptoms and the functional symptoms were significantly lower ( $P=0.041$  and  $P=0.006$ ) in the kiwifruit intervention compared to the banana intervention. Similarly, the total score of the parental impact scores were also lower for the kiwifruit compared to the banana intervention, but the difference was not significant. The difference between the total symptom scores, the physiological symptom scores, and function symptom scores, in spite of its significance, was small between the kiwifruit and banana interventions. To provide an alternative view of effect in addition to significance a meaningful effect size was assessed by partial eta squares ( $\eta^2$ ). According to Cohn (Cohen, 1988) the total symptoms scores and physiological symptoms gave at least a "medium effect", the functional symptoms gave a "strong effect" and the parental impact gave a "small effect" when kiwifruit was consumed compared to banana.

Table 4.3 Symptom scores comparison for the banana intervention and the kiwifruit intervention

Symptom scores	Banana intervention (N=66)	95% CI	Kiwifruit intervention (N=66)	95% CI	P-value	Effect size ( $\eta^2$ )
Total symptoms scores	22.1 ± 0.15	(21.8, 22.4)	21.6 ± 0.15	(21.3, 21.9)	<b>0.015</b>	0.08
Physiological symptoms	8.53 ± 0.07	(8.39, 8.67)	8.34 ± 0.07	(8.21, 8.484)	<b>0.041</b>	0.06
Functional symptoms	5.26 ± 0.04	(5.22, 5.35)	5.12 ± 0.04	(5.03, 5.21)	<b>0.006</b>	0.1
Parental impact (burden of illness)	5.19 ± 0.04	(5.11, 5.28)	5.12 ± 0.04	(5.08, 5.16)	0.146	0.03

Results expressed as mean ± SE

Evidence of a treatment effect was assessed at the 5% level using type-3 sums of squares

Effect size was assessed by partial eta square ( $\eta^2$ ) with 0.010 relating to a Cohen “small effect” (0.2, 0.059 relating to a Cohen “medium effect” (0.5) and 0.138 relating to a Cohen “large effect” (0.8)

### 4.2.3 Individual upper respiratory tract infection symptoms measured by CARIFS

#### 4.2.3.1 The odds of having individual CARIFS measured upper respiratory tract infection symptoms

Further analysis was carried out on the individual URTI symptoms of the CARIFS instrument. The probability of a symptom occurring over a 28 day period was calculated for each individual symptom within each intervention. The odds ratio of each symptom for the kiwifruit intervention versus that of the banana intervention was also calculated (table 4.4). Results were significant for a number of the symptoms. The odds of having a poor appetite (functional symptom) was nearly half as much (OR=0.5, 95% CI (0.29, 0.86),  $P=0.013$ ) during the kiwifruit intervention compared to the banana intervention. The odds of feeling unwell (functional symptom) during the kiwifruit intervention was significantly lower than the banana intervention (OR=0.66, 95% CI (0.44, 1.18),  $P=0.037$ ). The odds of other functional symptoms such as having low energy or being tired (OR=0.57, 95% CI (0.45, 0.98),  $P=0.014$ ) and crying more than usual (OR=0.65, 95% CI (0.47, 0.99),  $P=0.043$ ) were also significantly lower during the kiwifruit intervention compared to the banana intervention. There were also significant differences in the individual physiological symptoms. The odds of having headache was more than three times higher during the banana intervention when compared to the kiwifruit intervention (OR=0.33, 95% CI (0.13, 0.86),  $P=0.022$ ). The odds of having a sore throat was nearly three times higher when in the banana intervention compared to the kiwifruit intervention (OR=0.35, 95% CI (0.13, 0.95),  $P=0.039$ ). Vomiting was the only symptom that was more favourable for the banana intervention compared to the kiwifruit intervention, however the difference is not significant (OR=1.39, 95% CI (0.54, 3.57),  $P=0.501$ ).

Table 4.5 The probability of having a symptom over 28 days for each intervention group and the odds ratio of each symptom in the kiwifruit and banana interventions

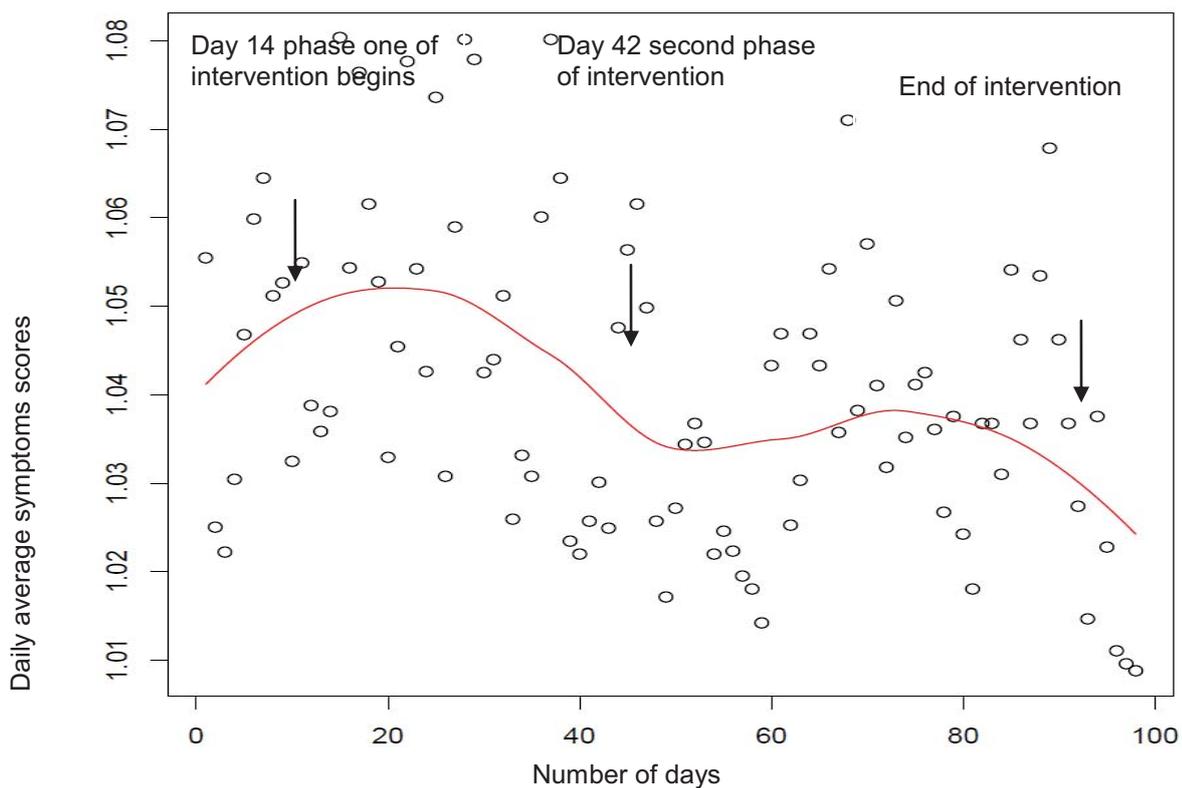
Symptoms	Kiwifruit intervention % probability* (N=66)	95% Confidence interval	Banana intervention % probability* (N=66)	95% Confidence interval	Odds ratio of kiwifruit / banana interventions	95% Confidence interval	P-value
Poor appetite	4.79	(2.94, 7.72)	9.50	(6.57, 12.4)	0.50	(0.29, 0.86)	<b>0.013</b>
Not sleeping well	6.14	(3.84, 9.68)	6.18	(3.88, 9.72)	0.99	(0.64, 1.53)	0.973
Irritable. Cranky.	5.65	(3.54, 8.92)	7.70	(5.38, 10.9)	0.72	(0.44, 1.18)	0.186
Fussy	3.80	(2.62, 5.50)	5.64	(3.85, 8.19)	0.66	(0.45, 0.98)	<b>0.037</b>
Feels unwell	4.01	(2.66, 6.03)	6.80	(4.62, 9.91)	0.57	(0.37, 0.90)	<b>0.014</b>
Low energy tired	2.13	(1.23, 3.68)	2.82	(1.71, 4.63)	0.75	(0.47, 1.20)	0.231
Not playing well	2.11	(1.36, 3.29)	3.25	(2.08, 5.07)	0.65	(0.42, 0.99)	<b>0.043</b>
Crying more than usual	3.15	(1.82, 5.39)	3.75	(2.40, 5.82)	0.84	(0.51, 1.37)	0.468
Needing extra care	3.46	(1.67, 7.03)	3.85	(2.24, 6.56)	0.89	(0.46, 1.72)	0.736
Clinginess	0.49	(0.19, 1.30)	1.46	(0.79, 2.68)	0.33	(0.13, 0.86)	<b>0.022</b>
Headache	2.16	(1.08, 4.28)	5.95	(3.15, 10.9)	0.35	(0.13, 0.95)	<b>0.039</b>
Sore throat							

Table 4.5 (continue)

Symptoms	Kiwifruit intervention % probability* (N= 66)	95% Confidence interval	Banana intervention % probability* (N= 66)	95% Confidence interval	Odds ratio of kiwifruit banana interventions	95% Confidence interval	P-value
Muscle aches or pains	1.86	(0.88, 3.92)	2.12	(1.18, 3.80)	0.88	(0.40, 1.96)	0.742
Fever	1.79	(0.95, 3.37)	2.79	(1.55, 4.98)	0.64	(0.31, 1.30)	0.211
Cough	16.9	(11.7, 23.7)	21.8	(14.9, 30.8)	0.73	(0.46, 1.15)	0.172
Nasal congestion and runny nose	15.1	(10.6, 21.1)	18.1	(12.4, 25.6)	0.81	(0.52, 1.23)	0.309
Vomiting	0.58	(0.31, 1.12)	0.42	(0.15, 1.19)	1.39	(0.54, 3.57)	0.501
Not interested in what's going on	0.62	(0.26, 1.48)	0.02	(0.76, 3.84)	0.36	(0.12, 1.09)	0.06
Unable to get out of bed	1.02	(0.39, 2.70)	1.14	(0.43, 3.01)	0.91	(0.53, 1.54)	0.69

\*Probability % of having a symptom on a given day over the 28 days intervention Results analysed using Proc Genmod to fit a Generalised Estimating Equation (GEE) and significance tests were carried out at the 5% level

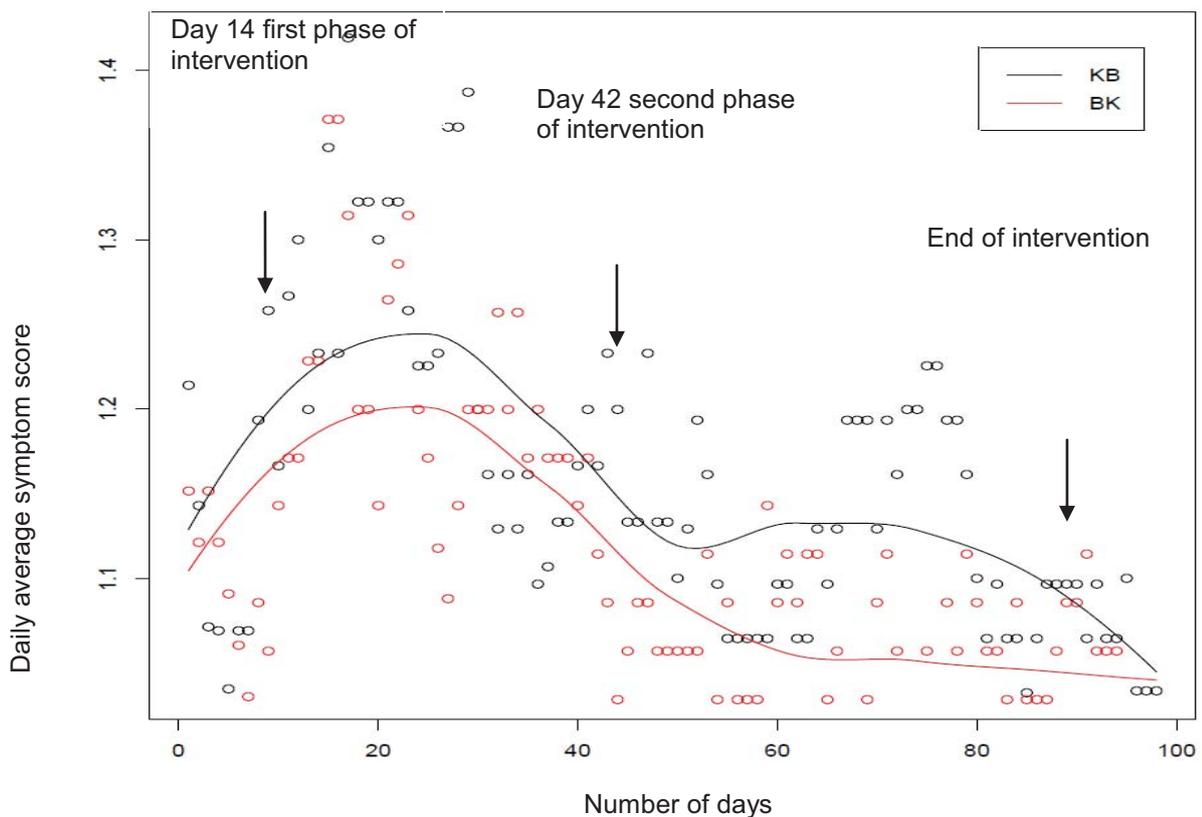
The plots of selected symptoms are represented below (figures 4.2-4.5) to show the overall trend of those symptoms over the course of the intervention. The intervention started late August (towards the end of the New Zealand winter and early spring) with a 14 day washout period followed by phase one of the intervention (lasting for 28 days). Upon completion of this phase, it was followed by another 14 days washout period. On day 42 of the intervention, about mid-October, the second phase of the intervention commenced (groups crossed-over), lasting for another 28 days, and upon conclusion it was followed with another 14 days washout period. The study concluded in early December (the beginning of the New Zealand summer). A seasonal effect can be observed (figure 4.2) over the duration of the study with symptoms declining as the study approaches summer.



*The black open circles represent the average score of all upper respiratory tract infection symptoms on a given day (N=66). Seasonal effect is shown by a red line as represented by LOESS smoother plot.*

Figure 4.2 Seasonal effects of upper respiratory tract infection symptoms throughout the intervention study.

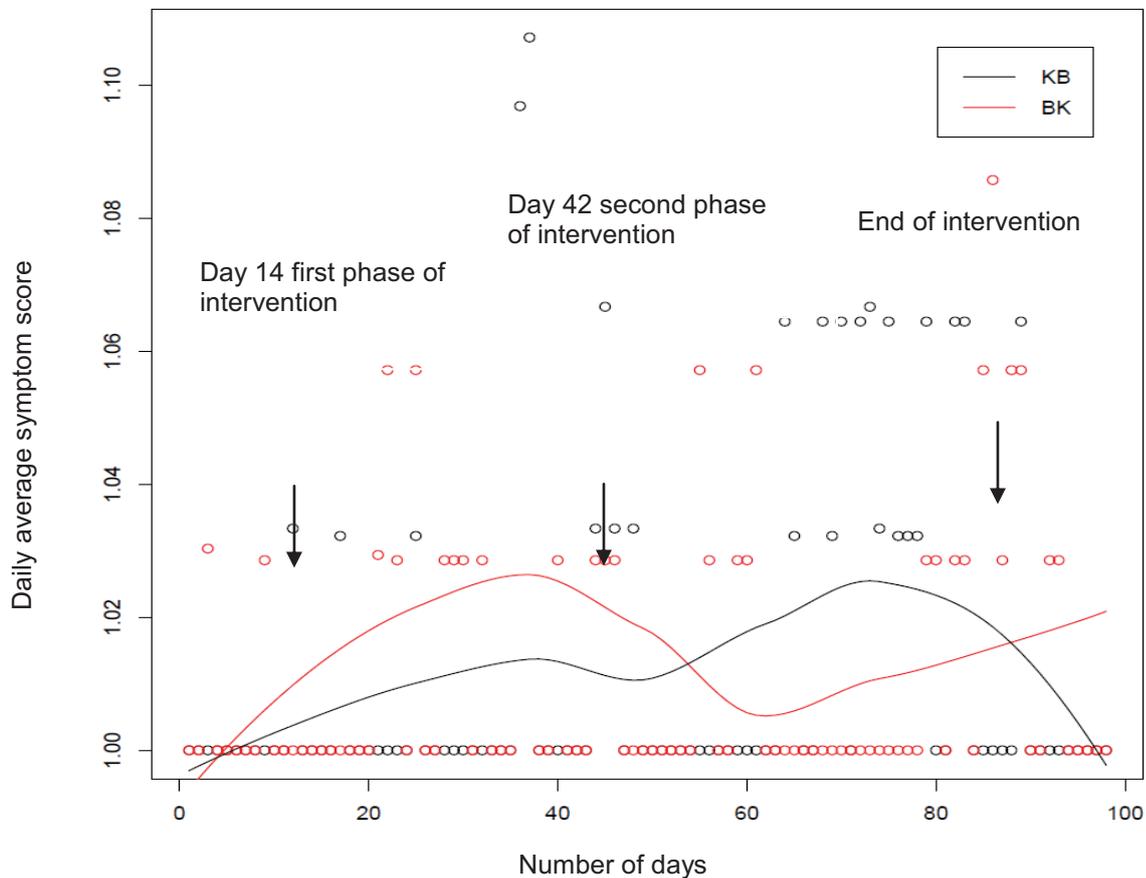
The LOESS smoother plot was used to graphically represent the symptom scores. Each dot represents the average score of symptom or symptoms on a given day. The black open circles represent the kiwifruit followed by banana intervention (n=32 children), while the red dots represent the banana followed by kiwifruit intervention (n=34). The cough symptom scores were plotted for the duration of the intervention (figure 4.3).



*Cough symptom of children in kiwifruit followed by banana group (KB), black open circles. Cough symptom of children in banana followed by kiwifruit group (BK), red open circles. Seasonal effect shown by red line (BK) and black line (KB) as represented by LOESS smoother plot.*

Figure 4.3 Comparison of seasonal effects of cough symptom throughout the two phases of the intervention.

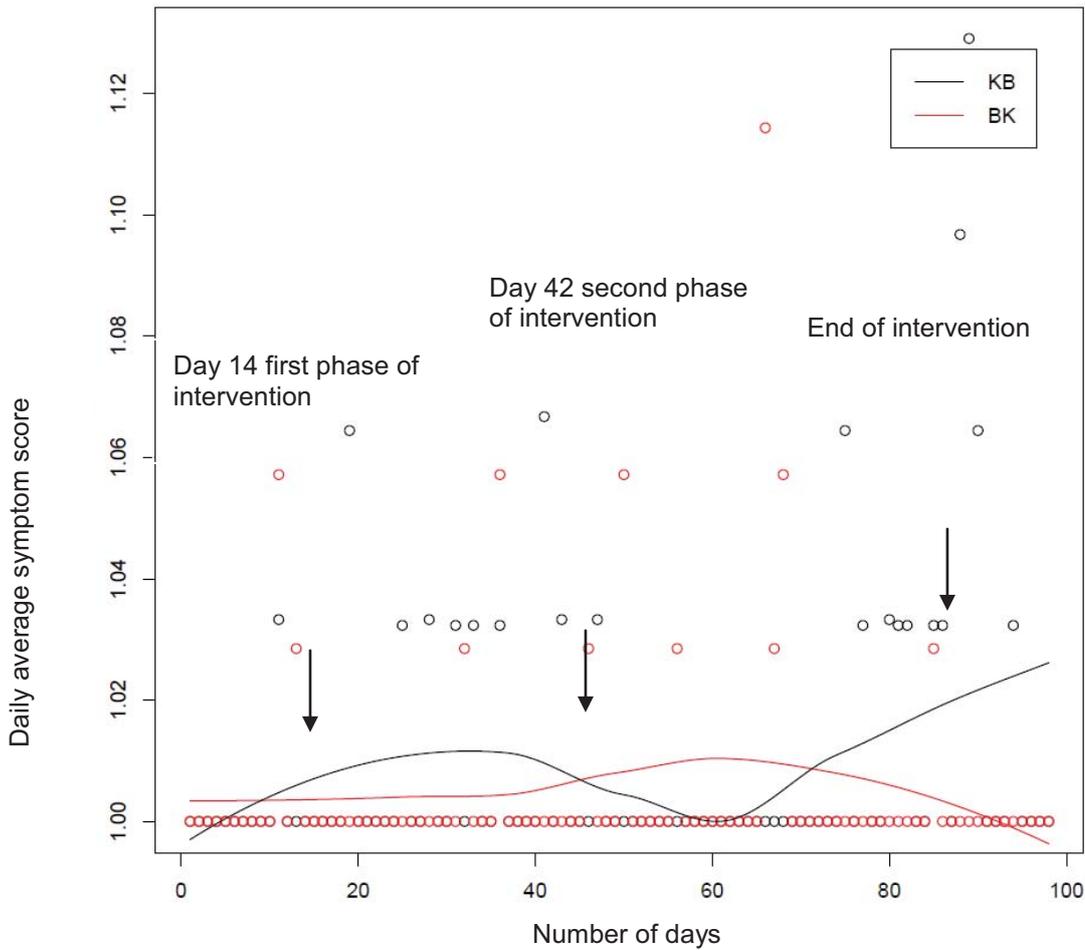
No difference in effect was observed over the first part of the intervention. However, during the second phase of the intervention the cough symptom scores were lower for the children consuming kiwifruit compared to those consuming banana. Overall, the differences between the kiwifruit and the banana intervention were not significant ( $P=0.172$ ). There is a general decline in the symptom scores over the treatment period; this is likely to be a seasonal effect over both intervention groups. The headache symptom scores were plotted for the duration of the intervention (figure 4.4).



*Headache symptom of children in kiwifruit followed by banana group (KB), black open circles. Headache symptom of children in banana followed by kiwifruit group (BK), red open circles. Seasonal effect shown by red line (BK) and black line (KB) as represented by LOESS smoother plot.*

Figure 4.4 Comparison of seasonal effects of headache symptom throughout the two phases of the intervention.

In general the headache symptom scores were lower over both phases of the trial during the kiwifruit intervention when compared to the banana intervention, and the difference was significant ( $P=0.022$ ). No seasonal effect was observed with the headache symptom. The vomiting symptom scores were plotted for the duration of the intervention (figure 4.5).



*Vomiting symptom of children in kiwifruit followed by banana group (KB), black open circles. Vomiting symptom of children in banana followed by kiwifruit group (BK), red open circles. Seasonal effect shown by red line (BK) and black line (KB) as represented by LOESS smoother plot.*

Figure 4.5 Comparison of seasonal effects of vomiting symptom throughout the two phases of the intervention.

As mentioned earlier the vomiting was the only symptom that scored lower overall for the banana intervention compared to the kiwifruit intervention. The LOESS smoother plot shows that the vomiting symptom was lower for the banana intervention during the first phase of the study. During the second phase of the study the vomiting symptom scores are lower for the banana intervention to begin with; however, there is an increase in symptom scores on about day 63 which continues until the completion of the study. No seasonal effect in this symptom was observed and the difference between the kiwifruit and the banana intervention was not significant ( $P=0.501$ ).

### **4.3 Fruit and vegetable consumption**

#### **4.3.1 Total fruit and vegetable consumption before and after intervention**

A fruit and vegetable FFQ was completed by the parents of the children with the aim to investigate whether the intervention influenced their fruit and vegetable consumption (see table 4.7). The FFQ was non-interviewer based. Fruit consumption did not differ between baseline and end. However, vegetable consumption increased by about one serving of vegetables ( $P=0.001$ ) at the end of the trial. This led to an overall increase in fruit and vegetable consumption values at the end of the trial ( $P=0.009$ ).

Table 4.6 Total fruit and vegetable consumption of children as reported by parents and as calculated from FFQ

	Median serving (per-day)	Lower quartile	Upper quartile	<i>P</i> -value
Fruit consumption before treatment (calculated from FFQ)	3.86	2.98	5.05	
Fruit consumption after treatment (calculated from FFQ)	3.86	3.28	4.57	0.379
Vegetable consumption before treatment (calculated from FFQ)	3.46	2.43	4.54	
Vegetable consumption after treatment (calculated from FFQ)	4.29	3.57	5.36	0.001
Fruit & vegetable consumption before treatment (calculated from FFQ)	7.21	6.21	9.09	
Fruit & vegetable consumption after treatment (calculated from FFQ)	8.43	7.00	9.68	0.009

*Results expressed as median (25<sup>th</sup>, 75<sup>th</sup> percentile)  
Differences between baseline and end was assessed using Wilcoxon signed ranks test*

### 4.3.2 Fruit and vegetable consumption at baseline

The data from the fruit and vegetable FFQ were also used to describe the fruit and vegetable intakes of the children. The frequencies for individual fruit and vegetable items reported by the parents were used to calculate daily intakes (table 4.7). In addition, parents also completed a section in the eating habits questionnaire where they were asked to report on how many servings of fruit and vegetables their children ate every day (table 4.8). It was anticipated that parents would be able to estimate the fruit and vegetable consumption of their children in terms of overall number of portions consumed compared to possibly overestimating their intakes from the individual judging of total items from the non-interviewer based FFQ.

Differences were indeed apparent between the two methods used. The median consumption of fruit and vegetables per day as reported by the parents was 3.28 (25<sup>th</sup>, 75<sup>th</sup> percentiles 1.8, 5). Fruit consumption alone had a median of 2.5 servings per day (25<sup>th</sup>, 75<sup>th</sup> percentiles 1, 2.5). Vegetable consumption had a mean of 1 serving per day (25<sup>th</sup>, 75<sup>th</sup> percentiles 0.78, 2.5). Results from the FFQ revealed that the fruit consumption after treatment have decreased, although not significantly ( $P= 0.379$ ), while vegetable consumption has increased after treatment by just over one serving per day and the increase was significant ( $P=0.001$ ). The total fruit and vegetable consumption have increased post intervention significantly ( $P=0.009$ ). The fruit and vegetable consumption calculated from the FFQ was much higher than that reported by the parents which indicate that overestimation may have occurred with the FFQ reporting.

Table 4.7 Total fruit and vegetable consumption of children before the intervention as reported by parents from the eating habits questionnaire

	Median serving (per-day)	Lower quartile	Upper quartile
Fruit consumption (parents-reported)	2.5	1.00	2.50
Vegetable consumption (parents-reported)	1.00	0.78	2.50
Fruit and Vegetable consumption (parents-reported)	3.28	1.80	5.00

*Results expressed as median (25<sup>th</sup>, 75<sup>th</sup> percentile)*

In an attempt to describe their preferences, the fruits and vegetables consumed by children, as reported in the FFQ at baseline, were ranked from highest consumption to lowest according to number of serves per week (table 4.8). Banana was the most frequently consumed fruit, followed by apple. Green kiwifruit was ranked fifth in terms of frequency of consumption and it was consumed more frequently than gold kiwifruit (which is ranked 11<sup>th</sup>). Nectarine, apricots and plums were the least frequently consumed fruit at baseline.

Carrots were by far the most frequently consumed vegetable at baseline (table 4.8) followed by tomatoes. Potato was ranked fourth in terms of consumption. Cucumber, kumara, cauliflower, pumpkin, cabbage, and silverbeet were the least consumed vegetables at baseline. Vegetable juice was also not consumed often and it ranked last on the vegetable list.

Table 4.8 Children's fruit and vegetable consumption (servings per week) at baseline, ranked from highest to lowest

	Median	Lower quartile	Upper quartile
<b>FRUIT</b>			
Banana	3.00	1.00	7.00
Apple	3.00	3.00	5.00
Dried fruit	3.00	1.00	5.50
Fruit juice	3.00	0.50	7.00
Green kiwifruit	0.50	<0.5	5.87
Mandarin	1.00	<0.5	3.00
Canned or cooked fruit	0.50	<0.5	3.00
Orange	1.00	0.50	3.00
Grapes	1.00	<0.5	3.00
Pear	1.00	<0.5	3.00
Gold Kiwifruit	<0.5	<0.5	1.00
Strawberries	0.50	<0.5	1.00
Berries	<0.5	<0.5	0.63
Nectarine	<0.5	<0.5	<0.5
Apricots	<0.5	<0.5	<0.5
Plums	<0.5	<0.5	<0.5
<b>VEGETABLES</b>			
Carrots	3.00	0.87	5.50
Tomato	1.00	<0.5	5.50
Broccoli	1.00	0.50	3.00
Potatoes	1.00	0.50	3.00
Lettuce/Green salad	0.50	<0.5	3.00
Peas	1.00	<0.5	3.00

Table 4.8 (continued)

VEGETABLES (continued)	Median	Lower quartile	Upper quartile
Green beans	1.00	<0.5	3.00
Frozen vegetables	0.75	<0.5	3.00
Spinach	1.00	<0.5	1.00
Corn	0.50	<0.5	1.50
Cucumber	0.50	<0.5	1.00
Kumara	0.50	<0.5	1.00
Cauliflower	0.50	<0.5	1.00
Pumpkin	0.50	<0.5	1.00
Cabbage	0.50	<0.5	1.00
Silverbeet	<0.5	<0.5	1.00
Vegetable juice	<0.5	<0.5	<0.5

*Results expressed as median (25<sup>th</sup>, 75<sup>th</sup> percentile)*

The intake of vitamin C rich fruit (including tomatoes) (Gourly, 2007) is shown in table 4.9 because of the strong association of vitamin C and symptoms and incidence of URTI (Douglas *et al.*, 2007). This provides some indication to the children's vitamin C consumption at baseline. Vitamin C rich fruit provided just under a third of the children's total fruit consumption.

Table 4.9 Number of serving per day of vitamin C rich fruit compared to other fruit

	Median serving (per day)	Lower quartile	Upper quartile
Vitamin C rich fruit	1.46	0.69	2.30
Other fruit	2.28	1.57	3.35

*Results expressed as median (25<sup>th</sup>, 75<sup>th</sup> percentile)*

## 4.4 Fruit and vegetable eating habits and behaviour

### 4.4.1 Food liking questionnaire

#### 4.4.1.1 Food liking at baseline

A visual analogue scale was used to measure children's fruit and vegetable likings (see appendix A). The scale has a range of 100 points ranging from positive to negative with the midpoint on the scale being zero, (no preference or neutral), and the extremes being -50 (extremely does not like) and 50 (extremely like).

The scale contained six fruits and six vegetables, seven hedonic and four non-food items for control / reference. Table 4.10 shows food liking at baseline ranked from most liked items in descending order. As expected, ice cream (hedonic control item) was the most liked item on the scale ( $43.2 \pm 0.52$ ) and loud siren (non-hedonic control item) was the least liked item on the scale ( $-15.1 \pm 1.85$ ).

All fruit were well within the liking range (liking range 0-50), with strawberries being the most liked fruit ( $40.58 \pm 1.108$ ) and kiwifruit being the least liked fruit at baseline ( $29.3 \pm 1.06$ ) but still well within the liking range (liking range=0-50). In general, vegetables were less liked than fruit. The most liked vegetable at baseline was corn ( $22.5 \pm 1.47$ ) followed closely by carrots ( $20.3 \pm 1.11$ ), however, both were below the lowest scoring fruit (kiwifruit) on the liking scale. Spinach, collard greens were the least liked vegetables at baseline ( $-3.4 \pm 1.8$ ) scoring below zero and within the dislike range (0 to -50).

Table 4.10 Food liking at baseline, items ranked in descending order

Items	Baseline liking (Mean $\pm$ SD)
Ice cream	43.2 $\pm$ 0.52
Strawberries	40.6 $\pm$ 1.12
Cookies, cake	40.2 $\pm$ 0.59
Chocolate (candy) bar	40.0 $\pm$ 1.04
Taking a bath	39.1 $\pm$ 1.08
Whole milk	37.5 $\pm$ 1.05
Apple juice	35.4 $\pm$ 0.82
Hot chips	34.2 $\pm$ 0.77
Banana	32.6 $\pm$ 1.14
Apple	32.5 $\pm$ 0.99
Watermelon	29.4 $\pm$ 0.97
Kiwifruit	29.3 $\pm$ 1.06
Getting dressed	23.4 $\pm$ 1.28
Corn	22.5 $\pm$ 1.47
Brushing teeth	21.7 $\pm$ 1.36
Fizzy drink*	20.8 $\pm$ 1.78
Carrot	20.3 $\pm$ 1.11
Lunch meat, hot dogs	18.6 $\pm$ 1.09
Butter, margarine	15.5 $\pm$ 1.17
Broccoli	8.40 $\pm$ 1.94
Kumara	2.70 $\pm$ 1.97
Spinach, collard greens	-3.40 $\pm$ 1.80
Loud siren	-15.1 $\pm$ 1.85

\* Fizzy drink liking was not rated by 40% of the parents as the children had never tried it before.  
Results expressed as mean  $\pm$  SD

#### 4.4.1.2 Fruit and vegetable liking before and after intervention

Repeated measures ANOVA were performed on the food liking questionnaire before and after the intervention. Kiwifruit was the only food item that changed on the liking scale and resulted in significant increase in liking after treatment (before intervention  $29.32 \pm 1.063$ , after intervention  $33.62 \pm 1.063$ ,  $P=0.006$ ) and it also moved in ranking from the fifth most liked fruit to third most liked fruit.

Table 4.11 Fruit and vegetable liking before and after intervention

Items	Before treatment	After treatment	<i>P</i> value
	Mean $\pm$ SD	Mean $\pm$ SD	
Strawberries	40.6 $\pm$ 1.12	39.2 $\pm$ 1.11	0.383
Apple juice	35.4 $\pm$ 0.82	36.8 $\pm$ 0.82	0.256
Banana	32.6 $\pm$ 1.14	33.3 $\pm$ 1.14	0.656
Apple	32.5 $\pm$ 0.99	32.8 $\pm$ 0.99	0.842
Kiwifruit	29.3 $\pm$ 1.06	33.6 $\pm$ 1.06	0.006
Watermelon	29.4 $\pm$ 0.97	29.2 $\pm$ 0.97	0.929
Corn	22.5 $\pm$ 1.47	20.6 $\pm$ 1.47	0.349
Carrot	20.3 $\pm$ 1.11	17.9 $\pm$ 1.11	0.151
Broccoli	8.40 $\pm$ 1.94	5.80 $\pm$ 1.94	0.337
Kumara	2.70 $\pm$ 1.97	4.00 $\pm$ 1.97	0.645
Spinach, collard greens	-3.40 $\pm$ 1.80	-6.10 $\pm$ 1.80	0.285
Loud siren (control item)	-15.1 $\pm$ 2.61	-18.1 $\pm$ 2.61	0.265

*Results expressed as mean  $\pm$  SD*

*Differences between baseline and end was assessed using repeated measures ANOVA*

#### 4.4.1.3 Fruit liking at baseline compared to fruit consumption

Results from the food liking questionnaire were compared to results from the FFQ i.e. fruit liking and fruit consumption were compared. Table 4.11 shows the fruits in terms of their liking scores and the frequency of their consumption.

Table 4.12 Fruit liking and consumption of specific fruits

Liking <sup>a</sup>	Consumption <sup>b</sup>			
	Liking scale (mean $\pm$ SD)	Median consumption (serving/week)	Lower quartile	Upper quartile
Strawberries	40.6 $\pm$ 1.108	0.50	<0.5	1.00
Banana	32.6 $\pm$ 1.137	3.00	1.00	7.00
Apple	32.5 $\pm$ 0.988	3.00	3.00	5.00
Kiwifruit	29.3 $\pm$ 1.063	0.50	<0.5	5.87

\*Fruits listed above appeared in both the liking questionnaire and FFQ

<sup>a</sup> Results expressed as mean  $\pm$  SD

<sup>b</sup> Results expressed as median (25<sup>th</sup>, 75<sup>th</sup> percentile)

In general fruits that were well liked were frequently consumed and the fruit liking ranks were the same as the fruit consumption rank (table 4.13). Strawberries were the only exception. While they were liked, they were not consumed often.

Table 4.13 Fruit ranked in descending order from the most liked/consumed fruit.

Liking	Consumption
Strawberries	Banana
Banana	Apple
Apple	Kiwifruit
Kiwifruit	Strawberries

*\* Fruits listed above appeared in both the liking questionnaire and FFQ*

#### 4.4.1.4 Vegetable liking at baseline compared to vegetable consumption

Table 4.14, shows the vegetables in terms of their liking scores and the frequency of their consumption. Corn was the most liked vegetable followed closely by carrot, and spinach was the least liked vegetable. However in terms of consumption, carrots were the most consumed vegetable by far (median=3 servings per week, (25<sup>th</sup>, 75<sup>th</sup> percentiles 0.8, 5.5). Starchy vegetables such as corn (median=0.5 servings per week, (25<sup>th</sup>, 75<sup>th</sup> percentiles 0.5, 1.5) and kumara (median=0.5 servings per week, (25<sup>th</sup>, 75<sup>th</sup> percentiles 0.5, 1) were the least consumed vegetables.

Table 4.14 Vegetable liking and vegetable consumption

	Liking <sup>a</sup>	Consumption <sup>b</sup>		
	Liking scale mean $\pm$ SD	Median consumption (serving/week)	Lower quartile	Upper quartile
Corn	22.5 $\pm$ 1.47	0.50	<0.5	1.50
Carrot	20.3 $\pm$ 1.104	3.00	0.87	5.50
Broccoli	8.4 $\pm$ 1.94	1.00	0.50	3.00
Kumara	2.7 $\pm$ 1.97	0.50	<0.5	1.00
Spinach	-3.4 $\pm$ 1.80	1.00	<0.5	1.00

*\*Vegetables listed above appeared in both the liking questionnaire and FFQ*

*<sup>a</sup> Results expressed as mean  $\pm$  SD*

*<sup>b</sup> Results expressed as median (25<sup>th</sup>, 27<sup>th</sup> percentile)*

Table 4.15 Vegetable liking and vegetable consumption in descending order from the most liked/consumed vegetables.

Liking	Consumption
Corn	Carrot
Carrot	Broccoli
Broccoli	Spinach
Kumara	Corn
Spinach	Kumara

*\*Vegetables listed above appeared in both the liking questionnaire and FFQ*

#### 4.4.2 Fruit and vegetable eating habits of the children in study

Only 30% of the children met their 5+ a day MOH recommendation of intake according to the parents (table 4.16). The percentage of children who consumed two or more servings of fruit per day was 60% and only 30% of children consumed three or more servings of vegetables per day as reported by parents. It was reported that 23.7 % of children usually ate fruit with their breakfast cereal every morning, and 20% of the children usually consumed fruit as part of their dessert. It was also reported that 52.2% of the children consumed fruit as a snack at least once per day and 27.1% of children consumed vegetables as a snack at least once per day.

Table 4.16 Fruit and vegetable eating habits of the children in study

Children's Eating habits	% children N=66
Children consuming 5 or more serving of fruit and vegetables per day	30.0%
Children consuming 2 or more servings of fruit per day	60.0%
Children consuming 3 or more servings of vegetable per day	30.0%
Children consuming fruit with their breakfast cereal every morning	23.7%
Children consuming fruit as part of dessert every day	20.0%
Children consuming fruit as a snack at least once per day or more	52.5%
Children consuming vegetables as a snack at least once per day or more	27.1%

*Results expressed as percentages*

## 4.5 Motivation of parents to provide their children with fruits and vegetables

### 4.5.1 Parents' self-efficacy

A self-efficacy questionnaire was used to determine parents' motivation to provide their children with fruit and vegetables. The questionnaire comprised six fruit self-efficacy questions, and six vegetable self-efficacy questions. Each question had a six point scale. A total score was calculated with a maximum score of 36. A total score of 18 or less indicates low-self efficacy, and higher than 18 indicates high self-efficacy. The median score for fruit self-efficacy was 30 (25<sup>th</sup>, 75<sup>th</sup> percentiles 26, 34) and the median score for vegetable self-efficacy was 29.5 (25<sup>th</sup>, 75<sup>th</sup> percentiles 22, 36) (table 4.17).

Table 4.17 Parents' self efficacy scores relative to motivation of parents to provide their children with fruits and vegetables

	Median self efficacy score (N=66)	Lower quartile	Upper quartile
Fruit self-efficacy	30	26	34
Vegetable self-efficacy	29.5	22	36

*Results expressed as median (25<sup>th</sup>,75<sup>th</sup> percentile)*

In general, the parents who took part in the study had a high self-efficacy with only 8.0% scoring below 18 on the fruit self-efficacy score, and 18% scoring below 18 on the vegetable self-efficacy score. This also indicates that our participants' (parents) had a higher self-efficacy for fruit than for vegetables.

Table 4.18 Parents' self-efficacy profile

	% Parent (N=66)
Parents with low fruit self-efficacy rating (less than 18 total score)	8.5%
Parents with low vegetable self-efficacy rating (less than 18 total score)	18%

*Results expressed as percentages*

#### 4.5.2 Correlation between parents' motivation to providing their children with fruits and vegetables (self-efficacy) and fruit and vegetable eating habits of children

Correlations between parents' motivation to provide their children with fruit and vegetables (parents' self-efficacy) and reported (by parents) children's fruit and vegetable consumption were analysed (table 4.19) by correlating the results from the eating habits questionnaire and the self-efficacy questionnaire. There was a strong significantly positive correlation between parents' vegetable self-efficacy and the amount of vegetables consumed by children (as reported by parents) ( $r=0.603$ ,  $P<0.0001$ ) and children's consumption of vegetables as an in-between meal snack ( $r=0.853$ ,  $P<0.0001$ ). There was a strong positive correlation between parents' fruit self-efficacy score and children's fruit consumption ( $r=0.54$ ,  $P<0.0001$ ), and parents fruit self-efficacy score and the consumption of fruit as an in-between meal snack by children ( $r=0.57$ ,  $P<0.0001$ ). Parents who had a high fruit self-efficacy score were likely to have a high vegetable self-efficacy score ( $r=0.61$ ,  $P<0.0001$ ).

Table 4.19 Correlation between parents' self-efficacy scores and the children's fruits and vegetables eating habits.

	Parents commitment to providing their children with fruit (self-efficacy) (N=66)	Parents commitment to providing their children with vegetables (self-efficacy) (N=66)		
	Correlation coefficient (r)	P-value	Correlation coefficient (r)	P-value
Servings of fruit consumed by child (as reported by parents)	0.54	<0.0001	0.60	<0.0001
Servings of vegetables given to child by parent not including potatoes (as reported by parents)				
Serving of fruit with breakfast	0.22	0.09		
Serving of fruit as part of dessert	0.22	0.09		
Fruit as in between meal snack	0.57	<0.0001		
Vegetable as in between meal snack			0.58	<0.0001
Parents commitment to providing their children with fruit (self-efficacy)			0.61	<0.0001

Results expressed as correlation coefficient (r)  
Correlations between groups assessed using Spearman's rank correlation coefficient test

## 4.6 Summary

The children in the study had a lower incidence of cold- and flu-like illnesses during the kiwifruit intervention phase of the trial compared to the banana phase. Some of the URTI symptoms were significantly less during the kiwifruit intervention phase compared to the banana phase. The children's fruit consumption did not change from baseline to the end of the study. However, their vegetable consumption increased at the end of the trial compared to baseline. The children's food liking did not differ between baseline and the end of the study with the exception of kiwifruit (liking increased at the end of the study). Fruit and vegetable consumption was associated with liking, and children's fruit and vegetable consumption habits were strongly correlated with their parents' motivation to provide their children with fruit and vegetables.

## Chapter 5

### 5. Discussion

The main purpose of the study was to investigate if regular consumption of gold kiwifruit by pre-schoolers can reduce the incidence and severity of upper respiratory tract infections (URTI). This was determined by using the validated CARIFS survey instrument to record the incidence and severity of URTI symptoms in a randomised cross over controlled trial where the consumption of two servings of gold kiwifruit per day for five days a week was compared to the consumption of two servings of banana per day for five days a week in children aged three to five years attending local (Auckland) crèches and play-centres.

The two servings of fruit given to subjects in the study were based on serving sizes specified by the New Zealand Ministry of Health guidelines for children aged 2-12 years (Ministry of Health, 2010) and therefore a realistic expectation of a fruit intake that this age group can meet. The kiwifruit and banana intake is likely to have displaced other fruit intake at the crèche/ play-centres but theoretically it should not displace fruit intake at home. Gold kiwifruit was selected as the “intervention” fruit due to its high vitamin C content and it also contains vitamin E, copper, carotenoids and folate (Ferguson, 2003). Previous *in vitro* cell based studies and *in vivo* animal studies have demonstrated that kiwifruit may have an immune support function (Chang, 2009, Collins *et al.*, 2001, Farr *et al.*, 2008, MA *et al.*, 2006, Molan *et al.*, 2008, Prior *et al.*, 2007, Shu *et al.*, 2008, Hunter *et al.*,

2008). An alternative fruit (banana) was provided during the control intervention period to control for any changes to symptoms or incidence of URTI due to improved wellbeing through the incorporation of fruit into the diet or they are fruit specific. Banana was selected as the control fruit because it has a relatively, similar energy content, but lower nutritional value than gold kiwifruit with respect to most minerals, vitamins and carotenoids <http://www.nal.usda.gov/fnic/foodcomp/search/> (accessed 11/08/2010)}. This study also investigated the fruit and vegetable eating habits of participating children. Discussion will include the effect of the intervention on the incidence of cold- and flu-like illnesses, and on URTI symptoms. Children's fruit and vegetable consumption habits, fruit and vegetable likings, and parents' self-efficacy will also be discussed.

## **5.1 Characteristics of study children**

On the commencement of the study the subjects were required to be between the ages of two to five years of age and attending a crèche or a play-centre. The reason for recruiting this population is the high incidence of cold- and flu-like illness in this particular age group (Eccles, 2005). In New Zealand, children between the ages of one and four who are attending a crèche or play-centre have the highest rate of cold-and flu-like illnesses (Blackmore, 2005, Ardagh, 2006, Curry *et al.*, 2006). Children under the age of five years are a relatively neglected group possibly due to the associated difficulties and limitations in designing a study for that age group. Children under five have limited language abilities that preclude self-report measures and necessitate the use of parents, clinicians, or

other proxies. In addition, issues such as the stage of development are also crucial factors in this age group and they have to be accounted for (Stein and Jessop, 1990).

A total of 100 children were recruited even though only 50 children were needed for the study to be sufficiently powered to detect a statistically significant difference between the kiwifruit and the banana interventions. It was anticipated that there would be some drop-out because this was somewhat of a high burden study for the parents as they were required to fill the CARIFS diary daily for three months. Additionally, as this was the first study of its kind, it was unclear as to how the children would comply with consuming two servings of gold kiwifruit or banana for five days a week for four weeks. Therefore, twice the number of children required was recruited to allow for any possible drop-outs. The majority of children who dropped out of the study (22 children) were due to the parents not completing their diaries, rather than the children not consuming their allocated fruit. Only 12 children were excluded due to compliance issues. Most children had no issues or difficulty consuming two servings of kiwifruit per day or two servings of banana per day.

As this was intended to be a true “free-living” situation, allergies to gold kiwifruit or banana were the only medical exclusion criteria. However, the detailed medical history questionnaires that were collected about the children allowed for assessment to ensure that the children with illnesses such as asthma, wheezing problems, or food allergies did not behave differently from other healthy subjects in the study.

## **5.2 The effects of the intervention on Upper Respiratory Tract Infection**

### **5.2.1 Incidence of cold-and flu-like illness**

The primary outcome was assessed by a common cold- and flu-like illnesses diary (CARIFS diary). The Canadian Acute Respiratory Illness and Flu Scale is valid in the target age of the study and has been used in other English speaking countries besides Canada. To our knowledge this is the first time it has been used in New Zealand (Jacobs *et al.*, 2000, Shepperd *et al.*, 2004, Butler *et al.*, 2002).

Findings from the study indicated that the odds of having a cold- and flu-like illness was almost half as much (OR= 0.55, 95% CI (0.32, 0.94), P=0.0293) during the kiwifruit phase of the intervention compared to the banana phase of the intervention. This yielded a subjective measure of what defines a cold- and flu-like episode. However, due to the blind crossover study design, the effect of the intervention was not expected to be biased. Children, parents, and crèches/ play-centres' staff were not aware which fruit was of interest and equal importance was placed on banana and gold kiwifruit consumption. Currently there are no studies in the literature that have investigated the effect of kiwifruit on URTI in children. Studies showing the effects of vitamins, antioxidants and nutrients such as vitamin C, vitamin E, and carotenoids on the common cold have been inconsistent (Douglas *et al.*, 2007, Hemila, 2004, Hemila and Kaprio, 2004, Hemila *et al.*, 2002). Some studies showed improvement (Douglas *et al.*, 2007, Hemila, 2004), while others showed no effect (Hemila, 1992, Meydani *et al.*, 2004, van der Horst-Graat *et al.*, 2004). We predict that the lower incidence of cold- and flu-like episodes during the kiwifruit intervention is due to our whole fruit approach.

Vitamin and nutrients such as vitamin C, vitamin E, and carotenoids, which might have showed inconsistent results in previous URTI trials, may provide a better outcome when consumed as part of a whole fruit. Gold kiwifruit provides a natural balance of these vitamins and nutrients along with other bioactives that may act in a combined complementary and synergistic manner to support host immune defences. Similarly, a recent study in pregnant women suggested that high consumption of both fruit and vegetables was associated with moderate reduction in the risk of URTI (Li and Werler, 2009). Our findings are comparable to some of the findings from vitamin C supplementation and URTI trials (Douglas *et al.*, 2007). Vitamin C, the main nutrient in gold kiwifruit, although possibly not the only bioactive present (Ferguson, 2003) was only effective at decreasing the risk for developing the common cold in subjects with a compromised immune system such as subjects under physical stress (athletes) or subjects under extreme environmental conditions (Douglas *et al.*, 2007).

A meta-analysis conducted by Douglas *et al.* in 2004 (Douglas *et al.*, 2004) found that athletes who supplemented with 200 mg or more per day of vitamin C show a relative risk of 0.5 (95% CI (0.38, 0.66)) of developing the common cold (Douglas *et al.*, 2004). Another five trials (one trial of military personnel, two trials of students in crowded lodging, and two trials of marathon runners) found a statistically significant reduction in the incidence of the common cold ranging from 45% to 91% (Hemila, 2004). With the exception of the groups mentioned above, the majority of placebo controlled vitamin C trials in the literature found no difference in the incidence of the common cold between those who regularly supplemented with vitamin C and those who did not (Douglas *et al.*, 2007). No

reduction in the incidence of common colds were reported in children (< 14 years of age) either, although a significant decrease in the number of missed school days (unspecified number) were reported by those supplementing with vitamin C ( $P=0.02$ ) compared to placebo (Douglas *et al.*, 2007). Our target population for this study was children under the age of five. This age group is characterised by their developing immune system (Adkins *et al.*, 2004) which may also explain some of the positive outcomes observed.

It is worth noting that there is a heavy domination by adult studies, and studies including children are scarce and mostly date back to the late 70s and early 80s. The authors of the most recent (2007) Cochrane review “Vitamin C for preventing and treating the common cold” have stated that more studies with children are needed. In addition, the authors have recommended that these studies should have an increased number of subjects with varied family educational background, socioeconomic levels, and immune status (Douglas *et al.*, 2007). This is what we intended to accomplish in our current study and is one of the rationales for our broad inclusion criteria. The positive outcomes observed during our kiwifruit intervention are probably not limited to the effects of vitamin C. Vitamin E and  $\beta$ -carotene are two other micronutrients found in gold kiwifruit and have demonstrated positive outcomes against the common cold in supplementation studies (Graat *et al.*, 2002, Meydani *et al.*, 2004), although these outcomes were not always consistent (Harman and White-Miller, 1986, Hemila and Kaprio, 2004, Hemila *et al.*, 2002, Cser *et al.*, 2004).

The number of unspecified infections were higher among elderly subjects (<60 years of age) with low vitamin E levels (Chavance *et al.*, 1989). In another elderly study (Girodon *et al.*, 1999) the total number of infections were 30% lower (compared to placebo) when they were given a combination of 15mg/day vitamin E, 6 mg/day  $\beta$ -carotene, and 120 mg/day vitamin C compared to placebo. This is in agreement with the proposal that vitamins and nutrients may be more effective when consumed together than singularly and in a food source rather than a supplement (Halliwell, 1996) which may further explain the outcomes of our study.

### 5.2.2 Upper respiratory tract grouped symptoms score (CARIFS scores)

The overall average total symptom score over the kiwifruit phase of the intervention was lower than the average symptom score over the banana phase of the intervention and the difference was significant ( $P= 0.015$ ). When the analysis was broken down further into the three domains measured by CARIFS, it revealed that there was a significant difference in the physiological symptom scores and the functional symptom scores, however there was no difference in the parental impact scores. These results indicated that the kiwifruit intervention had a beneficial effect on children's physiological symptoms which translated into a beneficial effect on functional symptoms. While there was a reduction in the burden of illness to parents, this reduction was not significant.

A possible explanation could be that the beneficial effect on physiological symptoms was moderate although statistically significant and the measurable follow-through effects were diluted down when translated into reduction of parental impact. In a study by Sheppard et al (Sheppard *et al.*, 2004) the

performance of CARIFS was evaluated in children in a European primary care setting. It was found that CARIFS appeared to be a good measure of functional severity and burden of illness to the parent but not a good measure of physiological severity (Shepperd *et al.*, 2004). This result appears to be in contrast to our findings that CARIFS was a good measure of physiological symptoms.

### 5.2.3 Individual upper respiratory tract infection symptoms measured by CARIFS

The probabilities of having a symptom over a 28 day period were calculated for each symptom. The odds ratios of having a certain symptom were also calculated in reference to the banana intervention. Daily consumption of two servings of gold kiwifruit were associated with a significant reduction in the odds ratio for three physiological symptoms (feels unwell, headache, and sore throat), and two functional symptoms (poor appetite, and low energy / feeling tired), and one parental impact symptom (crying more than usual). The most significant reduction in probabilities of having a symptom, over a 28 day period, were those for sore throat and headache. The odds ratios of having these symptoms during the kiwifruit intervention phase were a third of that of the banana group. More importantly, poor appetite and sore throat are early symptoms of cold-and flu-like illnesses, while headache tends to be experienced later as illness progresses (Eccles, 2005). Therefore, gold kiwifruit consumption may reduce the burden of symptoms at all stages of infection. While CARIFS was validated as a measure of URTI illness in children, it has mostly been used in observational studies evaluating URTI illness severity, determinants and impact on children and families (Butler *et al.*, 2002, Jacobs *et al.*, 2000, Shepperd *et al.*, 2004). Parents or health care professionals (such as doctors or nurses) are able to evaluate a child's

URTI illness severity by acting as proxies and completing CARIFS on behalf of the child. This yields a subjective measure of the URTI symptoms and their severity. However, previous studies using CARIFS have demonstrated that parents are accurate reporters of their children URTI symptoms (Shepperd *et al.*, 2004). While CARIFS may be completed by teachers or health care professionals as an illness evaluation measure it is parents who appeared to be the most accurate reports of their children's illness (Shepperd *et al.*, 2004). As far as we are aware of only one study that used CARIFS as an intervention evaluation measure for URTI (Vohra *et al.*, 2008). Vohra et al (Vohra *et al.*, 2008) investigated the safety and tolerability of a ginseng extract in the treatment of paediatric (3- 12 years old) URTI in a randomised controlled phase II trial. The treatment effect i.e. the severity of URTI episodes were evaluated using CARIFS. In the Vohra study (Vohra *et al.*, 2008) subjects were referred to a medical practitioner for confirmation of URTI episodes, and then they were administered the treatment or placebo and required to fill in the CARIFS for the three days of the treatment/placebo. Our study is unique because the subjects were required to record their CARIFS scores every day for the duration of the study.

All studies using vitamin C as a treatment for the common cold in children used their own URTI survey measures, which had varying definitions of what constitutes a common cold episode or the severity of the episode measured (Carr *et al.*, 1981, Coulehan *et al.*, 1976, Ludvigsson *et al.*, 1977, Martin *et al.*, 1982, Miller *et al.*, 1977). Also, those surveys mostly accounted for physiological symptoms only and were structured differently from CARIFS, which complicates comparison with them (Carr *et al.*, 1981, Miller *et al.*, 1977, Ludvigsson *et al.*, 1977, Coulehan *et al.*, 1976, Martin *et al.*, 1982). Furthermore, the recorded

symptoms were not analysed individually in those studies as they were in our study. Rather, those symptoms were used collectively to determine an overall score regarding the severity of the cold-and flu-like episodes (Carr *et al.*, 1981, Miller *et al.*, 1977, Ludvigsson *et al.*, 1977, Coulehan *et al.*, 1976, Martin *et al.*, 1982). Overall, vitamin C studies in children eight to nine years of age (Ludvigsson *et al.*, 1977) and vitamin C studies that included children (<14 years of age) among their adult subjects (Carr *et al.*, 1981, Martin *et al.*, 1982, Miller *et al.*, 1977) showed a slight reduction in the severity of cold- and flu-like episodes which may indicate a reduction in the individual symptoms should they be analysed separately.

We were well aware of these difficulties prior to commencing our study, and that there are no “normal” CARIFS scores or reference points to compare our study to. This was taken into account when designing the study. Thus, a randomised controlled cross over trial design was chosen and analysis was carried out by comparing the two interventions to each other i.e. using banana as the control. The intervention lasted for three months; commencing late August 2009 and concluding early December 2009. Therefore the study took part in late winter to early summer. This was not the original intention. Most of the cold and flu episodes occur in NZ during the winter months (June-October) (Arroll and Kenealy, 2002, Blackmore, 2005, Cross *et al.*, 2009) but due to logistical issues we were unable to commence earlier. The average yearly incidence of cold- and flu-like illnesses in children in this study can be extrapolated from the reported cold and flu episodes. Children had an average of three cold- and flu-like episodes per year. This is below the reported values in the literature which estimates that

children have six to eight cold- and flu-like episodes per year (Proud and Chow, 2006). This reduced number of cold- and flu-like episodes reported in this study is probably due to the study taking place at the end of the cold and flu season.

A seasonal effect was observed. Irrespective of the order of intervention (kiwifruit followed by banana, or banana followed by kiwifruit), there was a gradual decline in the proportion of subjects reporting one or more symptoms of URTI during each treatment or washout period as the trial progressed. No specific mention of seasonal effect was reported in the cold and flu studies reviewed earlier; however, those studies generally took part over the winter season or over an entire year, indicating that the authors are well aware of the seasonality of illness (Arroll and Kenealy, 2002, Blackmore, 2005, Cross *et al.*, 2009).

As reported in chapter 4 the cough symptom showed a general decline in scores as the study progressed towards summer and the scores tended to be less severe in early summer, compared with late winter. However, the seasonal effect did not change the direction of the effects of the intervention. The odds of experiencing the cough symptom (for which we saw reduced odds as the study progressed towards summer) were lower when consuming kiwifruit in either phase of the study compared to banana. The headache symptom plot showed no seasonal effect probably because it is not a commonly or frequently occurring symptom of URTI (Eccles, 2005). Even though a preschool aged child may be able to report this symptom to their parents, headache maybe one of the least identifiable symptom in small children. Vomiting was the only symptom that scored better during the banana intervention phases compared to the kiwifruit phases, although the difference was not significant ( $P=0.501$ ). This is possibly due to the acidic taste of kiwifruit (Ferguson, 2003) compared to the mild taste of banana. One

might expect that a milder tasting fruit might improve a gut associated symptom such as vomiting better than an acidic tasting fruit.

### **5.3 Fruit and vegetable consumption**

#### **5.3.1 Total fruit and vegetable consumption before and after intervention**

There was no change in fruit consumption between baseline and post intervention, while vegetable consumption increased after intervention ( $P=0.001$ ) resulting in an overall increase in total fruit and vegetable consumption post intervention ( $P=0.009$ ). Parents of subjects were asked to maintain their children's normal fruit and vegetable consumption at home during the intervention. However, the effect of studies/interventions on subjects' habitual behaviour (although it might be subconscious) is well documented (Gibson, 1990, Willett, 1998) and it is plausible that the addition of two servings of fruit during the intervention period may have affected normal fruit and vegetable consumption by "displacing" or "changing the type of" normally consumed fruits and vegetables in the diet. As this was a fruit intervention study and parents were aware that their children were receiving two servings of fruit per day at their crèche/play-centre, it is possible that this has led to parents focusing their attention on their children's vegetable consumption leading to the increase observed in vegetable consumption. Other school-based fruit and vegetable interventions also reported effects on subjects fruit and vegetable consumption habits at home (Sandie *et al.*, 2005). The School Fruit and Vegetable Scheme (SFVS), conducted in the United Kingdom provided a free piece of fruit or a vegetable to children aged four to six years, in more than 500 schools between 2000 and 2001. In contrast to the present

study they found that over the lifespan of the intervention, fruit and vegetable consumption of children declined at home (Ransley *et al.*, 2007, Sandie *et al.*, 2005) although the overall result was an increase in total fruit and vegetable consumption (Sandie *et al.*, 2005, Ransley *et al.*, 2007).

A seasonal effect can also be another possible explanation to the observed increase in vegetable consumption. The study commenced in late winter (when there is less choice of fresh vegetables available) and concluded in early summer (when a wider variety of fresh vegetables at more affordable prices become available) (Gourly, 2007).

At baseline parents were required to report their children's fruit and vegetable consumption (the "eating habits survey" (see appendix H) in addition to completing the fruit and vegetable FFQ. Parental reporting (from the "eating habits survey") of both total daily fruit-and-vegetable intakes and fruit-only intakes were nearly 50% lower, and vegetable intakes were nearly 25% lower than those estimated from the FFQ.

The discrepancy between the values reported from the two methods can either be as a result of parents under-estimating the servings of fruit and vegetables consumed by their children or over-reporting with the FFQ. The FFQ used in the study was non-interviewer based, and previous studies (Serdula *et al.*, 2001) report over-estimation of food consumption with self-filled FFQs. This was the primary reason for the addition of further questions, about fruit and vegetable consumption to the "eating habits survey" filled out at baseline.

According to findings from the FFQ the median fruit and vegetable consumption is about seven serves per day at baseline, and eight serves at the end of the study. This number of serves is a lot higher than reports from the NZ CNS (Ministry of Health, 2002) where only two out of five children consumed two or more servings of fruit per day, and three out of five children consumed three or more servings of vegetable per day. It is possible that over reporting occurred at baseline and at end of the study, due to the consistently high numbers from beginning to end.

Therefore the FFQ findings are still valid particularly in relation to baseline and end comparison and the type of fruits and vegetables consumed and the frequency of their consumption. However, it must be noted that the parents who took part in this study were highly motivated (as will be discussed later on with regard to their self-efficacy scores) and the high serves of fruit and vegetable consumption calculated from the FFQ cannot be ruled out to be accurate although highly unrealistic.

### 5.3.2 Fruit and vegetable consumption at baseline

The most commonly consumed fruits at baseline were banana or apples, which is somewhat comparable with the NZ CNS (Ministry of Health, 2002). According to the latest 2002 NZNS apples or pears were the most commonly consumed fruit by New Zealand children. However, pears were not consumed frequently by the children in this study and neither were oranges or mandarins compared to NZ CNS. Seasonal effects may account for this observation because the intervention started in late winter and concluded early summer, possibly resulting in lower availability of citrus fruit towards the end of winter. The NZ CNS took place over

the span of an entire year and therefore seasonal effects are accounted for compared to our three month long study.

The most commonly consumed vegetable group at baseline were carrots, followed by tomato and broccoli. These findings are very similar to the findings from the NZCNS with the exception of the frequency of potato consumption. In the NZCNS (Ministry of Health, 2002), potatoes were the most frequently consumed vegetable group followed by carrots and broccoli. In this study potatoes were the fourth most commonly consumed vegetable. The FFQ included less potato groups than the NZCNS, and no fried potato preparations were. The NZNS (Ministry of Health, 2002) included potatoes that were boiled, mashed, baked or roasted (as in the study FFQ) and in addition it included fried potatoes such as hot potato chips, kumara chips, French fries, wedges or hash browns. The differences in those extra potato preparations included may explain the slight variation in vegetable results between the study FFQ and NZCNS.

The association between vitamin C consumption and URTI illnesses and symptoms are well known (Douglas *et al.*, 2007). Therefore the consumption of fruit rich in vitamin C (including tomatoes) was calculated to assess intakes of vitamin C at baseline. On average vitamin C-rich fruits consumed at baseline were one third of all fruits consumed. Therefore it is possible that the intervention improved the children's' vitamin C intake leading to the observed positive outcomes.

## 5.4 Fruit and vegetable eating habits and behaviour

Children aged two to five years have less autonomy in making food choices, therefore, it is likely that environmental factors play just as much of an important role as personal preferences in determining their fruit and vegetable consumption (Sallis and Owen, 2002). When investigating the fruit and vegetable eating habits of our subjects we focused on two primary determinants of food consumption behaviour, namely: taste (food liking) and parents' motivation in providing their children with fruit and vegetables (environmental factors).

### 5.4.1 Food liking Questionnaire

#### 5.4.1.1 Food liking at baseline

In general, taste preferences or food liking, are regarded as key determinants of food choices (Capaldi, 2001), and in young people may be more so (Birch, 1999). Children tend to eat what foods they like, and avoid foods that they dislike, and certain tastes seem to be innate for them such as liking for sweet and salt, and disliking for bitter and sour (Birch, 1999, Rozin, 1990). However with age, taste preferences can be learned or unlearned (Rozin, 1990). It is argued that children are programmed to like the taste of energy-dense foods as an evolutionary advantage to recover their nutrient requirement and caloric needs during this period of their lives, which is characterised by a high growth rate (Brug *et al.*, 2008). Most fruits and especially vegetables have low energy densities, and many vegetables have some bitter taste, therefore preferences for these foods may need to be learned (Brug *et al.*, 2008).

A food liking questionnaire that was developed and validated by Kennedy et al (Kennedy *et al.*, 2008) at the University of Connecticut in the USA was utilised in our study to assess the participants liking of food items. Although the survey had 23 items containing all food groups the analysis was focused on fruit and vegetables. The survey was filled out by the parents/ guardians of participating children. As expected, rich-tasting energy dense foods were most liked, with ice cream being the most liked item on the questionnaire. Strawberries were the most liked fruit and it was followed closely by cookies, cake, and chocolate. Overall, sweet-tasting foods were more liked than salty-tasting foods. Apple juice was liked (ranked number 7) considerably more than fizzy drinks (ranked number 17), even though both items are sweet drinks. However, fizzy drinks were only tried by 40% of subjects, and it is possible that parents are likely to offer their children apple juice more commonly than fizzy drink.

#### 5.4.1.2 Fruit and vegetable liking before and after intervention

Fruit liking did not differ/ change between baseline and post-intervention, with the exception of kiwifruit. At baseline, kiwifruit ranked sixth, and was the least scoring fruit on the liking scale; however, post intervention kiwifruit ranked third and was only preceded by strawberries and apple juice in terms of liking.

Kiwifruit was not a commonly consumed fruit at baseline, and it is possible that after the intervention children became more familiar with kiwifruit and acquired a liking for it. Evidence suggest that taste preferences can be learned and unlearned at a young age (Rozin, 1990, Birch and Ventura, 2009), which highlights the importance of teaching children healthy eating habits from a young age as this is likely to have lifelong implications onto their eating habits and food preferences.

#### 5.4.1.3 Fruit liking at baseline compared to fruit consumption

Fruit liking and fruit consumption were closely comparable. As expected, fruits that were most liked were also most consumed. Strawberries were the only exception. While they were the most liked fruit, they also were the least consumed. Strawberries are a seasonal fruit (summer), and they were not commonly available at baseline of this intervention (late winter). In addition, strawberries are more expensive than other fruit such as apples, which might further explain the difference between their liking values and consumption values. Price of a fruit or a vegetable and their availability are both two important environmental determinants of children's food consumption (Sallis and Owen, 2002). Both gold and green kiwifruit were not as commonly consumed as other fruits at baseline. Kiwifruit had the lowest liking scores compared to other fruits at baseline. Although, kiwifruit is a readily available fruit in New Zealand, factors such as its price may influence its consumption.

#### 5.4.1.4 Vegetable liking at baseline compared to vegetable consumption

There were some differences between vegetable liking and vegetable consumption at baseline. Carrots were the second most liked vegetable but it was consumed the most. Possibly, because carrots are a vegetable that can be easily offered as an in-between meal snack (Ministry of Health, 2010). Corn was the most liked vegetable but it ranked second to last in terms of consumption. One can speculate that this is because parents thought of corn on the cob (rather than frozen corn, as it was not specified in questionnaires), and corn on the cob is only available in New Zealand during the summer months (January, February, and March) (Gourly,

2007) and was not available during our study period (August to December). This could explain the difference between liking and consumption observed with corn.

#### 5.4.3 Eating habits of Children in the study

Only 30% of children in the study met the recommended 5+ a day (Ministry of Health, 2010) as reported by their parents. The New Zealand Ministry of Health recommends at least two serves of fruit per day (Ministry of Health, 2010) and 60% of the children in this study met those recommended guidelines. This is well above the national average when compared with the latest 2002 NZ CNS (Ministry of Health, 2002), where only 40% (two out of five children) met the recommended number of serves of fruit per day. On the other hand, the vegetable consumption of children in this study was below the national average. Only 30% of children consumed at least three or more servings of vegetables per day (NZ recommended guidelines) compared to 60% of children in the 2002 NZ CNS (Ministry of Health, 2002). The children who were surveyed in the NZ CNS (Ministry of Health, 2002) were an older age group, five to twelve years of age, and the literature indicates that fruit and vegetable consumption changes with the age of the children (Ransley *et al.*, 2007, Rasmussen *et al.*, 2006). Additionally, this was a fruit based study and the bias towards fruit consumption observed might be a reflection of selection bias of subjects i.e. parents with children who like fruit are more likely to enrol them in a fruit study. The most common way of fruit consumption by children were as an in-between meal snack, and around 20% of children consumed fruit as a dessert or with their breakfast cereal. Just under a third of the children consumed vegetable as an in between meal snack. Fruit was more commonly consumed as a snack than vegetables were.

## **5.5 Motivation of parents to provide their children with fruits and vegetables**

### **5.5.1 Parents' self-efficacy**

In general, the majority of the participants (parents) had medium to high self-efficacy scores for both fruit and vegetable provision to their children. The self-efficacy scores for fruit were much higher than that for vegetables. This is consistent with the findings that fruit consumption was higher than vegetable consumption in children who took part in the study. It also re-affirms the assumption that the study had a bias or attracted participants that have a preference for fruit due to the nature of the intervention. Motivation and intentions are important determinants of nutrition behaviours and they are the results of subjective weighing of expected positive and negative consequences of behaviour (Brug *et al.*, 1995, Brug *et al.*, 2008). Self-efficacy (or also referred to as perceived behavioural control *PBC*), refers to one's confidence and abilities and skills to engage in certain behaviours (Brug *et al.*, 1995). Perceived behavioural control is behaviour and content specific and studies indicate that nutrition-related self-efficacy is associated with healthy food choices (Resnicow *et al.*, 1997, Neumark-Sztainer *et al.*, 2003). Parents have a significant impact on their children's food choices and particularly so in the case of subjects of this study, as pre-school aged children are likely to have less autonomy in their food choices (Neumark-Sztainer *et al.*, 2003). This is evident by the strong positive correlation between mothers' and children's intake of specific foods reported in the literature (Cooke *et al.*, 2004). Understanding the correlates of dietary intake of fruit and vegetables is necessary in order to guide the development of an effective intervention and to promote healthy eating behaviour among children.

### 5.5.2 Correlation between parents' motivation to providing their children with fruits and vegetables (self-efficacy) and fruit and vegetable eating habits of children

Parents' fruit self-efficacy scores significantly correlated with the servings of fruits consumed by children and with consumption of fruit as an in-between meal snack. Similarly, parents' vegetable self-efficacy scores significantly correlated with the servings of vegetables consumed by children and with consumption of vegetables as an in-between meal snack. This finding is consistent with other studies that showed self-efficacy for fruit and vegetable consumption is strongly and consistently associated with higher fruit and vegetable intake across all socio-economic levels of the population (Luszczynska *et al.*, 2007). Parents' fruit self-efficacy scores correlated with their vegetable self-efficacy scores i.e. if parents were motivated towards providing their children with fruit then they are also likely to be motivated to provide their children with vegetables as well.

The strong association between parents' motivation to provide their children with fruits and vegetables and children's fruit and vegetable consumption observed in this study is consistent with findings from the literature. In a recent Belgian-Flemish study by Neumark-Sztainer (Neumark-Sztainer *et al.*, 2003) it was shown that parents' demand and facilitation of their 11 year old children's fruit and vegetable consumption was associated with higher fruit and vegetable intake in the children (Neumark-Sztainer *et al.*, 2003).

Parents are both elements and determinants in young children's environment (Brug *et al.*, 1995). They influence young children's eating habits in several ways either by dictating which foods are available (as stated earlier), or how foods are prepared and in what quantities they are consumed at (Luszczynska *et al.*, 2007).

This study establishes a strong significant association between parent's motivation to providing their children with fruits and vegetables (self-efficacy) and the children's fruit and vegetable consumption. Suggesting that strategies aimed at developing strong parental motivations towards providing their children with fruits and vegetables may be worth incorporating into interventions aimed at increasing young children's fruit and vegetable consumption.

## Chapter 6

### 6.1 Summary of the study

Evidence suggests that diets rich in fruits and vegetables boost the body's natural defences against diseases caused by infection and several studies investigated the effects of components of these diets on infections such as cold-and flu- like illnesses (Douglas and Muirhead, 1983, Chatzi *et al.*, 2007, Cook *et al.*, 1997, Gleeson *et al.*, 2004). The treatment and prophylaxis of vitamin C against the common cold have been studied in a number of human trials (Douglas *et al.*, 2007). Regular consumption of vitamin C may reduce the cold symptoms in both adults and children in a dose dependant manner (Douglas *et al.*, 2007). Vitamin C may also be effective at preventing the common cold in certain groups of the populations such as athletes, or subjects exposed to strenuous environmental conditions (Hemila, 1999, Hemila, 2004, Hemila, 2006). Vitamin E and  $\beta$ -carotene have also been investigated in relation to the common cold in a hand full of studies mostly conducted in the elderly or male smokers (Hemila and Kaprio, 2004, Hemila and Kaprio, 2008, Hemila and Kaprio, 2009, Hemila *et al.*, 2004, Hemila *et al.*, 2006). The outcomes from these studies have been largely variable, with some showing benefits (Meydani *et al.*, 2004, Liu *et al.*, 2007), while others not (Graat *et al.*, 2002, Hemila *et al.*, 2006). It is possible that closely related antioxidant vitamins and precursors (vitamin C, E, and carotenoids) may affect the common cold incidence and symptoms more effectively when they are

consumed as part of a whole fruit that is rich in them and possibly other bioactives (Lampe, 1999). This natural balance found in fruits or vegetables may allow these vitamin and precursors to act in a combined or synergistic manner to improve the host immune system against common ailments like the URTI (Lampe, 1999).

Zespri® GOLD kiwifruit is a rich source of vitamin C and other vitamins, minerals and precursors such as vitamin E, copper, folate, and carotenoids that may influence immune function (Ferguson, 2003). Therefore, Zespri® Gold Kiwifruit provides an excellent candidate as a nutritional intervention for preventing and reducing the symptoms of the common cold.

The aim of this research was to investigate the effects of consuming gold kiwifruit on the incidence and severity of URTI symptoms in preschoolers. Furthermore, consideration was given to children's fruit and vegetable consumption and liking during the trial and their parents' motivation (self-efficacy) towards providing their children with fruits and vegetables.

In a randomised, crossover trial, 66 children (aged two to five years) were randomised into one of two groups following a 2-week washout period and consumed an equivalent of two servings of gold kiwifruit (group A) or two servings of banana (group B) daily for 4 weeks. This was followed by a 2-week washout period and a cross-over of the treatments i.e. "group A" consumed two servings of banana and "group B" consumed two servings of gold kiwifruit for a further four weeks, followed by a final 2-week washout period. Parents completed a daily questionnaire of URTI symptoms, the validated Canadian Acute Respiratory Illness and Flu Scale (CARIFS) (Jacobs *et al.*, 2000), which assessed the incidence of cold-and flu-like illnesses and the severity of those symptoms.

Children's fruit and vegetable consumption and liking information were collected (via fruit and vegetable FFQ and liking questionnaires completed by the parents) at baseline and upon completion of the intervention. Additionally, information regarding parents' motivation towards providing their children with fruits and vegetables were also collected via a self-efficacy questionnaire completed by the parents.

A mixed effects model was fitted using SAS 9.2 Proc Mixed to measure the various symptom scores produced by CARIFS. The probabilities of individual symptoms were analysed using Proc Genmod. Differences within groups were analysed using either repeated measures ANOVA or the Wilcoxon Ranked-Sum Test, for parametric variables and non-parametric variables Spearman's Correlations Coefficients were used to determine any significant mono-tonic relationships. A *P* value of <0.05 was considered to be statistically significant. The odds of having a cold-and flu-like illness was almost half as much (OR= 0.55, 95% CI (0.32, 0.94), *P*=0.0293) during the kiwifruit phase compared to the banana phase of the intervention. The overall total symptom scores for the kiwifruit intervention ( $21.6 \pm 0.15$ ) were significantly (*P*= 0.015) lower than that for the banana intervention ( $22.1 \pm 0.15$ ). The total score of the physiological symptoms were significantly lower (*P*= 0.041) in the kiwifruit intervention ( $21.6 \pm 0.15$ ) compared to that of the banana intervention ( $22.0 \pm 0.15$ ). The total score of the functional symptoms were also significantly (*P*= 0.006) lower in the kiwifruit intervention ( $8.34 \pm 0.07$ ) compared to that of the banana intervention ( $8.53 \pm 0.07$ ). While the parental impact scores were also lower for the kiwifruit intervention ( $5.12 \pm 0.04$ ) compared to the banana intervention ( $5.19 \pm 0.04$ ),

although the difference was not significant. Results also showed significant differences in individual symptoms between the kiwifruit and banana interventions. The odds ratio (over a 28 day period) of not feeling well, having a headache, sore throat, poor appetite, low energy, feeling tired, and crying more than usual were all significantly lower for the kiwifruit intervention compared to the banana intervention. Vomiting was the only symptom that was better for the banana intervention compared to the kiwifruit intervention but the difference was not significant. A seasonal effect in URTI incidence and symptoms were observed over the course of the study, although this did not affect the outcome of the study. There were no changes in fruit consumption between baseline and at the end of the intervention, however, vegetable consumption increased at the end of the intervention compared to baseline ( $P=0.001$ ). Children's daily fruit and vegetable intakes as reported on the FFQ appeared to be over reported (seven to eight portions per day) when compared to the NZ CNS (Ministry of Health, 2002). When intakes were assessed based on the fruit and vegetable eating habits reported by the parents (three portions per day), the intakes appeared to be more in line with the NZ CNS (Ministry of Health, 2002) indicating that children's fruit and vegetables intake is lower than the recommendation in NZ. Children's fruit and vegetable liking did not differ from baseline to end with the exception of an increase in liking for kiwifruit ( $P=0.006$ ). There was an association between liking and fruit and vegetable consumption and in general fruits or vegetables that were most liked tended to be the most consumed. The parents who took part in the study tended to have a high motivation towards providing their children with fruit and vegetables and as expected there was a strong association ( $r$  values

ranging from 0.22 to 0.6) between children's fruits and vegetables eating habits and their parents' self-efficacy.

## **6.2 Conclusion**

The final conclusions of this study will be presented according to the research objectives stated in chapter 1. This chapter concludes with the attempt to answer the research objectives, highlighting the strengths and weaknesses of the study. Based on this recommendations are made for future studies.

The primary objective was stated as follows; to compare the effect of consuming either two servings of gold kiwifruit or banana per day for five days a week over a four week period on the incidence and symptoms of URTI in preschool children aged two to five years attending crèches or play centres in Auckland.

Based on the results of this study the alternative hypothesis (H1) is accepted. The consumption of two servings of Zespri® Gold Kiwifruit per day for five days a week over a four week period may reduce the incidence or symptoms of URTI illnesses.

The secondary objectives were stated as follows; to investigate the fruit and vegetable consumption habits of the children in the study, along with their fruit and vegetable liking and their parents' motivation towards providing fruits and vegetables.

Based on the results of the study, the fruit and vegetable consumption of the preschool children were described and compared with the NZ CNS and current recommendations, revealing intakes less than optimal for vegetables. The children's fruit and vegetable liking compared well with those most consumed and in turn also with their parent's motivation towards providing them with fruits and vegetables.

In conclusion, children experienced a lower incidence of cold- and flu-like illnesses during the kiwifruit intervention phase of the trial compared to banana phase. Some of the URTI symptoms were significantly less during the kiwifruit intervention phase compared to the banana phase. Regular consumption of Zespri® GOLD kiwifruit during the cold & flu season may reduce the incidence or symptoms of URTI in children. Children's fruit and vegetable consumption was associated with their liking and their parents' motivation to providing them with fruits and vegetables. Regular exposure to a new food item such as gold kiwifruit in the daily diets of preschool children improved the liking of the fruit considerably. This is an important finding that could assist parents and caregivers in improving children's liking of fruit and vegetables in order to improve number of portions consumed daily. This may provide a mechanism to expand the range and the liking of fruit and vegetables in preschool children's diets.

### **6.3 Strengths of the present study**

This was a randomised cross over intervention. Children were randomised into one of two groups (receiving either gold kiwifruit or banana) and after a wash out

period they were crossed over. The randomised cross over design allowed each subject to act as a control for themselves accounting for any variability/inaccuracy that might arise from subjective perception of incidence and symptoms of URTI illnesses (Gwaltney, 2000). The participants (parents, children, and crèche staff) were not informed that kiwifruit was the fruit of interest and equal emphasis was placed on banana consumption and kiwifruit consumption.

The study was set to be a true “free-living” intervention, and the only medical exclusion criterion was allergy to gold kiwifruit or banana. Therefore, even though a proportion of the self-reported cold symptoms may not have been due to cold-and-flue etiology, regardless of that, the gold kiwifruit intervention improved symptoms related to URTI compared to banana. In fact, we predict that these wide inclusion criteria may have rendered the differences observed smaller than they actually were.

The incidence and symptoms of URTI illness was measured using the Canadian Acute respiratory Infection Flu Scale (CARIFS). This is a valid measure for URTI illnesses in our age group of interest (two to five years) (Butler *et al.*, 2002, Jacobs *et al.*, 2000, Shepperd *et al.*, 2004).

Finally, the majority of the study took place during crèche or play-centre hours where the fruit was provided and consumed in a regulated environment, and compliance was largely reported by the staff at the participating crèches and play-centres, which allows for extra assurances to the accuracy of reported compliance. In spite of early reservation prior to the commencement of the study regarding

children's ability to comply with consuming two serves of gold kiwifruit per day for five days a week over a four week period, there were no issues in that regard. While the rewards system offered to foster compliance might have played a role, ultimately it was the children's interest in taking part in the study and willingness to consume kiwifruit everyday that accounted for the success in compliance. The pre-school setting, the positive peer pressure, and enthusiasm of parents and crèche/play-centre staff were also major contributing factors to the success of the study. Future studies with children should use a school setting and ensuring the cooperation of teachers and parents is paramount for a successful intervention. Additionally, we predict that the use of a food-based approach rather than a nutrient supplementation approach has ultimately led to better outcomes and compliance in this study, and would thus be recommended for future studies.

#### **6.4 Limitations of the present study**

The primary end point measured in this study was URTI infections. The incidence of our primary end point was left largely to chance, furthermore URTI are highly seasonal illnesses with the highest incidence in New Zealand occurring over the late autumn and early winter months to late winter/ early spring (Early June- to late September) (Arroll and Kenealy, 2002, Blackmore, 2005, Cross *et al.*, 2009, Curry *et al.*, 2006). However, due to logistical issues, we were not able to commence our study until late winter/ early spring (mid August 2009) and the intervention concluded in early summer (early December 2009). Despite this, significant differences between the kiwifruit and the banana interventions were observed in regards to the incidence and symptoms of URTI. We envisage that the

significant differences observed may have been larger should the study have taken place earlier in the season (during the peak of URTI incidences). A no intervention arm (i.e. no banana or kiwifruit diet) could have been added to the study as a control. However, upon further consideration, it was concluded that perhaps this third group might be associated with further confounders (i.e. increased subjective reporting of cold and flu episodes due to the knowledge that neither of the intervention fruits are being consumed). Furthermore, children will still consume other fruits that will be uncontrolled for.

## **6.5 Recommendations for future studies**

Future studies should investigate the effect of consuming gold kiwifruit on the incidence and severity of URTI incidence and symptoms throughout the whole cold and flu season (Ardagh, 2006, Arroll and Kenealy, 2002, Blackmore, 2005, Cross *et al.*, 2009, Curry *et al.*, 2006) i.e. from late autumn until late spring to capture as many cold and flu incidences as possible.

This study only included children between two to five years of age, which required the parents to act as proxy for measuring the incidence and severity of illness. Additional studies may be done to determine the response in other target groups such as older children or adolescents (who will be able to report their own URTI symptoms and illnesses) (Douglas *et al.*, 2007), or college students and athletes who may benefit from extra protection against the common cold (Douglas *et al.*, 2007, Hemila, 2004).

Generally, children under the age of five are a neglected age group in spite of the high incidence of respiratory infections in that age group (Ardagh, 2006, Blackmore, 2005, Cross *et al.*, 2009, Curry *et al.*, 2006). No studies investigating the effect of a food-based nutritional intervention on URTI in children under the age of five was found in the literature. There was a hand full of studies investigating the effect of vitamin C supplementation on URTI illnesses in children under the age of 14 years, but most of these studies dated to the late seventies (Coulehan *et al.*, 1976, Ludvigsson *et al.*, 1977, Miller *et al.*, 1977, Carr *et al.*, 1981). More studies in investigating the effects of food-based nutritional interventions on URTI illnesses should utilise this age group.

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# **Appendix A** *Letter to crèche/ play-centre, study information, and consent form*



Institute of Food, Nutrition and Human Health  
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## **Letter to the crèche and study information**

### **The effects of fruit on common colds**

#### **Researchers:**

**Aselle Adaim** (principal)

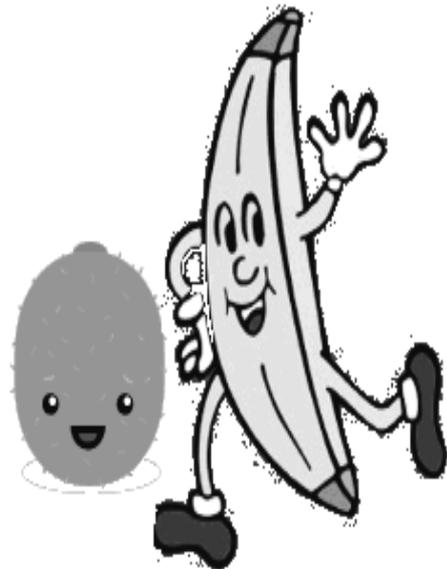
**Dr. Margot Skinner**

Plant & Food Research  
Mt. Albert Research Centre

**Dr. Rozanne Kruger**

**Assoc Prof Welma Stonehouse**

Institute of Food, Nutrition and  
Human Health  
Massey University- Albany Campus



#### **Introduction**

This study is collaboration between the Plant & Food Research Institute of New Zealand and the Institute of Food, Nutrition and Human Health at Massey University-Albany Campus. Aselle Adaim, the principal researcher, is a Research Associate at Plant and Food Research and this project is part of her studies towards a Masters of Science in Human Nutrition. The study is co-supervised by Dr. Rozanne Kruger, Dr. Welma Stonehouse, and Dr. Margot Skinner. All of the supervisors have a wealth of experience in designing and running interventions

both in New Zealand and internationally. This research is partially funded by Zespri International Ltd.

Your crèche is invited to participate in an interesting new research trial which seeks to examine the effect of gold kiwifruit or banana consumption on common ailments, such as colds and flu. We wish to recruit children aged between 2-5 years old, who are currently attending your crèche and we will also require your staff's co-operation during the study.

Upon completion of this study, and as a thank you, a \$40 Westfield gift voucher will be awarded to staff that help in the facilitation of the study, and books up to the value of \$50 will be donated to the crèche.

## **Study details and information**

### **Why is this research important?**

Each winter, cold and influenza outbreaks place an increased burden on both doctors' surgeries and hospitals within New Zealand. Children under the age of 5 are particularly susceptible to respiratory infection because of their young immune system. Including at least 2 servings of fruit, as part of daily food intake in the diet is considered a natural way to maintain health and it may enhance the natural defense system of the body, to cope with common ailments such as seasonal colds and flu. In this study, we will determine the feasibility of feeding young children two servings of kiwifruit or banana per day, and the effect that may have on the incidence or the symptoms of any common respiratory infections they might get. This study will also survey general fruit and vegetable consumption in this particular age group.

### **Who are we looking for?**

Children who take part in the study will be recruited through local crèches, such as yours. We aim to recruit about 100 children, aged 2-5 years old, who are not allergic to gold kiwifruit or banana.

If your crèche agrees to take-part in the study (signing the consent form provided), a presentation meeting, at your premises, will be given by the principal investigator to the parents and guardians to outline and explain this study. In addition, information sheets and consent forms will be handed out to the parents and guardians. As the participants in this study are minors, a legal guardian consent will be required for participation. If parents/ guardians are unable to attend the meeting but still wish for their child to participate in the study, they may receive copies of information sheets and the consent form from you and ring the investigator for an over the phone orientation. The contact details of the investigator and the research team are provided for any queries the parents or crèche staff may have.

## **How will your privacy be protected?**

No material that could personally identify your crèche or subjects in the study will be used in any reports or the labeling of survey material. All the records will be kept in a secure area at Massey University, for up to 10 years, with access limited to the principal investigator and specific researchers named on the study documents. The information gathered will be treated as confidential and will only be used for this project.

## **What are the costs involved in this research?**

There is no cost to you taking part, except your time in replacing regular morning and afternoon snacks with gold kiwifruit or banana and rewarding the children with a gold star once they eat their assigned fruit, and handing out and collecting forms at the beginning of the study. We estimate this time to average out to about 15-30 min/day throughout the study.

## **What happens in this research?**

Parents will be requested to hand back consent forms to you (staff at the crèche), and you will be provided with the following to hand back to the parents;

1. A general health questionnaire
2. Fruit and vegetable intake questionnaires
3. A booklet of multiple copies of the “respiratory infection symptoms survey”

A flow chart, outlining details of the study is highlighted on the next page.

Note; If a child is suspected of having an undiagnosed fruit allergy, the standard operating procedure for dealing with fruit allergy at the crèche will be implemented, the child will be disengaged from the study immediately, and advised to see their health practitioner. However, it must be noted that children usually receive meals at their crèche and fruit is part of those meals. This study merely exchanges fruit for gold kiwifruit or banana, both commonly eaten fruit in New Zealand.

## Flow-Chart of Study

Study Commences

### Stage 1

- Collect the fruit and vegetable survey questionnaires from parents.
  - Children will continue to eat their normal diets
- Parents will be monitoring and rating their child's respiratory symptoms.

Week  
1-2

### Stage 2

Children will be randomly divided by the researcher into one of the two groups below

#### Group A

*Gold Kiwifruit*

#### Group B

*Banana*

- Children (depending on their group) will receive at crèche (given by crèche staff) 2 servings of fruit (one during morning tea and one during afternoon tea).
- Children will be encouraged by crèche staff to eat their pieces of fruit, and once they have they will be rewarded with a gold star.
- If a child collects 5 stars/ week, they will be rewarded with a small toy at the end of the week.

Week  
3-6

### Stage 3

- Similar to stage one
- Children will resume their normal diets

Week  
7-8

### Stage 4

Similar to stage 2, except the groups will be swapped and children will receive the other fruit. E.g. if the child received kiwifruit during stage 2, then they will receive banana during stage 4.

#### Group A

*Banana*

#### Group B

*Gold Kiwifruit*

Week  
9-12

### Stage 5

- Similar to stage one and two
- Children will resume their normal diets
- Upon conclusion of the study you will collect the fruit and vegetable intake questionnaires, and the booklets containing the "respiratory infection symptoms surveys".

Week  
13-14

And as a thank you crèche staff who helped facilitate the study will be provided with a \$40 Westfield voucher, and books up to the value of \$50 donated to the crèche.

### **What are the benefits of this study?**

This will be the first study of its kind in this age group in New Zealand. The results will also enhance our knowledge about the health benefits of fruit. In addition, this study will provide us with information regarding the participants' general fruit and vegetable intake.

### **Will I receive feedback on the results of this research?**

Upon conclusion of the study and an analysis of the results, a brief report of the main findings will be posted to you. This study along with the findings will be written up in the form of a Masters thesis and submitted to Massey University. In addition the main overall findings may be presented at national/international conferences and published in a peer-reviewed international journal.

### **Participants' rights**

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

## Who do I contact for further information?

You are welcome to contact the principal investigator or any members of the research team with any questions or concerns you may have regarding the study

### **Aselle Adaim**

(principal investigator)

#### Contact details

Phone (direct): +64-9-925 7101

Phone (Site): +64-9-925 7000

Email; [aadaim@hortresearch.co.nz](mailto:aadaim@hortresearch.co.nz)

#### Address

Plant & Food Research

120 Mt Albert Road

Sandringham

Auckland 1025, New Zealand

### **Dr. Rozanne Kruger**

(main supervisor)

#### Contact details

Phone: +64-9- 414 0800 ext.41209

Email; [r.kruger@massey.ac.nz](mailto:r.kruger@massey.ac.nz)

#### Address

Institute of Food, Nutrition and  
Human Health

Te Kura Hangarau o Kai-orangā-ā-  
tāngata

Massey University -- Albany  
Campus

Private Bag 102 904

North Shore

Auckland, New Zealand

*“This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 09/29. If you have any concerns about the ethics of this research, please contact Professor Julie Boddy, Chair, Massey University Human Ethics Committee: Southern A telephone 06 350 5799 x 2541, email [humanethicsoutha@massey.ac.nz](mailto:humanethicsoutha@massey.ac.nz).”*

## The effects of fruit on common colds

### PARTICIPATING CRÈCHE CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree for crèche staff to participate in this study under the conditions set out in the Information Sheet.

**Position :**

**Date:**

**Crèche name:**

**Designated  
signature:**

.....

**Full Name - printed**

.....



# **Appendix C:** *Letter to parent, study information, and consent form*



Institute of Food, Nutrition and Human Health  
Massey University  
Private Bag 102-904  
North Shore Mail Centre  
Albany, Auckland  
New Zealand

## **Letter to parents and study information**

The effects of fruit on common colds

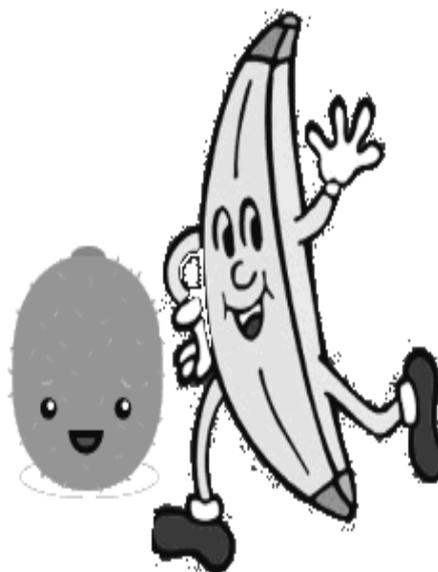
### **Researchers:**

**Aselle Adaim** (principal)  
**Dr. Margot Skinner** (supervisor)

Plant & Food Research  
Mt. Albert Research Centre

**Dr. Rozanne Kruger** ( main supervisor)  
**Dr. Welma Stonehouse** (supervisor)

Institute of Food,  
Nutrition and Human Health  
Massey University- Albany Campus



### **Introduction**

This study is collaboration between the Plant & Food Research Institute of New Zealand and the Institute of Food, Nutrition and Human Health at Massey University-Albany Campus. Aselle Adaim, the principal researcher, is a Research Associate at Plant and Food Research and this project is part of her studies towards a Masters of Science in Human Nutrition. The study is co-supervised by Dr. Rozanne Kruger, Dr. Welma Stonehouse, and Dr. Margot Skinner. All of the supervisors have a wealth of experience in designing and running interventions both in New Zealand and internationally. This research is partially funded by Zespri International Ltd.

You and your child are invited to take part in this interesting new research study which is happening at your child's local crèche. This research project aims to investigate the effects of

feeding 2 servings of either gold kiwifruit or banana per day to children between the ages of 2-5 years, on the incidence and the symptoms of respiratory tract infection.

We hope to commence the study by 31<sup>st</sup> of September 2009 at the latest. As a thank you for participation a \$40 Westfield gift voucher will be mailed out to participants upon completion of the study.

## **Study details and information**

### **Why is this research important?**

Each winter, cold and influenza outbreaks place an increased burden on both doctors' surgeries and hospitals within New Zealand. Children under the age of 5 are particularly susceptible to respiratory infection because of their young immune system. Including at least 2 servings of fruit, as part of daily food intake in the diet is considered a natural way to maintain health and it may enhance the natural defense system of the body, to cope with common ailments such as seasonal colds and flu. In this study, we will determine the feasibility of feeding young children two servings of kiwifruit or banana per day, and the effect that may have on the incidence or the symptoms of any common respiratory infections they might get. This study will also survey general fruit and vegetable consumption in this particular age group.

### **Participant Identification and Recruitment**

Children who take part in the study will be recruited through local crèches. We aim to recruit about 100 children, aged 2-5 years old. Interested guardians of children, who wish for their children to take part will be contacted by the principal investigator and screened to make sure they meet study criteria (needs).

Your child may partake if he/she is aged between 2 and 5 years old, and has no allergies to gold kiwifruit or banana. As the participants in this study are minors, consent will be required from the child's legal guardian for participation.

### **How will your privacy be protected?**

No material that could personally identify you, your child or your local crèche will be used in any reports or the labeling of survey material. All the records will be kept in a secure area at Massey University, for up to 10 years, with access limited to the principal investigator and specific researchers named on the study documents. The information gathered will be treated as confidential and will only be used for this project.

## **What are the costs involved in this research?**

There will be no cost for participating in this study beside your time. For parents or legal guardians of children, at the start of the study there will be a 10 min chat with the principal investigator to find out about your Childs eating habits. Then less than 5 min per day, to fill out a small survey that will helps us track the incidence and severity of the common cold or flu in children. As for the children, there is no anticipated extra time involved as we will be merely exchanging their morning and afternoon snack with either gold kiwifruit or banana.

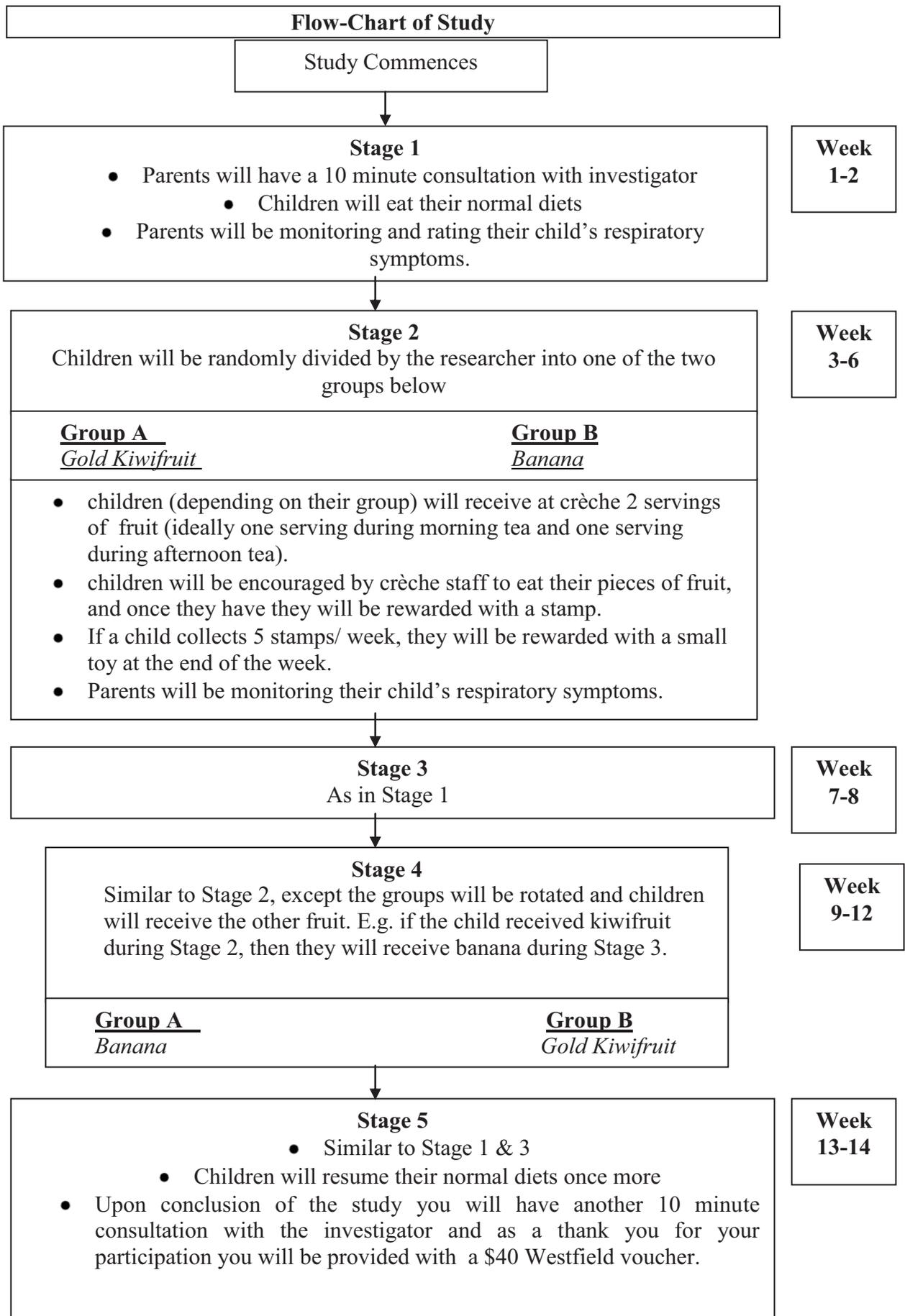
## **What happens in this research?**

- Once you have consented for your child to take part in this study, you will have a 10 minute chat with the investigator and answer some questions about your child’s eating habits.
- You will also be provided with a booklet of multiple copies of the “symptoms survey”, which you will have to complete every day for the duration of the study (it will take you less than 5min/day to complete)

A flow chart, outlining details of the study is highlighted on the next page

### Note:

- a. If your child does not attend crèche five days a week, or if you know that your child will be absent for a period of time, but you still wish for him/her to participate in the study, please let the staff at your crèche know or contact the investigator, and an arrangement can be worked out so that you may take the fruit home with you for your child to consume at home.
- b. If a child is suspected of having an undiagnosed fruit allergy, the standard operating procedure for dealing with fruit allergy at the crèche will be implemented, the child will be disengaged from the study immediately, and parents advised to see their health practitioner. However, it must be noted that children usually receive meals at their crèche and fruit is part of those meals. This study merely exchanges the fruit for gold kiwifruit or banana, both commonly eaten fruit in New Zealand.



### **What are the benefits of this study?**

This will be the first study of its kind in this age group in New Zealand. The results will also enhance our knowledge about the health benefits of fruit. In addition, this study will provide us with information regarding the participants' general fruit and vegetable intake.

### **Will I receive feedback on the results of this research?**

Upon conclusion of the study and analysis of results, a brief report of the main findings will be posted to you. This study along with the findings will be written up in the form of a Masters thesis and submitted to Massey University. In addition the main overall findings may be presented at national/international conferences and published in a peer-reviewed international journal.

### **Participants' rights**

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

## Who do I contact for further information?

You are welcomed to contact the principal investigator or any members of the research team with any questions or concerns you may have regarding the study

### **Aselle Adaim**

(principal investigator)

#### Contact details

Phone (direct): +64-9-925 7101

Phone (Site): +64-9-925 7000

Email; [aadaim@hortresearch.co.nz](mailto:aadaim@hortresearch.co.nz)

#### Address

120 Mt. Albert rd.  
Sandringham  
Plant & Food Research  
Auckland 1025, New Zealand

### **Dr. Rozanne Kruger**

(main supervisor)

#### Contact details

Phone: +64-9- 414 0800 ext.41209

Email; [r.kruger@massey.ac.nz](mailto:r.kruger@massey.ac.nz)

#### Address

Institute of Food, Nutrition and  
Human Health  
Te Kura Hangarau o Kai-orangā-ā-  
tāngata  
Massey University -- Albany  
Campus  
Private Bag 102 904  
North Shore  
Auckland, New Zealand

120 Mt Albert

*“This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 09/29. If you have any concerns about the ethics of this research, please contact Professor Julie Boddy, Chair, Massey University Human Ethics Committee: Southern A telephone 06 350 5799 x 2541, email [humanethicsoutha@massey.ac.nz](mailto:humanethicsoutha@massey.ac.nz).”*

## The effects of fruit on common colds

### PARENTAL CONSENT FORM - INDIVIDUAL

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to allow my child to participate in this study under the conditions set out in the Information Sheet.

**Signature:**

**Date:**

.....

**Full Name - printed**

.....

## **Appendix D: Eligibility questionnaire and demographics and medical questionnaire**

### Eligibility Questionnaire

- Is your child allergic to gold kiwifruit?       Yes       No
- Is your child allergic to banana?       Yes       No

*If you answered Yes to any of the questions above, then unfortunately your child is not eligible to take part in the study  
If you answered No to all of the above questions, then please proceed, your child is eligible to take part in the study*

---

**Project title:** A study investigating the effect of regular kiwifruit and banana consumption on the incidence and symptoms of respiratory infection in 2-5 year olds.

Your child's first name: \_\_\_\_\_

Your child's last name: \_\_\_\_\_

Legal guardian's first name: \_\_\_\_\_

Legal guardian's last name: \_\_\_\_\_

Relationship to child : \_\_\_\_\_

**Contact address:**  
(Note; the contact details provided will be merely used to post out a brief general overall findings of the study, and our “Thank you” gift voucher)

Postal address \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Contact phone number: \_\_\_\_\_

Email address : \_\_\_\_\_

**General health and demographics questionnaire**

Your child's date of birth: \_\_\_\_\_ day/\_\_\_\_\_ month/\_\_\_\_\_ year

Current age of child: \_\_\_\_\_

Your child's sex  male  female

Weight: \_\_\_\_\_ Height: \_\_\_\_\_

Ethnicity: \_\_\_\_\_

-----

**1. Does your child take any medication?      O Yes   O No**

*(If yes, please list with as many details as possible)*

Medication 1 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

Medication 2 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

Medication 3 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

Medication 4 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

---

**2. Does your child take any supplements?      O Yes   O No**

*(If yes, please list with as many details as possible)*

Supplement 1 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

Supplement 2 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

Supplement 3 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

Supplement 4 Name : \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

---

**3. Does your child have allergies to medications?      O Yes   O No**

*If yes please identify* \_\_\_\_\_

*Who diagnosed his/her allergy?* \_\_\_\_\_

4. Does your child have allergies to food?

If yes please identify \_\_\_\_\_

Yes  No

Who diagnosed his/her allergy? \_\_\_\_\_

5. Does your child have breathing problems?

Yes  No

Explain: \_\_\_\_\_

6. Has your doctor told you that your child has asthma?

Yes  No

7. Has your doctor told you that your child has wheezing problems?

Yes  No

8. Has your child had nervous system problems such as a seizure or epilepsy?

Yes  No

9. Does your child have any disease that could compromise their immune system ?

Yes  No

10. Is your child taking steroid medications such as cortisone or prednisone?

Yes  No

If yes, please identify \_\_\_\_\_ Dose \_\_\_\_\_ How often \_\_\_\_\_

-----  
**11. Has your child taken anti-cancer drugs  
or had radiation treatment?**

**O Yes O No**

-----  
**12. Does your child have kidney or bladder disorders?**

**O Yes O No**

-----  
**13. Does your child have diabetes?**

**O Yes O No**

-----  
**14. Is your child up to date with his/her vaccines?**

*If No, which one has he/she not received yet?*

\_\_\_\_\_

-----  
**15. Will your child be receiving any  
vaccines in the next 3 months?**

**O Yes O No**

*If yes, please identify* \_\_\_\_\_

*and when* \_\_\_\_\_

-----

**16. Is there anything else you think might be relevant, or that you would like to add?**  
(e.g. past illnesses such as hospitalisation for pneumonia, family history of an illness etc)

---

---

*Thank you*

# Appendix E: Canadian Acute Respiratory Illness flu scale (CARIFS) survey

**Parental Diary** **Today's date** \_\_ \_\_ \_\_ 2009

**Child's temperature (if measured)** \_\_ \_\_ \_\_ : \_\_ \_\_ C

Symptoms	No problem	Minor problem	Moderate problem	Major problem	Don't know or not applicable
Poor appetite					
Not sleeping well					
Irritable. Cranky. Fussy					
Feels unwell					
Low energy, tired					
Not playing well					
Crying more than usual					
Needing extra care					
Clinginess					
Headache					
Sore throat					
Muscle aches or pains					
Fever					
Cough					
Nasal congestion, runny nose					
Vomiting					
Not interested					
Unable to get out of bed					

	No	Yes
Need medical attention		
Unable to attend day care		
Need to take medication		

**Does your child have a cold or flu today?**  No  Yes

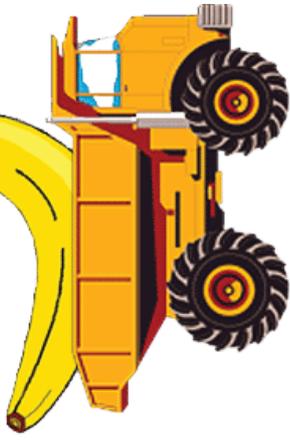
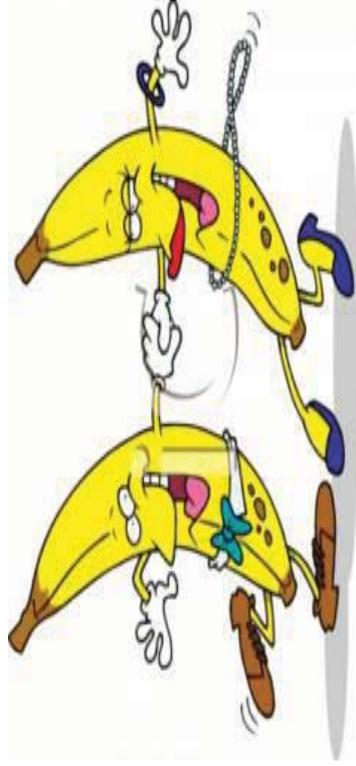
**Does your child feel unwell today due to another reason not including cold or flu? (e.g. teething, injury, another illness)**

No  Yes, please specify; \_\_\_\_\_

**This form was filled out by**  Mother  Father  Other  
**Parental Diary** **Today's date** \_\_ \_\_ \_\_ 2009

## **Appendix F:** *Banana and kiwifruit Compliance charts*

This chart belongs to \_\_\_\_\_



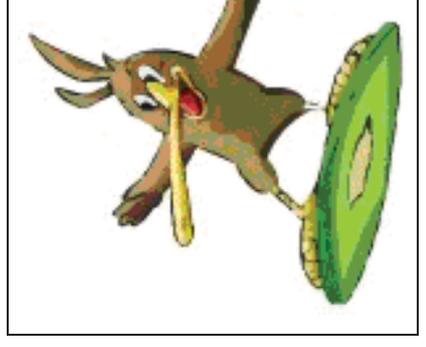
Week 1

Week 2

Week 3

Week 4


This chart belongs to \_\_\_\_\_






Week 1

Week 2

Week 3

Week 4



# Appendix G: *Food liking questionnaire*

ID# \_\_\_\_\_ Center Name: \_\_\_\_\_ Page 1: Generalized Degree of Liking scale

**Directions:** Please mark each line below using the smiley face scale. The ends of the scale represent the strongest liking or disliking of any kind. Place a vertical mark or an X anywhere on the line. An example is shown below.





Never tried or done | He/she loves it | Neither like nor dislike | He/she hates it

\_\_\_\_\_  
 EXAMPLE |

-  apple  \_\_\_\_\_
-  brushing teeth  \_\_\_\_\_
-  carrot  \_\_\_\_\_
-  fizzy drink  \_\_\_\_\_
-  kumara  \_\_\_\_\_
-  whole milk  \_\_\_\_\_
-  strawberries  \_\_\_\_\_
-  taking a bath  \_\_\_\_\_
-  chocolate candy bar  \_\_\_\_\_
-  apple juice  \_\_\_\_\_
-  lunch meat, hot dogs  \_\_\_\_\_



ID# \_\_\_\_\_

Never tried or done  He/she loves it  He/she thinks it's OK  He/she hates it

 kiwi  \_\_\_\_\_

 banana  \_\_\_\_\_

 broccoli  \_\_\_\_\_

 ice cream  \_\_\_\_\_

 cookies, cake  \_\_\_\_\_

 watermelon  \_\_\_\_\_

 hot chips  \_\_\_\_\_

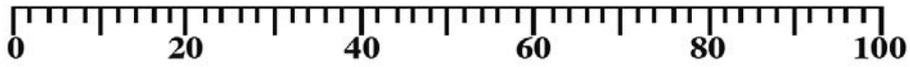
 getting dressed  \_\_\_\_\_

 loud siren  \_\_\_\_\_

 spinach, collard greens  \_\_\_\_\_

 butter, margarine  \_\_\_\_\_

 corn  \_\_\_\_\_



**Appendix H:** *Fruit & Vegetable Frequency Questionnaire, children’s eating habits questionnaire and parental self-efficacy questionnaire*

**Children Fruit & Vegetable Questionnaire**

*What fruit & vegetables has your child been eating lately?*

*“During the past four weeks, how often did your child eat a serving of the foods listed here?”*

**Mark only one X for each food**

**Example:**

	Last 4 weeks		Each week			Each day		
	0	1-3	1	2-4	5-6	1	2-3	4+
Number of times								
Fried potatoes, wedges, kumara	X							
A medium size baked or boiled potato					X			

During the past four weeks, how often did your child eat a serving of the fruit listed below? (Not including those given to him/her at crèche)

	Number of times	Last 4 weeks					
		0	1-3	Each week	Each day		
		1	2-4	5-6	1	2-3	4+
A medium size apple							
A small banana							
A medium size pear							
A medium size orange							
An average size mandarin							
A medium size gold Kiwifruit							
A medium size green Kiwifruit							
An average size nectarine							
Two small plums							
Two small apricots							
Strawberries (1/2 cup)							
Other berries (1/2 cup)							
Grapes (1/2 cup, 100g)							
Canned or cooked fruit (1/2 cup) e.g. Canned peaches							
Dried fruit e.g. raisins (2 table spoon)							
Fruit juice (1 cup/250ml)							
Other fruit (1) name _____							
Other fruit (2) name _____							

During the past four weeks, how often did your child eat a serving of the vegetables listed below? (Not including those given to him/her at crèche)

	Last 4 weeks		Each week			Each day		
	0	1-3	1	2-4	5-6	1	2-3	4+
Number of times								
A medium size baked or boiled potato								
Mashed potato (1/2 cup)								
Kumara or similar size root vegetable								
Carrots raw or cooked (1/2 cup)								
Pumpkin (1/2 cup)								
Mixed frozen vegetables (1/2 cup)								
Corn (1/2 cup)								
Peas (1/2 cup)								
Silver beet (1/2 cup)								
Spinach (1/2 cup)								
Green beans (1/2 cup)								
Broccoli (1/2 cup)								
Cauliflower (1/2 cup)								
Cabbage (1/2 cup)								
Lettuce or green salad (1/2 cup)								
Cucumber (1/2 cup or 10cm)								
Tomato medium size, raw or cooked								
Vegetable juice (1 cup/250ml)								
Other vegetable (1) name _____								
Other vegetable (2) name _____								

## General Fruit and Vegetable consumption

*This section concerns your child's general fruit and vegetable consumption. Think back over the past four weeks and answer the following questions:*

	Last 4 weeks				Each week			Each day		
	0	1-3	1	2-4	5-6	1	2-3	4+		
<b>Number of times</b>										
How often did you give your child a serving of fruit (not counting juices)?										
How often did you give your child a glass (250 ml) of 100% fruit juice (not including juice from concentrate)?										
How often did you offer a serving of fruit to your child with their breakfast cereal?										
How often did you offer a serving of fruit to your child as a snack between meals?										
How often did you offer fruit to your child as part of desserts (e.g. used it to make desserts or in baking)?										
How often did you give your child a serving of vegetables (not counting potatoes or kumara)?										
How often did you offer vegetables (e.g. carrot sticks) to your child as a snack between meals (not including potatoes or kumara)?										
How often did you give your child a glass (250 ml) of 100% vegetable juice?										

## Fruit and Vegetable intake pattern

*This section concerns your child's general fruit and vegetable consumption pattern. Think back over the past four weeks and answer the following questions:*

On a scale from 1-6 how certain are you that you will give your child fruit in the following situations ( <i>not counting fruit juice</i> )	Not Very Certain						Very Certain					
	1	2	3	4	5	6	1	2	3	4	5	6
In the evening, after your child is home from crèche												
During the weekends												
When your child is sick												
When the fruit is messy or requires preparation (e.g. peeling or need a spoon)												
During winter when there is less choice												
When you are really busy, in a hurry or having a hectic day												

On a scale from 1-6 how certain are you that you will give your child at least two servings of vegetables in the following situations ( <i>not counting potatoes or kumara</i> )	Not Very Certain						Very Certain					
	1	2	3	4	5	6	1	2	3	4	5	6
In the evening, after your child is home from crèche												
During the weekends												
When your child is sick												
When the vegetable preparation is time consuming												
During winter when there is less choice												
When you are really busy, in a hurry or having a hectic day												

Do you have any additional comments?

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## **Appendix I:**

*Nutritional contribution of the intervention fruit (two servings of gold kiwifruit (150g) or two servings of banana (110g))*

<b>Nutrient</b>	<b>Units</b>	<b>Gold kiwifruit (150gm)</b>	<b>% RDI</b>	<b>Banana (110g)</b>	<b>% RDI</b>
Water	g	125	10.0	82.4	6.50
Energy	kJ	376	15.6	408	17.0
Protein	g	1.85	9.25	1.35	6.75
Total lipid (fat)	g	0.84	–	0.36	–
Carbohydrate	g	21.3	–	25.1	–
Fiber, total dietary	g	3.00	17.0	2.86	15.8
<b>Minerals</b>					
Potassium, K	mg	474	20.0	374	16.2
Selenium, Se	mcg	4.65	15.5	1.10	3.60
<b>Vitamins</b>					
Vitamin C, total ascorbic acid	mg	158	451	9.57	27.0
Folate, total	mcg	51.0	25.5	22.0	11.0
Carotene, beta	mcg	64.5	16.1	22.0	5.50
Lutein + zeaxanthin	mcg	171	–	24.2	–
Vitamin E	mg	2.24	37.0	0.11	1.80
Vitamin K	mcg	8.25	23.6	0.55	1.57

**Kiwifruit, gold, raw; Refuse:** 26% (Skin); **Scientific Name:** *Actinidia chinensis*; **NDB No:** 9445 (Nutrient values and weights are for edible portion); Samples grown in New Zealand; cultivar is Hort 16A.

**Banana, raw; Refuse:** 36% (skin); **Scientific Name:** *Musa acuminata Colla* **NDB No:** 09040 (Nutrient values and weights are for edible portion)

USDA National Nutrient Database for Standard Reference, Release 22 (2009)

Nutrient Reference values For Australia and New Zealand, Release 1 (2005)