Fruit and vegetable intake and skin colour amongst young Australian women

Kristine B Pezdirc, BND (Hons I), APD, AN

A thesis submitted for the degree of PhD (Nutrition and Dietetics)

November 2015
Statement of originality

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.................................................

Kristine B Pezdirc
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I hereby certify that this thesis is in the form of a series of published papers of which I am joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Kristine B Pezdirc
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Conflict of interest

Kristine Pezdirc reports no conflict of interest.
Publications and presentations arising from this thesis

Manuscripts in peer-reviewed journals: Published


Manuscripts in peer-reviewed journals: Under review


Manuscripts in peer-reviewed journals: submitted

Conference abstracts: Published in conference proceedings or peer-reviewed journals


## Glossary of common abbreviations

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<thead>
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<th>Acronym</th>
<th>Description</th>
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<tr>
<td>AGTHE</td>
<td>Australian Guide to Healthy Eating</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary disease</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>mg</td>
<td>milligrams</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RRS</td>
<td>Resonance Ramon Spectroscopy</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>α</td>
<td>alpha</td>
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<tr>
<td>β</td>
<td>beta</td>
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<tr>
<td>μg</td>
<td>micrograms</td>
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Abstract

Higher fruit and vegetable intakes are associated with reduced risk of excess weight gain, type 2 diabetes, coronary heart disease, stroke and some specific cancers. Despite this, young women report low intakes of fruit and vegetables compared to Australian adults generally. Fruit and vegetables contain carotenoid pigments, which give them their bright colours. These accumulate in human skin, contributing to skin yellowness. There is some evidence to show that higher intakes of fruit and vegetables may have a beneficial impact on the appearance of health but the relationship between dietary intake and skin appearance has been studied infrequently.

The main aims of this research were to: 1) examine the association between fruit and vegetable intake and skin colour appearance in young women; 2) compare the consumption of high carotenoid fruit and vegetables versus low carotenoid fruit and vegetables on skin colour and plasma carotenoids in young women; 3) investigate Australian adult’s perceptions of health using standardised facial images associated with carotenoid-based skin colour. To meet these aims a series of four studies were conducted.

The first was a systematic review of the evidence examining the association between dietary intake and appearance and to also determine the effectiveness of dietary interventions on actual or perceived appearance. Nine observational studies examined the relationship between dietary intake and appearance and studies found significant associations between fruit and vegetable intake and skin colouration. The majority of dietary interventions (n=16) evaluated the effect of various dietary supplements on skin appearance outcomes among women and found significant improvements. Only one study examined the effect of actual food. This intervention evaluated the consumption of vegetables high in β-carotene versus β-carotene supplements on skin colour appearance. Overall this systematic review demonstrated that there is currently insufficient evidence to determine the association between actual food and skin appearance. Further studies are required in representative populations that examine
actual food intake on appearance, using validated tools and well-designed high-quality randomised control trials.

A cross-sectional study was conducted to examine the association between fruit and vegetable intake and skin colour in young women (n=91, 18-30 years) from the Hunter region. Fruit and vegetable intake was assessed by a validated food frequency questionnaire, with skin colour measured using spectrophotometry. The results showed that women who reported higher fruit and vegetable intakes had significantly higher overall skin yellowness (b*) ($\beta=0.8$, $p=0.017$).

The third study was the randomised cross-over trial conducted in young women (n=30, 18-30 years). This trial investigated whether consuming the same quantity of fruit and vegetables that were either high in β-carotene or low in β-carotene was associated with a difference in skin yellowness (b*) and in plasma carotenoid concentrations over four weeks. Skin colour was assessed by reflectance spectroscopy (CIE L*a*b*) and fasting plasma carotenoids were determined by high performance liquid chromatography, pre and post each four week intervention period. The results showed that there was a significantly greater increase in skin yellowness (b*) ($p<0.001$) following consumption of high carotenoid fruit and vegetables, with no change in skin lightness (L*) or redness (a*), compared to the low β-carotene intervention. Significantly higher plasma α-carotene ($p=0.004$), β-carotene ($p=0.001$) and lutein ($p=0.028$) concentrations were found following consumption of the high carotenoid fruit and vegetables. Overall skin yellowness (b*) correlated with α-carotene ($r=0.29$, $p<0.05$) and β-carotene ($r=0.35$, $p<0.001$).

The final study evaluated whether skin colouration attributed to fruit and vegetable consumption influences young adults perception of health. The results showed that Australian adults perceive facial skin colouration, associated with both carotenoid intake from fruit and vegetables and melanin as conveying the appearance of health. However carotenoid colouration was perceived as more important to health than melanin.
The body of research presented in this thesis provides further evidence that dietary
intake, in particular fruit and vegetables, has an impact on skin yellowness. This skin
colouration is also shown to be perceived as conveying the appearance of health.
As young adults, in particular women are motivated to change behaviour to improve
their appearance, this research provides further justification for a behavioural
intervention to improve fruit and vegetable intake that focuses on appearance.
Chapter 1  Introduction

This chapter begins with an overview of what is known about fruit and vegetables. Worldwide data related to fruit and vegetable consumption demonstrate the problem of low intakes. This is followed by a summary of the evidence of the health benefits attributed to fruit and vegetable consumption. The nutrient composition of fruit and vegetables, in particular the carotenoid content will then be explored in detail.

1.1 Fruit and vegetables

Fruit and vegetables are defined as “edible plant foods, excluding cereal grains, nuts, seeds, tea leaves, coffee beans, cacao beans, herbs and spices” (World Health Organisation, 2003)(p.1). The National Health and Medical Research Council (NHMRC) also include legumes and beans in the definition of vegetables as part of the Australian Dietary Guidelines (Australian Government Department of Health, 2013).

1.2 Low fruit and vegetable consumption; the worldwide problem

Fruit and vegetable consumption has been a priority of public health policy for several decades. Approximately 16 million (1.0%) disability adjusted life years (DALYs,) and 1.7 million (2.8%) deaths worldwide are attributable to low fruit and vegetable consumption (World Health Organisation, 2014). In addition, low fruit and vegetable intake is considered the sixth main risk factor for mortality (World Health Organisation, 2009). The World Health Organisation (WHO) recommends a minimum of 400g of fruit and vegetables be consumed daily to lower the risk of developing chronic diseases (World Health Organisation, 2014). The majority of individuals worldwide are not meeting recommended daily intake targets and the average population level consumption of fruit and vegetables is significantly lower than the WHO recommendations. For example, across Europe the average daily consumption of fruit and vegetables is approximately 220g per day (Elmadfa I (ed), 2009). In the US only 6-8% of individuals achieve the recommended targets for fruit and vegetable intakes (Produce for Better Health Foundation, 2010). The 2011-2012 Australian Health
Survey reported that 48% of adults aged 18 years or over met their daily fruit intake, while only 8.3% met their daily vegetable intake (Australian Bureau of Statistics, 2012).

### 1.3 Australian daily recommendations for fruit and vegetables

In Australia the national food selection guide, the Australian Guide to Healthy Eating (AGTHE), recommends the consumption of at least five servings of vegetables per day and at least two servings of fruit per day, with specific targets varying by age and sex (Australian Government Department of Health, 2013) (Table 1.1). One serve of vegetables is 75g, which is equivalent to ½ cup cooked vegetables or 1 cup of salad vegetables. One serve of fruit is 150g, which is approximately equivalent to one medium-sized piece of fruit (Australian Government Department of Health, 2013) (Table 1.2).

#### Table 1.1 AGHE recommended number of serves of vegetables, legumes, beans and fruit per day for adults aged 18-50 years

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Vegetables and legumes/beans target daily servings</th>
<th>Fruit target daily servings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>14-18</td>
<td>5 ¹⁄₂</td>
</tr>
<tr>
<td>Men</td>
<td>19-50</td>
<td>6</td>
</tr>
<tr>
<td>Girls</td>
<td>14-18</td>
<td>5</td>
</tr>
<tr>
<td>Women</td>
<td>19-50</td>
<td>5</td>
</tr>
</tbody>
</table>

#### Table 1.2 AGHE standard serve size equivalents for vegetables, legumes/beans and fruit

<table>
<thead>
<tr>
<th>Food group</th>
<th>Serve sizes (100-350kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables and legumes</td>
<td>75g (1/2 cup) cooked green vegetables</td>
</tr>
<tr>
<td></td>
<td>75g (1/2 cup) cooked orange vegetables</td>
</tr>
<tr>
<td></td>
<td>75g (1/2 cup) cooked dried or canned beans, chickpeas, lentils</td>
</tr>
<tr>
<td></td>
<td>75g (1 cup) raw green leafy vegetables</td>
</tr>
<tr>
<td></td>
<td>75g starchy vegetables</td>
</tr>
<tr>
<td></td>
<td>75g other vegetables</td>
</tr>
<tr>
<td>Fruit</td>
<td>150g (1 piece) medium sized fruit e.g. apple, banana, orange, pear</td>
</tr>
<tr>
<td></td>
<td>150g (2 pieces) of small fruit e.g. kiwi fruit, plums</td>
</tr>
<tr>
<td></td>
<td>150g (1 cup) diced, cooked or canned fruit (no added sugar)</td>
</tr>
<tr>
<td></td>
<td>125ml (1/2 cup) 100% fruit juice (no added sugar)</td>
</tr>
<tr>
<td></td>
<td>30g dried fruit e.g. 4 dried apricots (occasionally)</td>
</tr>
</tbody>
</table>
1.3.1 Health benefits of fruit and vegetable consumption

Epidemiological studies (Table 1.3) have reported the health benefits of regular consumption of adequate fruit and vegetables, which includes the potential reduction in risk of coronary heart disease (CHD) and stroke (Boeing et al., 2012), obesity (Boeing et al., 2012), adiposity (Ledoux, Hingle, & Baranowski, 2010), type 2 diabetes (Boeing et al., 2012), and specific cancers (American Institute for Cancer Research, 2007).

1.3.1.1 Coronary heart disease and stroke

There is convincing evidence that increasing regular fruit and vegetable consumption will reduce the risk of CHD, stroke and hypertension (Boeing et al., 2012). Fruits and vegetables that contain vitamins C and E and phytonutrients (such as carotenoids) act as antioxidants which may reduce the risk of inflammation and of cholesterol becoming oxidised and deposited into blood vessel walls to form plaque (Australian Government Department of Health, 2013). Fruit and vegetables contain the minerals magnesium and potassium, which have been linked to lower blood pressure (Australian Government Department of Health, 2013). In addition, green leafy vegetables and beetroot are particularly rich in inorganic nitrate and studies using dietary nitrate supplementation have shown that blood pressure and endothelial function have improved in healthy humans (Clements, Lee, & Bloomer, 2014).

1.3.1.2 Obesity

There is probable evidence that increasing consumption of fruit and vegetables will prevent weight gain (Boeing et al., 2012). There is also probable evidence that increasing fruit and vegetable intakes may lead to weight reduction, if consumed in place of food high in fat or energy. (Boeing et al., 2012). As vegetables are low in kilojoules, high in dietary fibre and contain water, there is a plausible mechanism by which consumption of these foods reduces the risk of weight gain (Australian Government Department of Health, 2013). Fruit and vegetables provide a higher degree of satiety and have a longer chewing time, both of which potentially facilitate a lower total energy intake (Australian Government Department of Health, 2013).
Two reviews have assessed the relationship between adiposity and fruit and vegetable intake (Hebden et al., 2015; Ledoux et al., 2010) with mixed evidence. Higher fruit and vegetable intake was associated with adiposity among overweight adults, however the evidence is weak (Ledoux et al., 2010). Fruit consumption excluding fruit juice was found to reduce the risk for long term weight gain in middle aged adults (Hebden et al., 2015).

1.3.1.3 Type 2 diabetes
There is probable evidence that the risk of developing type 2 diabetes is not influenced by fruit and vegetable consumption, as the majority of studies suggest a lack of association (Boeing et al., 2012). Being overweight or obese is a risk factor for type 2 diabetes, thus increasing fruit and vegetable intakes may indirectly contribute to a reduced incidence of type 2 diabetes (Boeing et al., 2012).

1.3.1.4 Cancer
According to the World Cancer Research Fund/American Institute for Cancer Research there is probable evidence that higher fruit and vegetable consumption has a protective effect against mouth, pharynx and larynx, oesophagus stomach, lung cancer and limited suggestive evidence for nasopharynx, lung, colorectum, ovary, endometrium, pancreas, liver cancer (American Institute for Cancer Research, 2007; Norat, Aune, Chan, & Romaguera, 2014). There is no convincing evidence that fruit and vegetables play a part in the cause of cancer (American Institute for Cancer Research, 2007).
1.4 Nutrient composition of fruit and vegetables

Fruit and vegetables are low in energy (kilojoules) and are a good source of dietary fibre, carbohydrates, folate, vitamins A, C & E, phytochemicals (carotenoids and bioflavonoids), magnesium, zinc, potassium and phosphorous (Australian Government Department of Health, 2013). Carotenoids are one of the most studied phyttonutrients found in fruit and vegetables due to their protective role as antioxidants. Carotenoids cannot be synthesised by the human body independently and must be taken up from the diet where they exist in high concentrations in a wide range of fruits and vegetables (Darvin, Sterry, Lademann, & Vergou, 2011).

The next section outlines the role of carotenoids in human metabolism.
1.4.1 Carotenoids

Carotenoids are lipid soluble red, orange and yellow pigments synthesised by a variety of plants and are found in many fruits and vegetables. Carotenoids give fruit and vegetables their bright colour. For example, $\beta$-carotene gives vegetables such as sweet potatoes, pumpkin and carrots their orange/yellow colour (Ermakov & Gellermann, 2015). The most common carotenoids are $\beta$-carotene, $\alpha$-carotene lycopene, lutein and zeaxanthin, and are present in human blood and tissues.

1.4.1.1 Function of carotenoids

Carotenoids are consumed as part of the diet. After absorption in the intestine they are transported by lipoproteins through the bloodstream and are dispersed to various organs (Alaluf, Heinrich, Stahl, Tronnier, & Wiseman, 2002; Scarmo et al., 2010) and into the skin. Carotenoids accumulate in all layers of human skin including the dermis, epidermis and the upmost skin layer, the stratum corneum (Alaluf et al., 2002; Scarmo et al., 2010). Carotenoids also accumulate in adipose tissue due to the large volume of adipose in the human body (Alaluf et al., 2002; Scarmo et al., 2010).

Carotenoids are present in the interior membrane of cells as well as lipoproteins. They possess nine or more conjugated double bonds that make them lipid soluble. Carotenoids have been classified as antioxidants, due to their ability to efficiently quench single molecular oxygen and neutralise free radicals, mainly reactive oxygen species (ROS) (Fiedor & Burda, 2014). This antioxidant potential is particularly important due to the uncontrolled generation and increase of ROS in the body resulting in oxidative stress (Fiedor & Burda, 2014). Oxidative stress is a contributor to the pathogenic process of many chronic diseases including cardiovascular disease, some cancers and age related macular degeneration (Fiedor & Burda, 2014).

In addition, human skin is directly exposed to a number of free radicals from the external environment, such UV radiation, tobacco smoke, ozone and nitrogen oxide (Darvin et al., 2011). Carotenoids serve as a protective role as they accumulate in all layers of this organ they are capable of neutralising these free radicals as these antioxidants form protective chains in the tissue (Darvin et al., 2011).
1.4.1.2 Bioavailability and bioconversion of carotenoids

Digestion and absorption can vary between individuals, with carotenoids having a half-life between 14 to 76 days (Burri, Neidlinger, & Clifford, 2001; Rock, Swendseid, Jacob, & McKee, 1992; Yeum et al., 1996). Humans cannot synthesise carotenoids, however they can metabolise some of them into vitamin A (retinol) (Fernandez-Garcia et al., 2012). Pro-vitamin A carotenoids such as β-carotene and α-carotene are converted to retinol via an oxidation process by the action of the enzyme β, β- carotene- 15,15'-mono-oxygenase. This action occurs mainly in the enterocytes (Castenmiller & West, 1998). Carotenoids are lipophilic compounds which upon digestion undergo solubilisation where they are released from the food matrix, interact with bile salts and are incorporated into micelles (Fernandez-Garcia et al., 2012). The uptake by enterocytes involves the assimilation of the lipid micelles contents through the diffusion process. Once the lipid material is internalised by the cells it is then packed into chylomicrons, which are excreted into the lymphatic system and taken up by the liver (Fernandez-Garcia et al., 2012).

There are a number of factors that determine the bioavailability of carotenoids and their bioconversion including the matrix composition, processing, as well as intake of dietary fat and fibre. (Fernandez-Garcia et al., 2012; van het Hof, West, Westrate, & Hautvast, 2000)

One of the first major factors that affect absorption of carotenoids is the type of matrix in which carotenoids are incorporated. Most studies have examined the effect of the food matrix on carotenoid availability on plasma response after supplementation into the diet delivered via whole fruit and vegetables, versus pure carotenoids (Fernandez-Garcia et al., 2012; van het Hof et al., 2000). The relative bioavailability of β-carotene from fruit and vegetables is lower compared to purified carotenoids, ranging from 3 to 34 % (van het Hof et al., 2000). Disruption of the food matrix, such as by heat treatment from cooking, has the potential to enhance the bioavailability of carotenoids from vegetables by sixfold, as more is extracted. (van het Hof et al., 2000). The type and amount of fat also influences bioavailability (Castenmiller & West, 1998; van het Hof et al., 2000). The addition of a small quantity of fat (3-5 grams per meal) greatly improves
the absorption from vegetables however it also depends on the type of fat and if it is absorbable (Castenmiller & West, 1998; van het Hof et al., 2000). Long chain-triacylglycerides appear to increase the bioaccessibility when ingested whilst short/medium chain-triacylglycerides tend to decrease bioaccessibility (Fernandez-Garcia et al., 2012). Whilst fat has a positive effect on carotenoid absorption, dietary fibre has a negative effect (Castenmiller & West, 1998; van het Hof et al., 2000). Dietary fibre and the fibre found in fruit and vegetables has the potential to reduce the bioavailability of carotenoids as it has been has been suggested that fibre interferes with micelle formation (van het Hof et al., 2000).

1.4.1.3 Detection and measurement of dietary carotenoids

The most common dietary carotenoids can be measured by biochemical methods in blood and in other tissues following extraction (Mayne et al., 2010). The gold standard assay for measurement of carotenoids in plasma or serum tissue is high performance liquid chromatography (HPLC) (Ermakov & Gellermann, 2010; Mayne et al., 2010). Research has demonstrated that plasma carotenoid concentrations are a reliable marker for usual fruit and vegetable intake (Burrows et al., 2015). HPLC has been used to validate plasma concentrations relative to measurement of dietary carotenoids, however HPLC is expensive with cost associated with phlebotomy, blood sample processing, storage and laboratory analysis. In addition, carotenoids have an estimated half-life of < 14 days for \( \beta \)-carotene so this method only works well for short-term dietary intake of fruit and vegetables (Ermakov & Gellermann, 2010; Mayne et al., 2010).

Carotenoid presence can be detected and concentrations measured in human skin by non-invasive optical methods such as Resonance Ramon Spectroscopy (RSS) and Reflectance Spectroscopy (Ermakov & Gellermann, 2015). RRS is a form of laser spectroscopy that detects the characteristic vibrational energy levels of a molecule (Hertz) (Ermakov & Gellermann, 2010, 2015; Mayne et al., 2010). An additional method is reflectance spectroscopy, where the spectrophotometer shines a light of specific wavelength using a filter and measures the intensity of the light reflected back by the skin. Reflectance spectroscopy operates on the tristimulus 1976 CIE L*a*b* space values.
to determine skin colour objectively. (Ermakov & Gellermann, 2010, 2015; Mayne et al., 2010). Each of these colour space components are detailed below (Figure 1.1):

- L* measures skin reflectance or lightness, values of 0 to 100 where 0 represents black and 100 represents white (perfect reflective diffuser)
- a* measures colour saturation from green to red, positive values indicate degree of redness
- b* measures colour saturation from yellow to blue, positive values indicate degree of yellowness
- both a* and b* have no specific numeric limits

![Figure 1.1 CIE L*a*b* colour space](image)

1.4.1.4 Carotenoids and skin colour

An individual’s skin colour in general has an influence on their appearance. The presence and/or absence of pigments in skin can have an impact on skin colour (Alaluf et al., 2002). Haemoglobin and melanin are considered the two major pigments that dominate the skin colour. Haemoglobin, provides red colouration whilst melanin provides the variability of brown colouration at the skin surface (Alaluf et al., 2002). There is also considerable evidence to show that carotenoids pigmentation, secondary to the consumption of fruit and vegetables, can also have a significant influence on skin colour, especially in terms of skin yellowness as defined by CIE b* value (Alaluf et al., 2002).
Skin colour change from consuming fruit and vegetables has been reported to be associated with greater perception of a healthy appearance (Whitehead, Re, Xiao, Ozakinci, & Perrett, 2012). If this is a universal phenomenon across population groups, it potentially could be tested as a motivator to improve fruit and vegetable intake in particular for young women.

The following section will explore fruit and vegetable intake in young women and the current evidence on interventions to improve fruit and vegetable intake in adults. This will be followed by an examination of young women’s motivations to change their dietary behaviour. The current evidence related to appearance based interventions will then be explored.

1.5 Fruit and vegetable intake in young women

Young women have low fruit and vegetable intake. In the United States, more than 90% of women aged 19 to 30 years do not meet recommended daily intake targets (Krebs-Smith, Guenther, Subar, Kirkpatrick, & Dodd, 2010). In Australia, women aged 19-30 years have one of the lowest overall female adult intakes, with only 44.7% meeting recommendations of two servings of fruit and 4.1% meeting recommendations of five servings of vegetables (Australian Bureau of Statistics, 2014).

1.6 Fruit and vegetable behaviour change interventions in adults

Current efforts to improve fruit and vegetable intakes have focused mainly on behaviour change interventions. Three reviews published from 2002 to 2011 evaluated the effectiveness of such interventions (Ammerman, Lindquist, Lohr, & Hersey, 2002; Pomerleau, Lock, Knai, & McKee, 2005; Thomson & Ravia, 2011) (Table 1.4). In 2004, a review was conducted by Pomerlau et al who identified 44 intervention studies that focused on promoting fruit and vegetable intake in adults. The review included interventions across various settings (e.g. worksite, supermarkets, churches), targeting specific groups (e.g. those with cardiovascular disease or risk factors) and involved different modes of delivery (e.g. face-to-face, individual or group counselling/education, computer-based (Pomerleau et al., 2005). The largest increases
in fruit and vegetable intake were observed in interventions targeting people with pre-existing health problems (range: +0.27-4.9 servings per day), whilst interventions targeting healthy adults (n=34) saw an increase of 0.1 to 1.4 servings per day (Pomerleau et al., 2005). Face-to-face counselling was shown to be consistently effective in increasing fruit and vegetable intake as well as individualised computer-based nutrition information and feedback when compared to a control group (Pomerleau et al., 2005).

The most recent review conducted by Thomson et al was an update of the previous two reviews (Ammerman et al., 2002; Pomerleau et al., 2005) and evaluated behavioural interventions from 2005 to 2010 (Thomson & Ravia, 2011). Twenty-seven of the 34 studies included in the review were conducted in adult populations and consisted of several sub-groups; healthy adults (n=11), low minority populations (n=11) and employees at worksites (n=7). Only three of the studies targeted younger adult populations (18-24 years) with an increased fruit and vegetable intake reported in the range of 0.9 to 1.16 servings per day. The mean increase in fruit and vegetables was highest in the healthy adult population (+1.13 servings per day). Even though this overall increase in this population group is quite significant, it is still well below recommended intake levels. The review concluded that in order for the population to sustain or achieve the recommended fruit and vegetable intake targets, other approaches are needed such as combining behavioural interventions with social marketing or technology based behaviour models (Thomson & Ravia, 2011).

A recent review conducted by Rehky et al specifically evaluated the success of worldwide campaigns to increase fruit and vegetable consumption (Rehky & McConchie, 2014). Overall the campaigns have resulted in increased awareness and short term modest increases in consumption (Rehky & McConchie, 2014). In Australia the “Go for 2&5 “ campaign conducted between 2005 and 2007 reported a 62.4% recall of awareness of the campaign after 3 years (Rehky & McConchie, 2014). The average increase in self-reported total fruit and vegetable consumption was 0.8 servings per day, which is equivalent to 11.4% increase of the total recommended intakes (Rehky & McConchie, 2014). The cost to roll out this campaign was AUD 7.6 million which is
high considering there has been no sustained long term impact as only 5.6% adults met
the daily recommendations for fruit and vegetable consumption in 2011-2012 (Rehky &
McConchie, 2014).

Although the studies included in the reviews are effective to some degree, few studies
have actually included young adults. Many of the interventions have focused on the
health benefits of the fruit and vegetables consumption which may not be relevant for
the younger adult population who are not usually motivated by their future health
outcomes (Rehky & McConchie, 2014). Further research is required to evaluate cost
effective interventions to improve fruit and vegetable intake amongst young adults. In
addition, an understanding of what motivates young adults needs to be considered
when developing strategies to change dietary behaviours (Rehky & McConchie, 2014).
Table 1.4: Summary of review articles examining effectiveness of fruit and vegetable interventions

<table>
<thead>
<tr>
<th>Study name</th>
<th>Inclusion</th>
<th>Reach</th>
<th>Efficacy</th>
<th>Suggestion for future research</th>
<th>Strengths and limitations</th>
</tr>
</thead>
</table>
| (Ammerman et al., 2002) | • RCT or non RCT’s
• Published 1975-1999
• Sample size n≥40
• All intervention types must include dietary component (FV or dietary fat intake)
• Follow-up data
• Healthy or high risk populations
• Human adults, children, adolescents
• Included F&V studies n=22 | • Included adults, children, adolescents
• NR on young adults/women
• NR: Age and sex
• Settings: Schools, health care facilities, communities, worksites | Overall n=12 studies that reported FV servings/day so no meta-analysis could be conducted
• 77% (17/22 studies) reported significant intervention effects
• Average increase of FV 17% (0.6 servings) compared to control groups | • Interventions need to access generalisability, response rate, elements of intervention intensity, individual's involved in intervention delivery and specific behavioural theories
• To included cost effectiveness | Strengths
• Critical appraisal
Limitations
• Adults and children included |
<table>
<thead>
<tr>
<th>Studies:</th>
<th>Characteristics</th>
<th>Findings</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| (Pomerleau et al., 2005) | • RCT or non RCT’s with control group  
• Published until April 2004  
• Adults who were free living, not actually ill,  
• Minimum follow up ≥3mo  
• Measured change in FV intake  
• Included studies n=44 | • Majority of studies included both men and women (n=36)  
• NR on young adults/women  
• 72.7% studies from USA  
• NR: Age  
• Settings: Supermarkets, Worksites, healthcare, Low SES, CVD or risk factors/cancer  
• Sample size: range 242-3737  
• Follow up ≥ 12 months (n=29) | Healthy adults  
• Increase of 0.1 to 1.4 FV servings per day  
Pre-existing health problems  
• Increase of 0.27 to 4.9 FV servings per day  
Face-to-face counselling  
• Consistent increases 0.62 to 1.4 FV servings per day | • Identify new cost effective and efficient ways to increase FV intake  
• Studies to examine further effectiveness of specific components of interventions |
| | | | Limitations  
• No critical appraisal |
| (Thomson & Ravia, 2011) | • RCT or clinical trials  
• Published 2005-2010  
• USA studies  
• Reported behaviour theory  
• Reported FV intake as an outcome measure  
• Sample size > 30  
• Included studies n=34 | • Demographic data provided but inconsistent  
• Young adults (18-24 years)  
• n=3 studies  
• Sample size: 80-2024  
• Females (n=1429, 56%) | Young Adults  
• Increase of 0.9 to 1.16 FV servings per day  
Healthy adults (n=11) studies  
• Average increase 1.13 FV servings per day  
Low income populations (n=9)  
• Average increase 0.97 FV servings per day  
Employee worksites (n=7)  
• Average increase 0.8 FV servings per day | • Promote enrolment of most challenged individuals with the greatest need to increase FV intake  
• Interventions to assess change FV intake with other health behaviours (diet, physical activity)  
• Behavioural interventions to be combined with social marketing, economics and technology |
| | | | Limitations  
• No critical appraisal  
• Adults and children included  
• Only included studies from USA |

**Abbreviations:** RCT: Randomised control trial; NR: not reported; FV: fruit and vegetables; Yr: year; mo: months; SES: Socio economic status; CVD: cardiovascular disease
1.7 Health versus appearance in motivating behaviour change in young women

Several studies have examined what motivates young women’s dieting behaviours (Putterman & Linden, 2004), diet quality (Traill, Chambers, & Butler, 2012), fruit and vegetable intake (Chung, Hoerr, Levine, & Coleman, 2006; Satia, Kristal, Curry, & Trudeau, 2001) and weight management behaviours (Holley, Collins, Morgan, Callister, & Hutchesson, 2015; LaRose, Leahey, Hill, & Wing, 2013).

Three studies have compared motivations for weight loss or behaviour change between young and older women (LaRose et al., 2013; Putterman & Linden, 2004; Traill et al., 2012). LaRose et al compared motivating factors for attempting weight loss in young adults (18 – 35 years, n=848) versus older adults (36-50 years, n= 2116) in participants of the US National Weight Control Registry who had been successful at long-term weight loss maintenance (LaRose et al., 2013). There were significant differences between the age groups for six of the nine motivating factors assessed (p<0.001). Young adults rated “improving your appearance” and “wanting to feel better about yourself” more important than health concerns, which was rated most important amongst the older adults (LaRose et al., 2013). These findings are consistent with recent data from a survey of 250 UK adults (18 to 85 years) who were asked questions on the importance of appearance and health in relation to their attitudes and constraints to healthy eating (Traill et al., 2012). The results found that health importance was highest amongst the older female age group (50-64 years, n=65) whilst 18-34 year old females (n=74) reported being more motivated to eat healthily by concerns about their appearance (Traill et al., 2012). Putterman & Linden found young female college students (aged 16-34 years, n=110) were more motivated to change their appearance through dieting compared to older women (35-67 years, n=96). Their mean appearance score (extent of appearance motivation) of 11.8 was significantly higher p<0.001 than older women (10.4) (Putterman & Linden, 2004). Dieting to improve their health was significantly more important (p<0.001) for older women (11.6) compared to young students (9.3) (Putterman & Linden, 2004).
One study conducted by Satia et al measured motivations for dietary behaviour change from both females (n=603) and males (n=602) adults (Satia et al., 2001). At baseline, data was collected on the motives for changing diet, including fruit, vegetable and fat intake. The study found that females aged 18-34 years (n=354) were more likely to change their diet for a better self-image compared to males (mean adjusted score 3.24 vs. 2.93 p<0.001) whilst older persons (aged 55+ years) and males were more motivated by personal health (p<0.001) (Satia et al., 2001).

Two studies examined motivators for behaviour change in young women only (Chung et al., 2006; Holley et al., 2015). Chung et al, conducted a cross-sectional study of 18-24 year old college women (n=236) to examine processes underlying their decisions to eat fruit and vegetables and examining their stages of readiness to change (Chung et al., 2006). The study found that one of the processes that influenced their decision to eat fruit and vegetables was due to their weight concerns and their appearance (Chung et al., 2006). On the contrary, a recent study conducted by Holley et al showed that improving appearance was not the key motivator for weight change in a group of young women aged 18-30 years (n=647) (Holley et al., 2015). Improving health outcomes was ranked the most important reason to change their weight amongst 24.4% of the young women, followed by feeling better about oneself (22.3%). Appearance (10.0%) was ranked the least important reason for young women to want to change their weight (Holley et al., 2015).

In summary, there is some evidence to suggest that young adults, especially women, may be more motivated to change their behaviour to improve their appearance particularly compared to older adults and males. Interventions that focus on improving appearance to motivate dietary change (including fruit and vegetable intake) rather than focusing on health outcomes need to be explored and evaluated to determine whether they could be effective for young women.
1.8 Appearance based behavioural interventions

A novel and effective strategy used in sun exposure and smoking cessation interventions is emphasising the negative effects of these behaviours on appearance (Table 1.5). Jones and Leary demonstrated that excessive sun exposure had negative consequences on the outward physical appearance in the long-term (Jones & Leary, 1994). They found that messages which focused on the effect of sun exposure on skin appearance, motivated sun protection intentions significantly more than messages which focused on the negative consequences of sunbathing on health (Jones & Leary, 1994).

More recently, Mahler et al conducted a Randomised Controlled Trial (RCT) to show the effects of Ultra Violet (UV) light on skin wrinkling and age spots by the use of UV photographs and a photo-ageing information video to a group of US university students (n=133) aged 18 to 44 years (Mahler, Kullk, Gerrard, & Gibbons, 2007). Sun protection behaviour improved significantly for a sustained period of up to four months for both the photo-ageing video and photographs interventions compared to the control group. Students also had significantly lighter skin and less melanin (increased L* values) after four months. The lighter skin continued to show after one year for those who were allocated to the photo-ageing information video intervention (Mahler et al., 2007).

A recent RCT conducted by Burford et al, examined the effectiveness of a computer generated photo-ageing software program that displayed images of “smokers faces” (wrinkling, ageing, gautness, grey complexion) to a group of young Australian smokers (n=160) aged 18-30 years (Burford, Jiwa, Carter, Parsons, & Hendrie, 2013). The control group received standardised smoking cessation advice from a pharmacist for two minutes (Burford et al., 2013). Effectiveness was assessed by comparing smoking cessation rates, number of quitting attempts and changes in smoking dependence between the intervention and control group. At six month follow-up, 13.8% of participants in the intervention group confirmed to have quit smoking (n=11) (via self-report and carbon monoxide breath test) and quit attempts were significantly
higher (p=0.03) than the control group (n=1). In addition their smoking dependence score had significantly dropped compared to the control group (Burford et al., 2013).
Table 1.5 Summary of appearance based behavioural interventions for sun protection and smoking cessation in young adults

<table>
<thead>
<tr>
<th>Study name</th>
<th>Study type</th>
<th>Objectives</th>
<th>Setting</th>
<th>Follow-up</th>
<th>Participant characteristics</th>
<th>Measures/outcomes</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun protection</td>
<td></td>
<td></td>
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<tr>
<td>(Mahler et al., 2007)</td>
<td>RCT</td>
<td>Examine the longer term effects of exposure of UV. Photographs and photo</td>
<td>University, USA</td>
<td>4-5 mo, &amp; 12 mo follow-up</td>
<td>n=107 female</td>
<td>Cognition outcomes</td>
<td>UV photo interventions further demonstrates the potential to motivate UV protection behaviours to reduce skin cancer risk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Assigned to one</td>
<td>ageing and photo ageing for increasing sun protection intentions and</td>
<td></td>
<td></td>
<td>n=26 male</td>
<td>• Assess potential cognitive mediators of interest</td>
<td></td>
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<tr>
<td></td>
<td>of the four</td>
<td>behaviours</td>
<td></td>
<td></td>
<td>Mean ± SD age: 20.1 ± 3.38 yrs</td>
<td>• Photo ageing video (p&lt;0.001) and UV photos (p&lt;0.03) had immediate significant greater intentions to engage in sun protection and felt more susceptible to photo ageing.</td>
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<tr>
<td></td>
<td>groups: 1. UV</td>
<td></td>
<td></td>
<td></td>
<td>45% Caucasian</td>
<td>• Using skin reflectance spectrophotometry CIE L<em>a</em>b* baseline, 4, 5 and 12mo</td>
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<tr>
<td></td>
<td>photos</td>
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<td></td>
<td></td>
<td>Sunscreen use face: 74.2%;</td>
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<td></td>
<td>2. Photoageing</td>
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<td></td>
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<td>body 56.3%</td>
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<tr>
<td></td>
<td>information</td>
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<td></td>
<td></td>
<td>Incidental use face: 45.1%;</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3. UV photos</td>
<td></td>
<td></td>
<td></td>
<td>body 21.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ photo ageing</td>
<td></td>
<td></td>
<td></td>
<td>23% at least 30 minutes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>information</td>
<td></td>
<td></td>
<td></td>
<td>sunbathing in prior week</td>
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<td></td>
<td>4. Control</td>
<td></td>
<td></td>
<td></td>
<td>93.9% at least 3 hours of</td>
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<td></td>
<td></td>
<td>incidental sun exposure</td>
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<td></td>
<td>27.1% family hx of cancer</td>
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</tr>
<tr>
<td>(Jones &amp; Leary, 1994)</td>
<td>Experimental:</td>
<td>To examine the effectiveness of health based versus appearance based</td>
<td>University, USA</td>
<td>No follow-up</td>
<td>n=65 female</td>
<td>Subjects beliefs on severity of the effects of sun exposure (range of scales)</td>
<td>Concerns about effects of significantly correlated with intended sunscreen use (r=0.49, p&lt;0.01)</td>
<td>The appearance based essay that focused on negative effectives of the sun on appearance was most effective. However more effective in those who had a lower appearance motivation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>messages on sun related belief and intentions of people who difference in</td>
<td></td>
<td></td>
<td>n=69 male</td>
<td>• Concern of harmful effects</td>
<td>The appearance based essay had a significant effect on the concerns of harmful effects on the sun compared to the health essay (p=0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>appearance motivation</td>
<td></td>
<td></td>
<td>age: 17 to 23</td>
<td>• Demotivating tanning behaviours</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All Caucasian</td>
<td>• Degree to which they intend to use sunscreen</td>
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</tr>
</tbody>
</table>
The appearance based essay had significant intentions to use sunscreen \((p<0.05)\) compared to health based essay.

### Smoking cessation

**(Burford et al., 2013)**

<table>
<thead>
<tr>
<th>RCT</th>
<th>Intervention group: digitally computer generated images of participants ((n=80))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control group ((n=80))</td>
</tr>
<tr>
<td>2.</td>
<td>Both groups received smoking cessation advice ((2) minutes)</td>
</tr>
</tbody>
</table>

To test the efficacy and cost effectiveness of an intervention based on illustrations of smokers faces among young smokers.

**Community pharmacies**  
Perth, Australia

<table>
<thead>
<tr>
<th>Control group:</th>
<th>Change in smoke behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD age: 25.1 ± 4.1 yrs</td>
<td>Nicotine dependence</td>
</tr>
<tr>
<td>33.8% 11-20 cigarettes/day</td>
<td>Using fagerstrom scale</td>
</tr>
<tr>
<td>Response rate 77.5%</td>
<td></td>
</tr>
</tbody>
</table>

**Intervention group**

| Mean ± SD age: 24.2 ± 4.1 yrs | Smoking dependence score |
| 36.3% 11-20 cigarettes/day | significantly dropped |
| Response rate 72.5% | compared to control group \((p=0.03)\) |

**6 mo follow-up**

### Cost effectiveness

- Incremental cost per additional quitter and per additional lifetime quitter

### Change in smoke behaviour

- Intervention group appeared to be concerned about their physical appearance \((82.5% p=0.03)\) compared to control group
- 13.8% confirmed non-smokers in intervention group, compared to 1.3% control group \((p=0.03)\)
- Smoking dependence score significantly dropped compared to control group \((p<0.001)\)
- Cost effective ratio was $46 AU per additional quitter

### Displaying the effects of facial appearance using computer stimuli images by be effective as well as cost effective to influencing young adults to quit smoking

**Abbreviations:** RCT: Randomised control trial; Yr: year; mo: months; SD: standard deviation; AU: Australian
1.9 Appearance based dietary interventions

In contrast to the interventions described in Section 1.8, an appearance based intervention could potentially be used to improve dietary behaviour by demonstrating the positive effects on appearance to young adults. However prior to developing an appearance based dietary intervention the impact of dietary intake on appearance needs to be examined. There is some evidence to suggest a relationship between dietary intake and appearance but this evidence has not yet been synthesised.

Several studies have examined the relationship between fruit and vegetables on skin colour appearance. Whitehead et al conducted a study in young adults (18-25 years) in Scotland examining the relationship between fruit and vegetable consumption and skin colour using reflectance spectroscopy (Whitehead, Re, et al., 2012). Over a six week period, self-reported changes in fruit and vegetable intake were significantly correlated with changes in overall skin yellowness ($b^*$) ($p=0.038$) and redness ($a^*$) ($p=0.045$) (Whitehead, Re, et al., 2012). In the same study, Whitehead et al examined the level of skin colour change associated with fruit and vegetable consumption on the perception of health and attractiveness. Using digitally manipulated facial images, participants were asked to view the images and select the face that appeared the most healthy and attractive (Whitehead, Re, et al., 2012). A consistent pattern was shown where increased skin redness ($a^*$) and yellowness ($b^*$) was rated as appearing healthier and more attractive (Whitehead, Re, et al., 2012). They also showed that to enhance apparent health and to improve perceive attractiveness a modest increases of 2.9 and 3.3 portions of fruit and vegetables are required (Whitehead, Re, et al., 2012). The study concluded that the beneficial effects on skin appearance due to increased fruit and vegetable consumption could potentially influence individuals who are motivated by appearance, typically young adults, to make changes to their dietary patterns (Whitehead, Re, et al., 2012).

Similar studies have also been conducted on the perceived health of human faces using colour calibrated Caucasian facial images (Figure 1.2)(Stephen, Coetzee, & Perrett, 2011; Stephen, Law Smith, Stirrat, & Perrett, 2009). The studies have shown that greater yellowness ($b^*$) and lightness ($L^*$) in facial skin colour is perceived by Scottish adults to
be healthier (Stephen et al., 2011; Stephen et al., 2009). These results suggest that humans express a preference for skin colour associated with higher intakes of fruit and vegetables (carotenoids), rather than skin colour associated with sun exposure (melanin).

Figure 1.2 Examples of facial transformations of carotenoid pigmentation from ± fruit and vegetables portions

1.10 Summary of the literature

In summary, young women have one of the lowest intakes of fruit and vegetables amongst Australian adults. Interventions promoting an increase in fruit and vegetable intakes in adults have shown some success. However, there have only been a few interventions that have solely focused on young adults, especially women. Current evidence suggests that young women may be motivated to change their behaviours if they believe it will positively alter their appearance rather than being motivated by improving their health outcomes. Therefore an intervention that focuses on appearance, as a motivator to improve fruit and vegetable intakes requires testing. Appearance based interventions have been evaluated to change smoking and sun exposure behaviours and have shown positive results. Several studies have
investigated the effect of fruit and vegetable intake on skin colour appearance in relation to health and attractiveness, however the evidence on dietary intake and appearance has not been synthesised.

Further research needs to be conducted before appearance based dietary interventions can be developed and tested. A high quality systematic review of the literature is required to: 1) examine the evidence on the association between dietary intake and appearance, and 2) evaluate the effectiveness of interventions that have focused on dietary intake and appearance. The association between fruit and vegetable intake and skin colour needs to be examined in a group of young Australian women to confirm results from previous studies. In addition the perceptions of health based on manipulation of skin colouration in facial images is required in an Australian population to compare findings to those published in other populations. Lastly, a high quality RCT need to be conducted to strengthen the evidence on the impact of fruit and vegetable intake on skin colour and plasma carotenoids.

### 1.11 Research aims and hypothesis

The aims and hypotheses of the research reported in this thesis are:

#### Study 1

1. To investigate the best available evidence on the association between dietary intake and physical appearance.

2. To investigate the best available evidence of the effectiveness of dietary interventions on perceived or actual physical appearance.

- Hypothesis: A healthy diet is associated with appearance of health
Study 2

3. To examine the association between fruit and vegetable intake and skin colour in young women aged 18-30 years in the Hunter New England region.

- Hypothesis: Young women who report higher fruit and vegetable intakes will have higher skin yellowness (CIE b* values) compared to those who consume low amounts.

Study 3

4. To compare the consumption of high carotenoid fruit and vegetables versus low carotenoid fruit and vegetables on skin colour and plasma carotenoids in young women aged 18-30 years.

- Hypothesis: The consumption of the high carotenoid fruit and vegetables will increase skin colour, in particular skin yellowness (b*), and plasma carotenoid levels, more than the consumption of the same amount of low carotenoid fruit and vegetables.

5. To examine the relationship between the change in skin colouration and the change in plasma carotenoids following a four week intervention period.

- Hypothesis: Changes in plasma concentrations will be correlated with changes in skin yellowness (b*).

Study 4

6. To investigate Australian adult’s perceptions of health using standardised facial images associated with carotenoid-based skin colour, relative to the skin colour conveyed by melanin.

- Hypothesis: The perception of health will be associated with a change in skin colour associated with increased skin carotenoid and melanin colouration.
1.12 Thesis structure

The thesis is comprised of a series of four research papers (Chapter two to five). Two of the research papers have been published and the remaining two have been submitted (one is currently under review). These papers present a body of research made up of four key components: a systematic literature review (Study 1: Chapter 2), a cross-sectional study (Study 2: Chapter 3), a randomised cross-over trial (Study 3: Chapter 4) and a computer based perception of health evaluation study (Study 4: Chapter 5). An overall discussion of the findings from the body of research, and the implications for research and practice are provided as the final chapter of the thesis (Chapter 6).
Chapter 2  Systematic review

This article was published in 2015


The work presented in the manuscript was also presented at The Annual Scientific Meeting Nutrition Society of Australia and New Zealand in November 2013, Brisbane, Australia (Poster presentation).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 20).
2.1 Abstract

Appearance-based interventions have had some success in reducing smoking and sun exposure. Appearance may also motivate dietary behaviour change if it was established that dietary improvement had a positive impact on appearance. The aims of this review are to evaluate the current evidence examining the relationship between dietary intake and appearance and to determine the effectiveness of dietary interventions on perceived or actual appearance. An electronic search of English language studies up August 2012 was conducted using Cochrane, MEDLINE, Embase, CINAHL, Web of Science, SCOPUS and PsycINFO databases. Studies that included participants at least 18 years, that observed or altered dietary intake from actual food or dietary supplement use and assessed appearance-related outcomes were considered eligible. Data from 27 studies were extracted and assessed for quality using standardised tools. Nineteen studies were assessed as being of “positive” and 4 of “neutral” quality. All observational studies (n=4741 participants) indicated that there was a significant association between various aspects of dietary intake and skin colouration and skin ageing. The majority (16 studies, 769 participants) evaluated the effect of dietary supplements on skin appearance amongst females. Only 1 study examined the effect of actual food intake on appearance. Significant improvements in at least 1 actual or perceived appearance-related outcome (facial wrinkling, skin elasticity, roughness and skin colour) following dietary intervention were shown as a result of supplementation. Further studies are needed in representative populations that examine actual food intake on appearance, using validated tools in a well-designed high-quality randomised control trial.

2.2 Introduction

Poor dietary intake is one of the most important risk factors for preventable disease and premature mortality (Australian Government Department of Health, 2013). Poor nutrition is responsible for around 16% of the total burden of disease worldwide and is associated with excessive intake of energy dense foods, saturated fat and added refined
sugar or salt (Australian Government Department of Health, 2013). Improving nutrient intake promotes good health, well-being and reduces the risk of many chronic diseases (Australian Government Department of Health, 2013). For instance, consuming an adequate amount of fruit and vegetables in our diet has been shown to reduce the risk of excess weight gain, type 2 diabetes, cardiovascular disease, and specific cancers (American Institute for Cancer Research, 2007; Australian Government Department of Health, 2013; Boeing et al., 2012). Despite the benefits of consuming adequate amounts of fruit and vegetables, intake of this food group is low amongst the adult population worldwide and is identified by the WHO as among the top 10 risk factors for global mortality (World Health Organisation, 2014). Only 2.2% of men and 3.5% of women (Kimmons, Gillespie, Seymour, Serdula, & Michels Blanck, 2009) in the United States meet their caloric-specific MyPyramid fruit and vegetable recommendations. In the United Kingdom 31% of adults meet their current combined fruit and vegetable intake recommendation of “5-a-day” (Department of Health, 2012). In Australia, only 5.5% of adults meet guidelines (2 serves of fruit and 5 serves of vegetables), with 18-34 year-olds least likely to meet recommendations (3.4%) (Australian Bureau of Statistics, 2012).

Current efforts to improve an individuals’ diet often involve behavioural interventions that aim to improve fruit and vegetable intake. Several reviews have evaluated the efficacy of such interventions (Ammerman et al., 2002; Pomerleau et al., 2005; Thomson & Ravia, 2011). The most recent was conducted in 2011 by Thomson and Ravia (Thomson & Ravia, 2011) who evaluated behavioural interventions to improve fruit and vegetable intake among individuals without pre-existing health conditions that were published between 2005 and 2010. Based on the 34 studies included, the mean increase in servings per day of fruit and vegetable post intervention was 1.13 in adults and 0.39 in children (Thomson & Ravia, 2011). This review concluded that in order for individuals to achieve recommended fruit and vegetable intake targets, additional approaches are required as the best available evidence indicates that it cannot be achieved solely by existing behavioural interventions (Thomson & Ravia, 2011).
To consider other approaches to promote dietary behaviour change, an understanding of what motivates individuals to change their health behaviours is required. A recent study conducted by LaRose et al (LaRose et al., 2013) compared motivating factors for weight loss and weight loss behaviours of young adults (18 – 35 years) versus older adults (36-50 years) who had been successful in long-term weight loss (LaRose et al., 2013). Young adults rated “improving your appearance”, “improving social life” and “wanting to feel better about yourself” more important than “health concerns”, which was rated as most important amongst the older adults (LaRose et al., 2013). These findings are consistent with a recent survey of UK adults who were asked questions on both the importance of “looking good” and the importance of “health” in relation to their diet (Traill et al., 2012). The results indicated that health was of highest importance amongst the older female age group, whilst younger women felt more motivated to eat healthily based on concerns about their appearance. The researchers concluded that interventions to promote healthy eating and improve diet quality among young women should focus on appearance rather than health (Traill et al., 2012). Therefore, appearance-based interventions may be a potential new approach to promote dietary behaviour change, particularly among younger adults.

Appearance-based interventions have had some success in motivating behaviour change, predominantly for smoking (Semer et al., 2005) and sun exposure behaviours (Mahler et al., 2007). Photographs demonstrating the effects of UV light exposure on facial images have been shown to precipitate a sustained period of behaviour change in sun tanning practices (Mahler et al., 2007). Images visualising the adverse effects of smoking on facial skin wrinkling and oral disfigurement have also motivated smoking cessation (Semer et al., 2005). Before appearance-based interventions are developed for dietary behaviour change we must first establish that what we eat influences our appearance. There is some evidence to suggest a relationship between dietary intake and appearance but existing evidence has not been systematically synthesised. Therefore, the primary objectives of this review are to evaluate recent evidence examining relationships between dietary intake and physical appearance and to determine the effectiveness of dietary interventions on perceived or actual physical
appearance. For the purpose of this systematic review Cochrane, Medline, Embase, CINAHL, Web of Science, Scopus and PsycINFO databases were searched using specific keywords for relevant studies published until August 2012 and by also reviewing the reference lists from retrieved articles. Study quality was assessed and data extracted using standardised appraisal tools. Data was analysed using narrative summary.

2.3 Methods

This review followed all Preferred Reporting Items for Systematic Reviews and Meta-analyses statement (PRISMA) guidelines except for protocol publication.

2.3.1 Eligibility criteria

2.3.1.1 Types of participants

Studies that included individuals aged 18 years and older were included. Studies in which participants had a history of eating disorders or chronic medical conditions or who were pregnant were excluded.

2.3.1.2 Types of interventions/exposure

Both experimental and observational studies that provided a dietary intervention or evaluated participants’ exposure to dietary components respectively were included. Included studies had to report dietary intake from either actual foodstuffs/food groups or dietary supplement use or both. The definition of dietary intake for the review was necessarily broad due to the researchers’ awareness that a low number of studies had specifically investigated the relationship between food intake and appearance.

2.3.1.3 Outcome measures

Studies that assessed physical appearance as the primary outcome were considered. For the purpose of this review, appearance is defined as either an individual’s or observer’s perception of physical appearance or an objective measure of an outward aspect of physical appearance, including skin colour and tone and body shape. Studies that examined the relationship between BMI and body image or shape were not included.
2.3.2 Search strategy

A 3-step search strategy was used for this review to identify published studies in the English language up to and including 2nd August 2012. An initial limited search was conducted in MEDLINE and CINAHL followed by analysis of titles, abstracts, and index terms used to describe articles. All identified keywords and index terms were used in a second search across the following databases: The Cochrane library, MEDLINE, PRE-MEDLINE, Embase, CINAHL, Web of Science, SCOPUS and PsycINFO. The reference lists of all included articles were also searched for additional relevant studies.

2.3.2.1 Search terms

The search terms were divided into 2 groups: (1) dietary intake (e.g. nutrition, fruit, vegetables, carotenoid*, diet quality, dietary supplement*) and (2) appearance (e.g. beauty/ vanity /appearance, skin pigmentation, complexion, skin colour/ skin color, skin colouration/ skin coloration, skin tone, attractiveness, self-image, body shape, body image, self-esteem, self-perception, self-concept.) The Boolean phrase “AND” was used between groups and “OR” for within groups.

2.3.3 Study selection

Following the search, duplicates were removed and articles were screened and identified for relevance to the review based on the title, abstract and description/MESH headings by 2 reviewers. Endnote was used for data management. The full article for studies that met the inclusion criteria were retrieved and examined independently by 2 reviewers to confirm inclusion. If it was unclear from the abstract whether the study met the inclusion criteria, the full article was also retrieved for clarification. If a disagreement occurred as to the inclusion or exclusion of a study a resolution was reached through a third independent reviewer.

2.3.4 Critical appraisal

Two independent reviewers assessed the included articles for methodological quality using the Academy of Nutrition and Dietetics Quality Criteria Checklist (Academy of Nutrition and Dietetics, 2012). Studies were appraised as “positive” if they answered
yes to all of the following criteria: selection of study participants “free from bias”, methods of assigning subjects/participants to groups described and unbiased “comparable study groups”, “interventions were described in detail”, were the primary and secondary “outcomes valid and reliable” plus at least one additional yes from the other 6 items (Academy of Nutrition and Dietetics, 2012). If 6 or more of the ten items were answered ‘no’ the study was labelled “negative”. Studies were labelled “neutral” if neither of these criteria were met (Academy of Nutrition and Dietetics, 2012).

2.3.5 Data extraction

Data were extracted from the included studies using a standardised form developed by the authors that included study characteristics such as number of participants, study duration, exclusion/inclusion criteria, retention rates, appearance outcomes and measures, results and exposure. One reviewer extracted all of the data, which was cross-checked by the second reviewer.

2.4 Results

2.4.1 Description of studies

The search identified 11,678 articles (Figure 2.1). Following the elimination of duplicates and assessment of the abstract for eligibility, 59 full text studies were retrieved of which 27 studies were included in the review. The primary reasons for exclusion on review of full text articles (after abstract/full article retrieval) were; not having relevant outcomes reported (n=30), failure to meet participant eligibility criteria (n=5) and not being an observational or experimental study (n=3).

Of the included studies, 9 observational studies evaluated the relationship between dietary intake and appearance, of which 4 were case studies, 4 were cross-sectional studies and 1 was a prospective cohort study. There were 18 experimental studies that evaluated effectiveness of dietary interventions on physical appearance outcomes, of which 17 were RCTs and one a pre-post single-arm study. The 27 studies were published between 1985 and 2012 and were conducted in 14 countries with the majority in the United States (n=7) and Europe (n=14) (Figure 2.2)
Figure 2.1 Flow diagram of studies included in the review
Figure 2.2 Key mechanisms between dietary intake and appearance
2.4.2 Association between dietary intake and appearance

Study characteristics, critical appraisal and results of the included observational studies are summarised in Table 2.1, Table 2.2 and Table 2.5 respectively. The total number of participants across all observational studies was 4741, of which 94% were female. The mean number of participants across all studies was 527 (range 1-4025). The age range of participants was 18 to 104 years.

Five observational studies were critically appraised for quality with all assessed as being “positive” (Table 2.5). The observational studies (n=5) had comparable study groups, participant selection free from bias and measured outcomes using valid and reliable methods. No studies reported or used blinding, which could introduce bias. The 4 case reports were not assessed for their quality as there is no current tool for this study type and their methodological quality is low. Results from these four studies have been interpreted with caution.

Appearance outcomes were grouped by the outcomes of skin colouration (n=6), skin ageing (n=2), and body image (n=1). The majority of the dietary intake variables examined in the included studies were reported as fruit and vegetable consumption (n=5), others included dairy, meat, fish oils, fats and sugar.

2.4.3 Relationship between dietary intake and appearance outcomes

2.4.3.1 Skin colouration

All 4 case reports (Table 2.1) (Caroselli, Bruno, & Manara, 2007; Nishimura, Ishii, Sugita, & Nakajima, 1998; Takita, Ichimiya, Hamamoto, & Muto, 2006; Vakil, Ayiomamitis, Nizami, & Nizami, 1985) reported orange colouration of the palms, soles of feet and face due to excessive consumption of foods, including kaki fruit (1kg daily) (Caroselli et al., 2007), nori rolls (50 sheets daily) (Nishimura et al., 1998), raw carrots (2kg daily) (Vakil et al., 1985) and tomatoes (0.5kg daily) (Vakil et al., 1985), or nutrient supplements containing carotene (4410ug/dl daily) (Takita et al., 2006). Skin colouration was reported to gradually subside after reducing or ceasing intake in each of the 4 case reports.
One study (Whitehead, Re, et al., 2012) reported significant associations between objectively measured skin yellowness (CIE Lab colour space b* values) at multiple body sites with changes in fruit and vegetable intake over six weeks (Table 2.2). In addition, it was found that diet-linked changes in spectrophotometer-assessed skin reflectance were correlated with absorption spectra of β-carotene, α-carotene and lycopene (Whitehead, Re, et al., 2012). Another study (Stephen et al., 2011) found that individuals with higher fruit and vegetable intakes had increased skin yellowness b*values.

### 2.4.3.2 Skin ageing

Two studies (Cosgrove, Franco, Granger, Murray, & Mayes, 2007; Purba et al., 2001) examined the relationship between nutrient intakes and skin ageing appearance. Purba et al (Purba et al., 2001) assessed dietary intake using a semi-quantitative food frequency questionnaire (FFQ) examined skin wrinkling via cutaneous microscopy and concluded that higher intakes of vegetables, fruit, olive oil and legumes may cause less skin wrinkling. Cosgrove et al. (Cosgrove et al., 2007) examined nutrient intake via 24-hour recalls and clinical examinations of the skin, finding that higher intakes of vitamin C and linoleic acid were associated with a lower likelihood of skin wrinkling, dryness and skin atrophy. Comparisons across these 2 studies were difficult due to demographic and methodological differences.

### 2.4.3.3 Body image

One study (George, Milani, Hanss-Nuss, & Freeland-Graves, 2005) examined compliance to dietary guidelines and found that those who were in the highest tertile of a measure of compliance to American dietary guidelines were more likely to have a positive body image.
Table 2.1. Design characteristics of Case studies included in the review

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Setting</th>
<th>Study Participants</th>
<th>Exposure</th>
<th>Signs and Symptoms</th>
<th>Results after follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caroselli et al, 2008</td>
<td>Rome, Italy</td>
<td>68 year old white woman.</td>
<td>Consumption of excessive amounts (~1kg/day) of Kaki fruit for 12 months.</td>
<td>Red-orange tinged skin of palms and soles of feet. Diagnosed with lycopenaemia.</td>
<td>Stop ingesting the Kaki diet and condition gradually subsided and serum lycopene levels returned to normal.</td>
</tr>
<tr>
<td>Nishimura et al, 1998</td>
<td>Yokohama City University Hospital, Japan</td>
<td>22 year old woman, Japanese, NIDDM (medications: glibenclamide), BMI 16.4, 14-17kg weight loss in previous 3 years on a ’diet’.</td>
<td>Patient reported eating average of 50 (range 10-60) sheets of Nori daily (50mg β-carotene) over a period of 5 months.</td>
<td>Orange-yellow colour changes in skin, notable palms, soles and face (Sclera were normal). Pigmentation began 3 months prior to presentation.</td>
<td>Skin colour returned almost to normal six months after ceasing Nori ingestion.</td>
</tr>
<tr>
<td>Takita et al, 2006</td>
<td>Japan</td>
<td>66 year old woman.</td>
<td>Ingested 6 x daily nutrient supplements (contained carotene) for 3 months, equivalent of 4410ug/dl.</td>
<td>Presented with yellow-orange discolouration of the skin and palms, diagnosed with carotenemia.</td>
<td>Stop ingesting supplements and condition gradually subsided.</td>
</tr>
<tr>
<td>Vakil et al, 1985</td>
<td>Clinical setting (country NR)</td>
<td>32 year old woman, Greek, BMI = 26.5 kg/m2 (overweight).</td>
<td>Voluntary consumption of up to 2kg raw carrot and 0.5kg tomato per day for several months. Additionally consumed a diet rich in green vegetables, salads and fruit. Consumption was driven by a weight loss motive.</td>
<td>Yellow-orange discolouration on hands, soles of feet and nasolabial folds.</td>
<td>6 weeks reduced intake, skin colour returned to normal.</td>
</tr>
</tbody>
</table>

Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; NR, not reported; BMI, body mass index
<p>| Author                  | Study Design  | Objectives/Hypotheses                                                                 | Setting                        | Duration | Sample Size | Participant Characteristics | Exclusion                                                                                   | Measures                                                                                          | Results                                                                                          |
|------------------------|---------------|--------------------------------------------------------------------------------------|--------------------------------|----------|-------------|-------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| (Whitehead et al, 2012) | Prospective Cohort | Investigate the effect of fruit and vegetables on skin colour over a six week period. | University of St Andrews, Scotland | 6 weeks  | n=35         | Male n=14, female n= 21. Mean age: 20.74 years, range: 18-25 years. Caucasian (n=34). | Recent sunbathing, use of self-tanning products, solariums or facial makeup, or skin lightness &gt; 2sd from mean. | Fruit and vegetable intake: 63 item FFQ baseline, 3, 6 weeks. Skin colour and reflectance, Konica Minolta Spectrophotometer, CIEL<em>a</em>b values, spectral wave lengths between 400 and 540 nm seven body sites. | Skin lightness decreased, redness and yellowness changes were significantly associated with increase fruit &amp; vegetable intake. Overall skin reflectance change was significantly correlated with the absorption spectra of beta carotene and lycopene. Higher daily intakes of fruit and vegetables and β-carotene had yellower skin (higher b* values) at multiple body sites (r=0.25 p&lt;0.026). |</p>
<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>Objectives/Hypotheses</th>
<th>Setting</th>
<th>Duration</th>
<th>Sample Size</th>
<th>Participant Characteristics</th>
<th>Exclusion</th>
<th>Measures</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>(Cosgrove et al, 2007)</td>
<td>Cross-sectional</td>
<td>To examine the relationships between nutrient intakes and skin ageing appearance.</td>
<td>Data sourced from NHANES I survey conducted in the United States 1971-1974.</td>
<td>NA</td>
<td>n=4025</td>
<td>Women aged 40 years or over who took part in the NHANES I (n=4025). (Mean ± SD) Age: 58.1±11.2 years. BMI 26.4±5.6 kg/m². White: 82.2%.</td>
<td>Exclusion: unsatisfactory/incomplete data from dermatologic examination and 24 hour dietary recall.</td>
<td>Nutrient intake (sourced from 24h recall) expressed as daily totals.</td>
<td>Higher vitamin C lower likelihood of wrinkled appearance (OR 0.89), senile dryness (0.93). Higher linoleic acid intakes - lower likelihood ofsenile dryness and skin atrophy.</td>
</tr>
<tr>
<td>(Purba et al, 2001)</td>
<td>Cross-sectional</td>
<td>To determine whether food and nutrient intakes are correlated with skin wrinkling in a sun exposed site.</td>
<td>Australia Greece Sweden</td>
<td>NA</td>
<td>n=453</td>
<td>Male n=247, female n= 206. Age range: 70 -104 years. n=177 Greek born living in Melbourne. n=69 Greek living in Greece. n=48 Anglo-Celtic Australian. n=159 Swedish living in Sweden.</td>
<td>None</td>
<td>Validated semi FFQ with cultural-specific foods and dishes. Grouped into minor (55, 77, 43) and major food groups (10). Skin wrinkling: using cutaneous microtopic method to assess actinic damage.</td>
<td>Swedish had the least skin wrinkling in a sun-exposed site, less actinic skin damage with higher intake of vegetables (r=-0.249, p&lt;0.0001) fish, olive oil and legumes. High intakes of vegetables, legumes and olive oil protective against cutaneous actinic damage.</td>
</tr>
<tr>
<td>Author</td>
<td>Study Design</td>
<td>Objectives/Hypotheses</td>
<td>Setting</td>
<td>Duration</td>
<td>Sample Size</td>
<td>Participant Characteristics</td>
<td>Exclusion</td>
<td>Measures</td>
<td>Results</td>
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<tr>
<td>(George et al, 2005)</td>
<td>Cross-sectional</td>
<td>To evaluate compliance with dietary guidelines in late postpartum in low income women.</td>
<td>USA</td>
<td>NA</td>
<td>n=146 female. Inclusion: 18 years or older, white, African, American or Hispanic, parity ≤ 3, read, write and speak English, telephone access, family incomes ≤ 185% of poverty guidelines.</td>
<td>None</td>
<td>Dietary intake: validated semi-quantitative 195 FFQ. Dietary guidelines index compliance score obtained from FFQs, BMI at 1 year and physical activity. Body dissatisfaction: Body cathexis scale: 30 item validated instrument that measures.</td>
<td>Greater compliance with dietary guidelines was associated with lower perceived barriers to weight loss and less body image (p&lt;0.05).</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CHO, carbohydrates; NA, not applicable, OR, odds ratio
2.4.4 Effectiveness of dietary interventions on appearance

Study characteristics, critical appraisal and results of included experimental studies are summarised in Table 2.3, Table 2.4 and Table 2.5 respectively. The total number of participants across the 17 RCTs and 1 pre-post study was 997, of which at least 82% were females (13% unclear). The mean number of participants across all studies was 56 (range 10 - 127). The age range of participants was 18 to 77 years, with one study not reporting the age of participants. The duration of studies ranged from 7 days to a 2-year intervention (Janjua et al., 2009). Fourteen interventions ranged from 8 to 14 weeks, two less than 8 weeks, two were 24 weeks (Cho, Won, et al., 2009; Skovgaard, Jensen, & Sigler, 2006) and one 2 years (Janjua et al., 2009). Only one study followed up participants after the end of the intervention which was an initial 6-week intervention with a further 6-weeks follow-up (Micozzi, Brown, Taylor, & Wolfe, 1988). Retention rates varied from 64 to 100% at post-intervention, the majority (16 of 18) of interventions had a retention rate greater than 80%.

Studies were appraised for methodological quality with 14 assessed as being of “positive” and the remaining four of “neutral” quality. Twelve studies reported blinding. The source of funding was not stated in six studies. The majority of the studies (n=16) had comparable study groups, used appropriate statistics and measured outcomes in a valid and reliable method. Ten studies (55%) did not consider or mention potential limitations in their study.

Thirteen studies had 2 intervention arms (control/intervention) (Bouilly-Gauthier et al., 2010; Chiu et al., 2005; Cho, Choi, et al., 2009; Cho, Won, et al., 2009; Dayan, Arkins, Sharma, Paterson, & Barnes, 2011; Heinrich, Moore, De Spirt, Tronnier, & Stahl, 2011; Heinrich, Neukam, Tronnier, Sies, & Stahl, 2006; Janjua et al., 2009; Lattimore, Walton, Bartlett, Hackett, & Stevenson, 2010; Postaire, Jungmann, Bejot, Heinrich, & Tronnier, 1997; Segger & Schonlau, 2004; Skovgaard et al., 2006; Udompataikul, Sripiroj, & Palungwachira, 2009), 2 had one arm (Bouilly-Gauthier et al., 2010), 1 had three (Bacci et al., 2003) and 1 had six intervention arms (Micozzi et al., 1988). The majority of studies (15 out of 17)
evaluated the effect of various dietary supplements with several active ingredients including Polyphenols (Bacci et al., 2003; Skovgaard et al., 2006), Omega 3 fatty acids (Bacci et al., 2003; Dayan et al., 2011; Skovgaard et al., 2006), carotenoids (Stephen et al., 2011), (Dayan et al., 2011; Postaire et al., 1997), (Segger & Schonlau, 2004; Skovgaard et al., 2006), vitamins E (Bacci et al., 2003; Postaire et al., 1997; Segger & Schonlau, 2004; Skovgaard et al., 2006) and C (Postaire et al., 1997) (Segger & Schonlau, 2004; Skovgaard et al., 2006), Lactobacillus (La1) probiotics (Bouilly-Gauthier et al., 2010), green tea polyphenols (Chiu et al., 2005; Heinrich et al., 2011; Janjua et al., 2009), squalene (Cho, Choi, et al., 2009), red ginseng (Cho, Won, et al., 2009), and flavanol cocoa (Heinrich et al., 2006), whereas only one measured the effect derived from whole foods (Micozzi et al., 1988) which used foods high in β-carotene.

Appearance outcomes included facial wrinkles (n=9), skin roughness and elasticity (n=7), skin colour (n=6), cellulite (n=1), and body image (n=1). Ten studies used exclusively objective methods (Bouilly-Gauthier et al., 2010; Cho, Choi, et al., 2009; Heinrich et al., 2011; Heinrich et al., 2006; Micozzi et al., 1988; Postaire et al., 1997; Segger & Schonlau, 2004; Skovgaard et al., 2006; Stephen et al., 2011) to measure appearance outcomes whereas 8 studies used a combination of objective and subjective evaluations (clinician and patient) (Bacci et al., 2003; Bouilly-Gauthier et al., 2010; Chiu et al., 2005; Cho, Won, et al., 2009; Dayan et al., 2011; Janjua et al., 2009; Lattimore et al., 2010; Udompataikul et al., 2009).

### 2.4.5 Effectiveness of interventions

#### 2.4.5.1 Facial wrinkles

Five of the 9 studies that investigated the effect of dietary supplementation found significant improvements in facial wrinkles (periocular (Cho, Choi, et al., 2009; Cho, Won, et al., 2009; Dayan et al., 2011; Skovgaard et al., 2006; Udompataikul et al., 2009), forehead (Skovgaard et al., 2006) and perioral (Skovgaard et al., 2006) areas). These studies investigated various active ingredients and doses, including dietary supplements (n=3), (Dayan et al., 2011;
Skovgaard et al., 2006; Udompataikul et al., 2009) red ginseng root (n=1) (Cho, Won, et al., 2009) and squalene (n=1) (Cho, Choi, et al., 2009) and measured facial wrinkles using skin replicas (impressions using silicon) or skin vision meters.

Three studies (Chiu et al., 2005; Heinrich et al., 2011; Janjua et al., 2009), which evaluated the effects of green tea supplementation orally or topically, found no significant differences in facial wrinkling. Another study investigating the impact of a high-flavanol cocoa drink (Heinrich et al., 2006) found no significant changes in skin wrinkling after 12 weeks.

2.4.5.2 Skin roughness and elasticity

Of 7 studies that examined intakes of various supplements on skin structure and texture, 4 found significant improvements in skin ‘roughness’ (Heinrich et al., 2011; Heinrich et al., 2006; Segger & Schonlau, 2004; Udompataikul et al., 2009) and 3 in skin elasticity (Chiu et al., 2005; Heinrich et al., 2011; Segger & Schonlau, 2004). Supplement intake from green tea and dietary supplements (with a variety of active ingredients) improved both skin elasticity and roughness whilst the intake of high-flavanol cocoa improved skin roughness only (Heinrich et al., 2006).

2.4.5.3 Skin colour / photoprotection

One study (Postaire et al., 1997) examined the effects of 2 supplements containing various doses “high” (13mg/day of β-carotene, 2mg lycopene) versus “low” (3mg/day of β-carotene, 3mg lycopene) on the pigmentation of skin not exposed to UV light. Both supplements contained Vitamin C (30mg) and Vitamin E (5mg). Carotenodermia (yellowing of the skin) was only detectable with the higher dosage. A further study measured skin colour change after eight weeks of β-carotene (15mg/day) supplementation (Stephen et al., 2011) and found significant increases in b* values in five skin regions (Stephen et al., 2011).

One experimental study investigated the effects of following a diet high in carotenoids or use of β-carotene supplements on skin colour (Micozzi et al., 1988). This was measured by visual examination of the skin and fasting plasma
carotenoids. The subjects who consumed the 30mg purified β-carotene supplement daily for 42 days developed carotenodermia.

Three clinical trials (Bouilly-Gauthier et al., 2010) evaluated the effect of a dietary supplement containing lactobacillus (La1) and 7.2mg carotenoids (β-carotene and lycopene) on UVR exposed skin, using 3 levels of exposure; extreme (UV-SSR), moderate (UV-DL) and natural summer sunlight. Trial 1 assessed early markers of UVR-induced skin damage using histology and immunohistochemistry (Bouilly-Gauthier et al., 2010). In trial 2, a chromameter was used to evaluate skin colour changes, while in trial 3 both dermatologists and subjects completed assessment questionnaires on skin resistance to sun exposure (Bouilly-Gauthier et al., 2010). There were significant increases in minimal erythema dose (MED) (threshold required to produce sunburn) after 6 weeks of dietary supplement (DS) intake (P<0.05). Skin colour significantly increased after DS intake (P<0.05). In the third trial, dermatologists and subjects reported that dietary supplementation led to improvement in skin resistance to sun exposure (Bouilly-Gauthier et al., 2010). Although MED isn’t directly related to appearance, a reduced sensitivity to sunburn is likely to have long-term ramifications for skin appearance.

2.4.5.4 Cellulite

One double-blinded study investigated the effects of 2 different dietary supplements on cellulite appearance (Bacci et al., 2003). Supplement 1 contained polyphenols, fatty acids, vitamin E, and extracts of ginkgo, ruscus, melilotus and Centella asiatica whilst supplement 2 had similar components except for omission of the fatty acids and vitamin E. Both clinical (completed by physicians) and patient subjective evaluations indicated a significant improvement from the two products compared to the placebo group.

2.4.5.5 Body image

One study conducted by Lattimore et al (Lattimore et al., 2010) investigated perception of body image satisfaction after eating either a cereal or muffin
breakfast for 1 week. Those in the cereal breakfast group were significantly more satisfied with their body (P < 0.001) after eating breakfast than the muffin group.

2.4.6 Side effects/adverse events

Adverse events or side effects from supplement or placebo consumption were reported in 6 RCTs (Chiu et al., 2005; Cho, Choi, et al., 2009; Cho, Won, et al., 2009; Janjua et al., 2009; Segger & Schönlau, 2004; Skovgaard et al., 2006) including upper respiratory tract infection, loose stools or other minor gastrointestinal problems (Janjua et al., 2009). In 1 study, 55% of participants reported frequent incidence of loose stools (1-3 times daily) after consuming a high dose of squalene (27g/day) (Cho, Choi, et al., 2009). In another study by Segger and Schönlau (Segger & Schönlau, 2004), 4 of the 58 participants reported gastric discomfort after taking an oral supplement which contained Pycnogenol (10 mg), vitamin C (30 mg), vitamin E (5 mg), biotin (75 mg), selenium (25 mg), zinc (7.5 mg), bio-marine complex (50 mg), horsetail and dietary carotenoids (34 mg) and did not continue with the study. A further study (Janjua et al., 2009) reported side effects of loose stools and upper respiratory tract infection either after consuming 2 supplement tablets daily that included 250mg green tea polyphenols or from having the placebo capsules.
<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>Setting</th>
<th>Duration</th>
<th>Age (years)</th>
<th>BMI (kg/m²) Weight (kg)</th>
<th>Sample Size</th>
<th>Ethnicity</th>
<th>Smoking</th>
<th>Inclusion</th>
<th>Exclusion</th>
<th>Retention</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bacci et al, 2003)</td>
<td>Double Blinded</td>
<td>Italy</td>
<td>47 days</td>
<td>Mean: 31.6</td>
<td>Range: 18-45</td>
<td>n=127 NR</td>
<td>NR</td>
<td>NR</td>
<td>Cellulite ≥ 2 years.</td>
<td>BMI&gt;30kg/m², adipose cellulite, menopause/pre-menopause, sagging skin.</td>
<td>87.60%</td>
<td>Nil</td>
</tr>
<tr>
<td>Bouilly-Gauthier et al, 2010</td>
<td>Clinical Trial 1</td>
<td>France</td>
<td>14 weeks</td>
<td>Mean ± SD: 31± 3</td>
<td>NR</td>
<td>n=16 NR</td>
<td>NR</td>
<td>NR</td>
<td>Healthy women, abstain from dairy products during study period.</td>
<td>Pregnant, breastfeeding, supplement/ vitamin intake and particular diet.</td>
<td>100%</td>
<td>Nil</td>
</tr>
<tr>
<td>Bouilly-Gauthier et al, 2010</td>
<td>Clinical Trial 2</td>
<td>France</td>
<td>68 days</td>
<td>Mean ± SD: 34 7</td>
<td>DS Group</td>
<td>n=43 NR</td>
<td>NR</td>
<td>NR</td>
<td>Healthy women, abstain from dairy products during study period.</td>
<td>Pregnant, breastfeeding, supplement/ vitamin intake and particular diet.</td>
<td>100%</td>
<td>Nil</td>
</tr>
<tr>
<td>Bouilly-Gauthier et al, 2010</td>
<td>Clinical Trial 3</td>
<td>France</td>
<td>6-8 weeks</td>
<td>Mean ± SD: 42 ± 12</td>
<td>NR</td>
<td>n=80 NR</td>
<td>NR</td>
<td>NR</td>
<td>Nil, not to change habits of sunbathing or sunscreen use.</td>
<td>Pregnant, breastfeeding, supplement/ vitamin intake, hx of skin cancer.</td>
<td>100%</td>
<td>Nil</td>
</tr>
<tr>
<td>Chiu et al, 2005</td>
<td>Double Blinded</td>
<td>USA</td>
<td>8 weeks</td>
<td>NR</td>
<td>NR</td>
<td>n=37 NR</td>
<td>NR</td>
<td>NR</td>
<td>Moderate photageing Fitzpatrick skin phototypes I – III.</td>
<td>Steroid or retinoid use, change in HRT &lt; 6mo.</td>
<td>92.50%</td>
<td>Nil</td>
</tr>
<tr>
<td>Cho et al, 2009</td>
<td>RCT</td>
<td>Korea</td>
<td>90 days</td>
<td>Mean: 57.8</td>
<td>Range: 50-72</td>
<td>n=37 NR</td>
<td>NR</td>
<td>NR</td>
<td>Corticosteroid or retinoid use 2 weeks prior study.</td>
<td>Age &lt;40y, pregnancy, lactation, infectious skin disorder on face, atopic dermatitis, photallergic or photosensitive skin, renal or chronic diseases.</td>
<td>92.50%</td>
<td>Nil</td>
</tr>
<tr>
<td>Cho et al, 2009</td>
<td>Double Blinded</td>
<td>Korea</td>
<td>24 weeks</td>
<td>Mean: 51.9</td>
<td>Range: 40-70</td>
<td>n=82 NR</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
<td>95.30%</td>
<td>Nil</td>
</tr>
<tr>
<td>Author</td>
<td>Study Design</td>
<td>Setting</td>
<td>Duration</td>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
<td>Sample Size</td>
<td>Ethnicity</td>
<td>Smoking</td>
<td>Inclusion</td>
<td>Exclusion</td>
<td>Retention</td>
<td>Follow up</td>
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<tr>
<td>(Dayan et al, 2011)</td>
<td>Double Blinded</td>
<td>USA</td>
<td>270 days</td>
<td>Mean ± SD:</td>
<td>NR</td>
<td>n=76</td>
<td>Caucasian (67%),</td>
<td>NR</td>
<td>NR</td>
<td>Chronic skin disease, tobacco smoker, vitamin</td>
<td>64%</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Placebo RCT</td>
<td></td>
<td></td>
<td>52 ± 12</td>
<td>Range: 30-77</td>
<td>Female n= 61 (80%)</td>
<td></td>
<td></td>
<td></td>
<td>supplement use within 30 days.</td>
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<td></td>
<td></td>
<td></td>
<td>Males n=15 (20%)</td>
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</tr>
<tr>
<td>(Heinrich et al, 2011)</td>
<td>Double Blinded</td>
<td>Germany</td>
<td>12 weeks</td>
<td>Range: 18-56</td>
<td>NR</td>
<td>n=24</td>
<td>Fitzpatrick skin</td>
<td>NR</td>
<td>NR</td>
<td>Pregnant, breastfeeding, smokers, medication</td>
<td>100%</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Placebo RCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female n= 24 (100%)</td>
<td></td>
<td></td>
<td></td>
<td>use sunbathing/ sunbed use.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Heinrich et al, 2006)</td>
<td>Double Blinded</td>
<td>Germany</td>
<td>12 weeks</td>
<td>Range: 18-56</td>
<td>NR</td>
<td>n=60</td>
<td>Fitzpatrick skin</td>
<td>NR</td>
<td>NR</td>
<td>Pregnant, breastfeeding, smokers, medications,</td>
<td>100%</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Placebo RCT</td>
<td></td>
<td></td>
<td>BMI: 18-25</td>
<td></td>
<td>Female n=60 (100%)</td>
<td></td>
<td></td>
<td></td>
<td>sunbathing/ sunbed use. Vitamin/ supplements</td>
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<td>kg/m²</td>
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<td></td>
<td>use.</td>
<td></td>
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<tr>
<td>(Janjua et al, 2009)</td>
<td>Double Blinded</td>
<td>USA</td>
<td>2 years</td>
<td>Range: 25-75</td>
<td>Healthy</td>
<td>n=34</td>
<td>Moderate photoageing</td>
<td>NR</td>
<td>NR</td>
<td>Systemic retinoid use 6 weeks prior, active</td>
<td>60.70%</td>
<td>Nil</td>
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<td></td>
<td>RCT</td>
<td></td>
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<td></td>
<td>Female n=34 (100%)</td>
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<td>facial dermatologic conditions, hx facial</td>
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<td>cosmetic procedure.</td>
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<tr>
<td>(Lattimore et al, 2010)</td>
<td>RCT</td>
<td>North West England</td>
<td>7 days</td>
<td>Range: 20-40</td>
<td>BMI Range:</td>
<td>n=123</td>
<td>Fitzpatrick skin</td>
<td>NR</td>
<td>NR</td>
<td>Food allergies/ gluten intolerance, current/</td>
<td>95.30%</td>
<td>Nil</td>
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<td></td>
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<td></td>
<td></td>
<td>BMI: 19.5-29.5</td>
<td></td>
<td>Female n=123 (100%)</td>
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<td>past hx eating /mental health disorders,</td>
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<td>kg/m²</td>
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<td></td>
<td>pregnant, diabetic, anti-depressants or wt</td>
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<td>loss medication.</td>
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<tr>
<td>Author</td>
<td>Study Design</td>
<td>Setting</td>
<td>Duration</td>
<td>Age (years)</td>
<td>BMI (kg/m2)</td>
<td>Sample Size</td>
<td>Ethnicity</td>
<td>Smoking</td>
<td>Inclusion</td>
<td>Exclusion</td>
<td>Retention</td>
<td>Follow up</td>
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<tr>
<td>(Micozzi et al., 1988)</td>
<td>RCT USA</td>
<td>12 weeks</td>
<td>Range: 20-45</td>
<td>Wt range: 64.1-92.7</td>
<td>n=30</td>
<td>NR</td>
<td>NR</td>
<td>No hx of chronic disease, non-smokers, within 10% of IBW, no unusual dietary patterns</td>
<td>None</td>
<td>100%</td>
<td>6 weeks</td>
<td></td>
</tr>
<tr>
<td>(Postaire et al., 1997)</td>
<td>Double Blinded RCT Germany</td>
<td>8 weeks</td>
<td>Mean ± SD: 32.4±7.5</td>
<td>Range: 22-46</td>
<td>n=20</td>
<td>Caucasian</td>
<td>NR</td>
<td>Healthy, skin phototype II</td>
<td>ds or drug study within previous 3 months, malabsorption problems, liver disease, obese, smokers, &gt;30g alcohol/day 15 days prior, ds use.</td>
<td>100%</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>(Segger et al., 2004)</td>
<td>Double Blinded RCT Germany</td>
<td>12 weeks</td>
<td>Range: 45-75</td>
<td>NR</td>
<td>n=58</td>
<td>NR</td>
<td>NR</td>
<td>Fitzpatrick skin, phototypes I- IV, Free of skin disease, warts, scabs little/no hair, no tattoos.</td>
<td>Chronic disease, pregnancy, breastfeeding.</td>
<td>93.50%</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Study Design</td>
<td>Setting</td>
<td>Duration</td>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
<td>Sample Size</td>
<td>Ethnicity</td>
<td>Smoking</td>
<td>Inclusion</td>
<td>Exclusion</td>
<td>Retention</td>
<td>Follow up</td>
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<tr>
<td>(Skovgaard et al, 2006)</td>
<td>Double blinded Placebo RCT</td>
<td>USA</td>
<td>6 months</td>
<td>Range: 45-65</td>
<td>NR</td>
<td>n=80 Female n=80 (100%)</td>
<td>NR</td>
<td>NR</td>
<td>1-5y post-menopause, Fitzpatrick skin type II-III, BMI 20-30kg/m², &lt;10 cigarettes/day avoided excessive sun/sun bed exposure</td>
<td>Skin/mental or uncontrolled metabolic disease, hx: breast/ovarian cancer, gastro-intestinal disease, impaired circulation, allergy or sensitivity to seafood or soy, prescription drug use for improving skin appearance, ds use of oral vitamin/nutritional supplement equal to &gt;1 multivitamin.</td>
<td>80%</td>
<td>Nil</td>
</tr>
<tr>
<td>(Stephen et al, 2011)</td>
<td>Pre and post</td>
<td>Scotland</td>
<td>8 weeks</td>
<td>Range: 19-22</td>
<td>NR</td>
<td>n=10 Male n= 2, Female n= 8</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>100%</td>
<td>Nil</td>
</tr>
<tr>
<td>(Udompataikul et al, 2009)</td>
<td>Double Blinded placebo RCT</td>
<td>Thailand</td>
<td>12 weeks</td>
<td>Range: 35-60</td>
<td>NR</td>
<td>n=60 Female n=60 (100%)</td>
<td>Thai</td>
<td>NR</td>
<td>No daily sun exposure, non-smoker, no hx of allergy to seafood, coenzyme Q10, vitamin E, green tea, grape seed extract, French maritime pine bark extract, β-carotene, selenium or zinc.</td>
<td>Received anti-ageing interventions &gt;3 months prior to consuming or antiageing compounds (e.g. vitamin E or C) within 3mo of study, oral retinoid &lt;6mo pre study, or applied topical AHA, BHA, retinoid, retinol, vitamin E, C or any other compounds that may interfere &lt;3mo pre study, pregnant or lactating.</td>
<td>100%</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Abbreviations: wt, weight; hx, history; NA, not applicable; HRT, hormone replacement therapy; IBW, ideal body weight
<table>
<thead>
<tr>
<th>Author</th>
<th>Research question/ Hypothesis</th>
<th>Intervention</th>
<th>Outcome and types of measures</th>
<th>Results</th>
<th>Significance difference between groups or changes in outcomes</th>
<th>Side effects</th>
</tr>
</thead>
</table>
| (Bacci et al, 2003)         | Evaluate the effect of two different dietary supplement/formulae on cellulite.                | Group A: supplement containing polyphenols (bioflavonoids), fatty acids (EPA, DHA, γ-linoleic acid), vitamin E, ginkgo biloba, ruscus, melilotus and centella.  
Group B: placebo containing inert substances (natural fibers and soya oil).  
Group C: cellulase Gold supplement containing polyphenols (bioflavonoids), Recaptacell, ginkgo biloba, ruscus, melilotus, centella and fucus. | Cellulite improvement: Clinical (Score 2 to 8) and self-assessment (scale 0-10) questionnaires.                                         | Higher scores given by treating physicians in for clinical and cosmetic appearance of participants post intervention for groups A and C (p<0.001). Overall improved subject improvement in groups A & C (p<0.001). | Y between groups                                                                                                                                  | NR           |
| (Bouilly-Gauthier et al, 2010) | To assess the effects of dietary supplements containing La1 and nutritional doses of carotenoids on early UV induced skin damage. | CT1: 18 day exposure to minimal erythema dose (0.75 MED) of UV daylight (UVA/UVB ration = 24) pre and post receiving supplement.  
Dietary supplement contained 5x10⁸ La1 (probiotic) and 7.2mg carotenoids (β-carotene & lycopene).  
Supplements were consumed daily for 6 weeks pre-UVR exposure. | Cell skin density skin condition: Biopsy sample taken from exposed and unexposed buttock areas before and after supplementation.  
Melanin: Fontanna masson stain used to revealed intraepidermal melanin. | After supplementation the melanin density was significantly lower p<0.05. | Y change in outcome                                                                                                                              | NR           |
<table>
<thead>
<tr>
<th>Author</th>
<th>Research question/ Hypothesis</th>
<th>Intervention</th>
<th>Outcome and types of measures</th>
<th>Results</th>
<th>Significance difference between groups or changes in outcomes Y/N</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouilly-Gauthier et al, 2010</td>
<td>To assess the effects of dietary supplements containing La1 and nutritional doses of carotenoids on early UV induced skin damage.</td>
<td>CT2: 4 day exposure to 0.9 MED UV-SSR (UVA/UVB ratio=10) and 10 day skin colour evaluation pre and post dietary Dietary supplement contained 5x10^8 La1 (probiotic) and 7.2mg carotenoids (β-carotene &amp; lycopene). Placebo: maltodextrin. Supplements were consumed daily for 6 weeks pre-UVR exposure.</td>
<td>Skin colour: Minolta Chromameter before and after supplementation. MED: Clinical evaluation and Chromameter.</td>
<td>Increase in MED (+19%, p&lt;0.05) after DS intake while no change in placebo. Clinical determination (+20 % p&lt;0.05). Colour difference Δ E* - an increase after DS intake but no change for placebo (p&lt;0.05).</td>
<td>Y between groups</td>
<td>Side effects: NR</td>
</tr>
<tr>
<td>Bouilly-Gauthier et al, 2010</td>
<td>To assess the effects of dietary supplements containing La1 and nutritional doses of carotenoids on early UV induced skin damage.</td>
<td>CT3: natural sunlight exposure during summer holidays (ax 3-4wk pre and post-holiday). Dietary supplement contained 5x10^8 La1 (probiotic) and 7.2mg carotenoids (β-carotene &amp; lycopene). Supplements consumed daily for 3-4 weeks pre-UVR exposure.</td>
<td>Skin resistance to sun exposure: Questionnaires on skin exposure to sun exposure one by subject &amp; the other by dermatologist.</td>
<td>Dermatologists reported DS prevented sunburn, sun intolerances and appearance of sunspots in participants that usually experience these phenomena. Participants noticed improved skin colour (intensity, evenness) and better skin condition.</td>
<td>Y change in outcomes</td>
<td>Side effects: NR</td>
</tr>
<tr>
<td>Chiu et al, 2005</td>
<td>Investigate oral and topical green tea supplementation and their possible effects on ageing skin.</td>
<td>Green tea treatment apply 10% green tea extract cream 2 x daily to face &amp; arms, 300mg green tea oral sup 2 x daily. Placebo group: placebo cream 2 x daily to face &amp; arms, placebo oral sup 2 x daily.</td>
<td>Facial skin (wrinkles, roughness, dryness, overall), clinical assessment and histologic grading of skin biopsies. Self-assessment on facial skin (scale 0 to 5).</td>
<td>No significant difference clinical grading between groups. Histologic grading of skin biopsies showed significant improvements in elastic tissues in treatment group (p &lt;0.05). Significant differences between groups, placebo group rating have less dry skin (p&lt;0.01) and superior overall appearance (p&lt;0.05).</td>
<td>N between groups and clinical grading Y between groups histologic grading s</td>
<td>Side effects: skin irritation from topical</td>
</tr>
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<tr>
<td>Cho et al, 2009</td>
<td>Investigate if squalene supplementation improves signs of photo ageing in human skin.</td>
<td>High dose squalene: 27g/day Low dose: 13.5g/day</td>
<td>Facial wrinkles, measured by Skin replica and Visionmeter. Facial erythema and pigmentation, measured by Derma Spectrometer.</td>
<td>Facial wrinkles decreased in both groups, however in high dose group all R values significantly decreased after 90 days supplementation (p&lt;0.05). Melanin indices increased and erythema indices decreased from the cheek area in both groups post squalene supplementation (p&lt;0.05)</td>
<td>Y between groups</td>
<td>Side effects: observed loose stool/diarrhoea 35%, 55% participants low and high dose respectively, High blood cholesterol for 3 participants</td>
</tr>
<tr>
<td>Cho et al, 2009</td>
<td>Investigate whether repeat oral administration of red ginseng extracts and herbal mixtures can reduce wrinkly formation.</td>
<td>Supplement: 3g/day (10 capsules) of KTNG0345 (45.3% Korean red gingseng extract &amp; 54.6% powder extract of torilus fructus &amp; corni fructus). Placebo: 226.5mg dextrin and 70mg caramel colour. FFQ 25 specific foods, baseline, 12 and 24 weeks.</td>
<td>Facial wrinkles (skin replicas and skin visiometer). Elasticity, measured by Cutometer. Facial erythema and pigmentation, measured by Derma Spectrometer on face cheek. Clinical assessment of facial wrinkles, elasticity and pigmentation from both investigators and subjects (scale).</td>
<td>Facial wrinkles R1 and R5 values significantly improved after 12 (14.7%, 19.0% respectively) and 24 weeks (14.1%, 23.4%). No significant differences were found between groups for elasticity and facial pigmations. No significant difference in responses on improvements (as per subjects and dermatologists at 12/24 weeks).</td>
<td>Y between groups</td>
<td>N between groups</td>
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<td>(Dayan et al, 2011)</td>
<td>To determine the effect of Skin Health Experimental Product (SHEP) on skin health.</td>
<td>Active Treatment: SHEP supplement food-based mixture of ascorbic acid, omega-3 fatty acids, mixed carotenoids, zinc rice chelate, lutein, pyridoxine, pantothenate, niacin, choline and coenzyme Q10. Control: identical appearing capsule.</td>
<td>Skin Profilometry: Major and minor skin lines measured by topographical analysis using silicon profilometry of the periorbital skin (SkinReplica) obtained at day 60, 90, 180 and 270. Skin Carotenoid concentration: measured by Resonance Raman Spectroscopy at 270 days of subjects stratum corneum obtained from palm and four interdigital webs of dominant hand. Self-image: Global Aesthetic Improvement Scale (GAIS) performed at 60, 90, 180 and 270 days.</td>
<td>Significant reduction in fine lines (p=0.0194) for active treatment group. Carotenoids were detectable at 2 peaks which correspond to the 2 strongest found in the skin (1159 and 1524 cm-1) Site 1 to Site 5. All significant except site 3 (third and ring finger) and 5 (palm). More subjects in the placebo group responded &quot;worse&quot; to facial changes post intervention (p=0.019). Active treatment subjects more likely to experience subjective improvement to facial comparisons post intervention (p=0.04).</td>
<td>Y between groups</td>
<td>Side effects: NR</td>
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<tr>
<td>(Heinrich et al, 2006)</td>
<td>To investigate the effects of intake of product rich in cocoa flavanols on skin sensitivity toward UV exposure.</td>
<td>HF group (n=12): cocoa beverage - 329mg of total cocoa flavonols (TCF). LF group (n=12): nutrient matched cocoa beverage -27mg of TCF. Consumed 1 x daily with morning meal for 12 weeks. Advised not to change dietary habits and no supps (vitamins/ polyphenols).</td>
<td>Sensitivity towards UV radiation (baseline, 6 12, skin colour was measured before and 24hours after irradiation) by chromametry. Skin structure and texture measured with high-frequency ultrasound B-scan with 2-D-configuration. Skin hydration by chromametry.</td>
<td>a* values, significant ↓ in HF group by 15 and 25%, no change in LF Group mod but significant ↑ HF Group density and thickness of skin p&lt;0.05. Moderate but significant ↑ HF Group density and thickness of skin p&lt;0.05. Skin surface profiles evaluated by SEL method; roughness, scaling, smoothness and wrinkles: significant ↓ in skin roughness and scaling for HF Group. Skin hydration significantly ↑ in HF Group.</td>
<td>Y between groups</td>
<td>Side effects: NR</td>
</tr>
<tr>
<td>(Heinrich et al, 2011)</td>
<td>To investigate the effects of repetitive intakes of a beverage enriched with green tea polyphenols on skin sensitivity toward UV exposure, skin structure and texture.</td>
<td>Green Tea group consumed 1L of green tea beverage each day (1402mg total tea catechins). Control group: ingested 1L of a constituent matched beverage with a replicate taste daily.</td>
<td>Sensitivity towards UV radiation (baseline, 6 12, skin colour was measured before and 24hours after irradiation) by chromametry. Skin elasticity based on suction method using Cutometer SEM 474 on inner forearm. Skin structure and texture measured with high-frequency ultrasound B-scan with 2-D-configuration. Skin surface (photos) was characterised by roughness, scaling, volume and wrinkles.</td>
<td>a*values ↓ 16-25% (6 wk. 12wk) in GT Group indicating improved photo protection (p &lt;0.05). Viscoelasticity ↓ by 21% 12 wks for GT group (p &lt;0.05). Skin density ↑ by 7.7% after 12 wks (p &lt;0.05). Wrinkles↓ by wk 12 p&lt;0.05 (p &lt;0.05).</td>
<td>Y between groups</td>
<td>Side effects: NR</td>
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<tr>
<td>(Janjua et al, 2009)</td>
<td>To evaluate the long term effects of oral green tea polyphenols on photo ageing skin.</td>
<td>Green tea: one capsule 2 x daily (contained 250mg polyphenols). Placebo group: placebo capsule 2 x daily.</td>
<td>Sun exposed arm skin: by histological assessment of arm skin samples by blinded dermatologists. Facial skin: clinical assessment using digital left sided facial photographs (wrinkles, hyperpigmentation, pore size, roughness and overall assessment of solar damage) scaled by a blinded dermatologist.</td>
<td>No significant differences between groups at 24 mo Overall solar damage was reduced from baseline to 6 mo in green tea group (p=0.02). No significant differences in any time points for hyperpigmentation, poor size, roughness or wrinkling between groups.</td>
<td>N between groups</td>
<td>Side effects: Adverse events upper respiratory tract infection (7 events in green tea group, 4 in placebo). Loose stools (5 in tea group, 3 in placebo).</td>
</tr>
<tr>
<td>(Lattimore et al, 2010)</td>
<td>To investigate the effect of consuming isocaloric breakfasts appearing in different in calorie content on appetite, mood and body image satisfaction.</td>
<td>Muffin breakfast: 1 commercially produced chocolate chip muffin and a pure apple juice for 7 days. Cereal breakfast: 392kcal with portion of breakfast cereal, semi-skimmed milk, whole meal bread toasted with low fat spread, and pure apple juice for 7 days.</td>
<td>Appetite, mood, body-image satisfaction, by a diary using Visual Analogue scale (VAS).</td>
<td>Cereal breakfast gave participants higher body and weight satisfaction than the muffin breakfast.</td>
<td>Y between groups</td>
<td>Side effects: NR</td>
</tr>
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</table>
| (Micozzi et al, 1988) | To evaluate the relation between plasma carotenoid levels and carotenodermia receiving supplementation for 42 days. | 30mg carotenoid:  
1. Purified β-carotene capsule  
2. Carrots - 272g  
12mg carotenoid:  
3. Purified β-carotene capsule  
4. Tomato Juice - 180g  
6mg carotenoid:  
5. Broccoli - 300g  
6. Placebo - BHA, BHT and sodium benzoate capsule  
Strictly controlled diet (3000kcal with constant, low carotenoid content 0.5-1.6mg/day). | Carotenodermia: blood carotenoids samples using HPLC.  
Plus physical examination of skin (zygomatic prominence, hands, elbows, knees and feet). | Group 1 (30mg supplement) was the only group to experience unequivocal carotenodermia with presences first noted day 25 of tx - 7d post supplementation, and remained yellow >14 days. | Y between groups | NR |
| (Postaire et al, 1997) | The role of antioxidant nutrient intakes on pigmentation of the skin without any UV exposure. | Supplement 1 (B13/L2): 2 capsules containing 5mg natural tocopherol, 30mg ascorbic acid, 13mg β-carotene and 2mg lycopene.  
Supplement 2 (B3/L3): 2 capsules containing 5mg natural tocopherol, 30mg ascorbic acid, 3mg β-carotene, 3mg lycopene. | Skin colour: Chromater CR200 for L"a"*B" values.  
Melanin and carotene concentration, multiple reflection Spectrophotometry (Multiscan -OS10) at selective sites. | No significant influence on skin colour in subjects consuming B3/L3 dosage. Only differences in subjects with B13/L2 dosage occurred with b-values on forehead (1.08) and inside of hand (2.27) as compared to baseline means (b-values represent yellow components of the skin).  
Increases in carotene concentration with B13/l2 group, melanin increases in both groups. | Y between groups and outcomes | NR |
<table>
<thead>
<tr>
<th>Author</th>
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<th>Significance difference between groups or changes in outcomes Y/N</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Segger et al, 2004</td>
<td>To investigate whether supplementation with Evelle, may improve skin roughness and elasticity in women aged 45 years &gt; over.</td>
<td>Verum group: two Evelle tables 2 x day (Vit C, E carotenoids, Se, Zn, amino acids, blueberry extract and pycnogenol). Placebo group: placebo tables identical with none of the above ingredients 2 x daily.</td>
<td>Skin elasticity using Cutometer. Skin roughness: 3D Microtopography imaging system.</td>
<td>Skin elasticity significantly ↑ by 9% after 6 weeks in the treatment group (p=0.0351). Roughness significantly ↓ by 6% in the treatment group after 12 weeks (p=0.0157).</td>
<td>Y between groups</td>
<td>Side effects: gastric discomfort from 4 subjects</td>
</tr>
<tr>
<td>Skovgaard et al, 2006</td>
<td>To quantify the effects on skin in post-menopausal women with a novel dietary supplement.</td>
<td>Active treatment: supplement containing 350mg soy extract, 210mg biomarine complex (fish protein polysaccharides), 118.7mg ViTea, 27.5mg grape seed extract, 28.8mg tomato extract, 60mg vitamin C, 10mg vitamin E, 5mg zinc, and 100mg chamomile (in evening tablets only). Placebo: 1115mg maltodextrin, 266mg starch, 95.8mg burnt sugar, 30.4mg silicon dioxide, 11.4mg magnesium stearate and 0.76mg riboflavin.</td>
<td>Clinical grading of skin (0−9 scale) and photo evaluation of wrinkles. Skin denisty using DUBplus 20 of crows foot area. Skin elasticity Cutometer SEM 575.</td>
<td>Significant improvements on skin grading (p&lt;0.05) compare to placebo for - wrinkles, hyperpigmentation, crepyness overall appearance (6mo) - decollete crepyness (2, 3, 6mo) and overall appearance (3, 6mo) - hand crepyness (3, 6mo), mottled hyperpigmentation (3mo) Significant improvement on the face after 3 and 6 months from photo evaluation (p&lt;0.05). Significant differences in skin density between groups at 6 months p&lt;0.05. No significant difference for skin elasticity.</td>
<td>Y/N between groups</td>
<td>Side effects: adverse event swollen tongue from one participant in active treatment group</td>
</tr>
<tr>
<td>Stephen et al, 2011</td>
<td>Establish carotenoid-skin colour relationship in response to dietary supplementation with carotenoids.</td>
<td>Beta-carotene supplementation 15mg/day.</td>
<td>Skin colour: using Spectrophotometer (CIE Lab values) in six location sites.</td>
<td>Significant increases of skin b*values in five of the six locations.</td>
<td>Y in outcome</td>
<td>Side effects: NR</td>
</tr>
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<tr>
<td>(Udompataikul et al, 2009)</td>
<td>To evaluate the potential benefits of an oral nutraceutical on cutaneous ageing.</td>
<td>Active treatment group: nutraceutical containing coenzyme Q10, antioxidants (beta-carotene, D-alpha-tocopheryl-acetate, grape seed extract, green tea extract, selenium, zinc and GAGs). Placebo: identical capsules without active ingredients.</td>
<td>Skin ageing: assessed by 3 dermatologists at baseline, 4, 8 and 12 wks of tx evaluation using a Visiometer SV600. Patient Satisfaction Questionnaire of tx - decrease &amp; homogeneity of skin colour, reduction in pore size, skin roughness and wrinkles.</td>
<td>Statistically significant reductions occurred in the active treatment group at week 4 (p = 0.001), 8 (p = 0.000) and 12 (p = 0.000) [21.2% improvement in comparison to 1.7% in control] No significant difference in satisfaction regarding pigmentation change, however significant differences existed in the level of satisfaction on reduction of pore size, skin roughness and fine wrinkles.</td>
<td>Y/N between groups</td>
<td>NR</td>
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</table>

**Abbreviations:** HPLC, high performance liquid chromatography; tx, treatment; Y, yes; N, no; ▲ increase ▼ decrease; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole
Table 2.5 Methodological quality of included studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>1. Was the research question clearly stated?</th>
<th>2. Was the selection of study subjects/patients free from bias?</th>
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<th>4. Was method of handling withdrawals described?</th>
<th>5. Was blinding used to prevent introduction of bias?</th>
<th>6. Were intervention/therapeutic regimens/exposure factor or procedure described in detail?</th>
<th>7. Were outcomes clearly defined and the measurements valid and reliable?</th>
<th>8. Was the statistical analysis appropriate for the study design and type of outcome indicators?</th>
<th>9. Were conclusions supported by results with biases and limitations taken into consideration?</th>
<th>10. Is bias due to study's funding or sponsorship unlikely?</th>
<th>Overall Quality*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Purba et al, 2001)</td>
<td>Cross-sectional</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>P</td>
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<tr>
<td>(Cosgrove et al, 2007)</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
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<td>Cross-sectional</td>
<td>Y</td>
<td>Y</td>
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<td>P</td>
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<tr>
<td>(Whitehead et al, 2012)</td>
<td>Prospective Cohort</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
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<td>P</td>
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<tr>
<td>(George et al, 2005)</td>
<td>Cross-sectional</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>(Bacci et al, 2003)</td>
<td>Double Blinded RCT</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
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<td>(Bouilly-Gauthier et al, 2010)</td>
<td>Clinical Trial 1</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
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<tr>
<td>(Chiu et al, 2005)</td>
<td>Double Blinded Placebo RCT pilot study</td>
<td>Y</td>
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<td>Y</td>
<td>N</td>
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<tr>
<td>(Cho et al, 2008)</td>
<td>RCT</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
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<tr>
<td>(Cho et al, 2009)</td>
<td>Double Blinded, Placebo RCT</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>(Dayan et al, 2011)</td>
<td>Double Blinded Placebo RCT</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>(Heinrich et al, 2011)</td>
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<td>Double Blinded RCT</td>
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<td>2. Was the selection of study subjects/patients free from bias?</td>
<td>3. Were study groups comparable?</td>
<td>4. Was method of handling withdrawals described?</td>
<td>5. Was blinding used to prevent introduction of bias?</td>
<td>6. Were intervention/therapeutic regimens/exposure factor or procedure described in detail?</td>
<td>7. Were outcomes clearly defined and the measurements valid and reliable?</td>
<td>8. Was the statistical analysis appropriate for the study design and type of outcome indicators?</td>
<td>9. Were conclusions supported by results with biases and limitations taken into consideration?</td>
<td>10. Is bias due to study’s funding or sponsorship unlikely?</td>
<td>Overall Quality*</td>
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<tr>
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<td>Pre–post study</td>
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<td>N</td>
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<td>Y</td>
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<td>Double Blinded</td>
<td>Y</td>
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<td>Y</td>
<td>P</td>
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</table>

Abbreviations: Y, Yes; N, No; P, Positive; n, neutral

* Studies were appraised as ‘positive’ if they answered “yes” (including criteria 2,3,6,7) and at least one additional “Yes”
2.5 Discussion

The current review identified 27 studies, 9 of which were observational studies that examined the relationship between dietary intake and appearance and 18 were studies that evaluated the impact of a dietary intervention on appearance. Observational studies indicated that there was a significant association between various aspects of dietary intake and skin colouration and skin ageing. The majority (n=15) of the dietary intervention studies were of positive methodological quality and found significant improvements in at least 1 actual or perceived appearance-related outcome following dietary intervention predominantly from supplement intake (n=15) or actual foodstuffs (n=1), with facial wrinkling, skin elasticity, roughness and skin colour being the outcomes most commonly evaluated and reported.

2.5.1 Associations between dietary intake and appearance

Two cross-sectional studies (Stephen et al., 2011; Whitehead, Re, et al., 2012) reported significant associations between skin yellowness and fruit and vegetable intake. They also showed that changes in skin yellowness caused by modest improvements in fruit and vegetable intake are perceived to improve the appearance of health and attractiveness in white persons. However, the studies used a self-reported validated questionnaire to determine fruit and vegetable intake, which is based on an estimate of participants’ intake rather than participants’ actual intake. In addition, the sample sizes in the studies were moderate (range, n=35 - 82) which could affect the generalisability of the results. The included participants were primarily white, further limiting external validity.

Two studies (Cosgrove et al., 2007; Purba et al., 2001) found an inverse association between higher dietary intakes of fruits and vegetables (which were self reported) and skin ageing appearance, independent of factors known to affect skin ageing such as smoking, age, sun exposure and race.
All case studies reported change in skin colour after consuming carotenoid rich foods and supplements containing β-carotene. However, the amounts consumed (which was self-reported) from both foods and supplementation was largely outside the range of usual consumption, in particular, supplement intakes were 50 times above the normal daily intake of 87μg/dl for β-carotene (Takita et al., 2006). Once consumption ceased from both the food and supplements, the skin returned to its normal colour within a time frame of 6 weeks to 6 months. This was also confirmed by reduced plasma concentrations of lycopene (Caroselli et al., 2007) or carotene (Vakil et al., 1985).

Based on the 9 observational studies there is some evidence to support that there is a relationship between dietary intake and appearance. However, the numbers of studies are limited and each study has used different methodologies to assess appearance outcomes and dietary intakes. In addition, as the majority of the observational studies were cross-sectional, temporality of associations is unable to be determined. Cross-sectional studies however can be informative for future longitudinal studies examining relationships between dietary intake and appearance.

2.5.2 Effectiveness of interventions

Almost all intervention studies (n=14) evaluated the effects of a dietary supplement on skin appearance in the context of skin ageing or UV damage. It is not surprising that many of the retrieved studies focus on skin as this organ plays a major role in physical appearance, with its functioning and attractiveness known to be dependent on nutrition, particularly the intake of antioxidant vitamins, minerals and essential fatty acids (Boelsma, Hendriks, & Roza, 2001).

Despite the lack of homogeneity across the studies in terms of the types of supplements used (individual supplements or complex mixtures), the majority were shown to have beneficial impacts on skin health and appearance in both the short and long term. Overall, we can conclude that supplements are effective in optimising appearance of skin ageing and protection against UV damage. However, as the interventions assessed here did not report follow-up results and
varied in sample size, study duration and appearance-related outcomes it is not possible to determine the optimum dosage or specific active ingredients required to improve skin health and appearance.

Only 1 experimental study examined the effects of dietary intake of foods containing carotenoids on skin colour and found that the participants consuming foods high in carotenoids did not develop carotenodermia and had lower plasma total carotenoid levels compared to those who consumed β-carotene supplements. However, this was conducted more than 25 years ago (Micozzi et al., 1988) and was a small sample of men. Therefore we cannot determine the actual impact of whole food intake on appearance. Further studies are needed to evaluate the effectiveness of food on appearance.

### 2.5.3 Study quality

The overall quality of the included studies was moderate. Twelve of the RCT’s were of moderate quality, 4 were classified as weak and there was only 1 strong-quality RCT that met all criteria. Risk of bias was reasonably low in most studies, with the majority receiving a “positive” classification. However, approximately half of the studies did not report their funding source, making it difficult to ascertain potential bias secondary to this. The studies that did report funding (n=8) were supported by skin, beauty, food or supplement corporations; and this could be seen as a conflict of interest potentially affecting reporting bias. Two studies (Chiu et al., 2005; Janjua et al., 2009) that examined the effects of green tea extracts or oral green tea on photoageing of skin were funded by a company that produces antiageing products. However, there were no significant improvements in the long-term skin status reported. Fourteen of the RCTs did not report handling of participant withdrawals. The clinical assessments and histologic grading of participants’ skin conditions in a number of studies were conducted by qualified dermatologists who were blinded to treatment allocation therefore reducing risk of bias of the outcome assessments.

The retention rate for most of the experimental studies was quite high, with the majority reporting retention rates greater than 80%, which could be explained by
the short duration of the studies, participant blinding, and the low participant burden. However, over half of the studies (8 of the 15) required participants to attend measurement sessions from 3 to 6 times throughout the study, so it is surprising that the retention rate was high in these studies, although the actual measurements were mostly non-invasive. Interestingly none of the 15 studies reported reimbursement for costs associated with travel.

The majority of studies (n=17) were conducted in female adults only. Thirteen of these studies specifically recruited female participants, therefore making the external validity of the studies uncertain and results may not be applicable to the general population, particularly for men. Hence, further research in more diverse population groups, including men is warranted to confirm the relationship between appearance and diet.

2.5.4 Side effects

Less than half of the studies that were supplement based reported whether there were any side effects following consumption of oral supplements, making it difficult to determine whether there were in fact no side effects or side effects were not monitored within the studies. Hence, statements about safety cannot be made. Only 1 study (Cho, Choi, et al., 2009) that reported positive results for skin ageing concluded that the side effects of taking the supplement outweighed the beneficial results, suggesting further study was needed to evaluate outcomes using a smaller dosage.

This review has some limitations. Some recent or unpublished studies may have been missed as may have those published in languages other than English. However, a predefined protocol was used; and a comprehensive search strategy employed across several databases along with a reference list search to identify the key studies in this area. In addition, 2 independent reviewers were used for study inclusion, data extraction and quality appraisal in order to increase objectivity.
2.6 Gaps in knowledge and future research

This review has demonstrated that there is currently insufficient evidence to determine the association between actual food intake or dietary patterns and appearance only 1 study has examined this relationship. There is some evidence to suggest that supplements may contribute to improving appearance in particular with skin ageing and UV damage; but these studies were heterogeneous in study design, appearance-related outcomes, supplement regimens and the majority of them were conducted in females only. Further studies are needed in representative populations that examine actual food consumption on appearance, using validated tools in a well-designed high-quality RCT. In addition, higher quality studies are required that examine the effects of supplements on appearance. There is also a need for high-quality large observational studies such as prospective cohort studies to examine the relationship between food intake and appearance and therefore, generate hypotheses for future RCTs.

Furthermore, as future research is undertaken systematic reviews may be conducted that focus only on high-quality RCTs to determine the effectiveness of dietary interventions on perceived or actual appearance.
Chapter 3  Cross-sectional study

This article was published in 2015


The work presented in the manuscript was also presented at The Annual Scientific Meeting Nutrition Society of Australia and New Zealand in December 2014, Tasmania Australia (Oral presentation).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 21).
3.1 Abstract
Fruit and vegetables contain carotenoid pigments, which accumulate in human skin, contributing to its yellowness. This effect has a beneficial impact on appearance. The aim was to evaluate associations between diet (fruit, vegetable and dietary carotenoid intakes) and skin colour in young women. Ninety-one Caucasian women (Median and Interquartile Range (IQR) age 22.1 (18.1–29.1) years, BMI 22.9 (18.5–31.9) kg/m²) were recruited from the Hunter region (Australia). Fruit, vegetable and dietary carotenoid intakes were estimated by a validated food frequency questionnaire. Skin colour was measured at nine body locations (sun exposed and unexposed sites) using spectrophotometry. Multiple linear regression was used to assess the relationship between fruit and vegetable intakes and skin yellowness adjusting for known confounders. Higher combined fruit and vegetable intakes (β=0.8, p=0.017) were associated with higher overall skin yellowness values. Higher fruit combined fruit and vegetable intakes (β=1.0, p=0.004) were associated with increased unexposed skin yellowness. Combined fruit and vegetables plus dietary carotenoid intakes contribute to skin yellowness in young Caucasian women. Evaluation of interventions using improvements in appearance as an incentive for increasing fruit and vegetable consumption in young women is warranted.

3.2 Introduction
Higher fruit and vegetable intakes are associated with lower risk of excess weight gain, type 2 diabetes, cardiovascular disease, and specific cancers (Australian Government Department of Health, 2013; Boeing et al., 2012; Center for disease control and prevention, 2013). Despite these benefits, many young women are not consuming adequate amounts of fruit and vegetables for overall health and to prevent future chronic disease. For instance, in the United States, more than 90% of women aged 19 to 30 years do not meet recommended targets (Krebs-Smith et al., 2010). In Australia, women aged 18 to 34 years have one of the lowest overall adult intakes, with only 3.7% meeting recommendations of two servings of fruit and five servings of vegetables a day (Australian Bureau of Statistics, 2012). Recent studies indicate that women are ambivalent about the importance of nutrition for their health (Chung et al., 2006).
Thus, finding novel strategies to motivate increased fruit and vegetables in this group is necessary to protect against chronic diseases.

Recent evidence has shown that young women are motivated to change their health behaviours based on improving their appearance or looking good rather than health concerns (LaRose et al., 2013; Traill et al., 2012), which are more important amongst older females (36–50 years old). Appearance-based interventions focusing on other health risk behaviours (smoking and sun exposure) in young adults have been successful in motivating behaviour change (Mahler et al., 2007; Semer et al., 2005). Similarly, a recent appearance-based intervention displaying the effects of fruit and vegetable intake on participants own facial skin colour found that this approach motivated increased consumption of fruit and vegetables (Whitehead, Ozakinci, & Perrett, 2014). Interventions that focus on appearance could be a novel way of motivating young women to improve dietary intake, including fruit and vegetable intakes.

Our recent systematic review (Pezdirc et al., 2015a) examined research evidence on the relationship between food intake and appearance. Nine observational studies found significant associations between aspects of dietary intake and appearance. Findings indicated that well-conducted large observational studies using validated methods to assess exposure and outcomes are needed to further examine these relationships and to inform hypotheses and the design of future RCTs (Pezdirc et al., 2015a).

Fruit and vegetables contain yellow/red carotenoid pigments that, once consumed, enter the bloodstream and are distributed to various organs, including the skin (Alaluf et al., 2002; Mayne et al., 2010). The majority of these fat-soluble carotenoid pigments accumulate in adipose tissues, where they can be utilised as antioxidants or released back into the circulation (Tanumihardjo, 2013). Carotenoid pigments also accumulate in all layers of skin, including the dermis, epidermis, and stratum corneum (Alaluf et al., 2002; Mayne et al., 2010). This accumulation contributes to normal skin yellowness (Alaluf et al., 2002; Mayne et al., 2010). Carotenoid colouration (yellowness) has been
reported by young adults as appearing more healthy and attractive than melanin colouration (tanning) (Lefevre & Perrett, 2015; Whitehead, Ozakinci, & Perrett, 2012).

Carotenoid colouration of human skin can be measured objectively using non-invasive optical methods such as RRS and reflectance spectroscopy (Ermakov & Gellermann, 2015). A recent review conducted by Ermakov et al. (Ermakov & Gellermann, 2015) on these two optical methods highlighted their potential as objective markers of fruit and vegetable intake. Both methods have been validated against plasma carotenoid concentrations (Jahns et al., 2014; Mayne et al., 2010; Scarmo et al., 2010) and found to be valid. RRS uses laser spectroscopy, which probes the vibrational energy levels of a molecule whilst reflectance spectroscopy involves measuring skin colour using CIE L*a*b* colour space values and skin spectral reflectance. Evidence from previous studies using reflectance spectroscopy has shown that changes in fruit and vegetable intake (Whitehead, Re, et al., 2012) and use of carotenoid dietary supplements (Stephen et al., 2011) over a period of time led to changes in skin redness and yellowness. However, the studies were conducted on relatively few participants (n = 35–82), and did not focus on young women. Further, although this previous work utilised a validated FFQ to estimate fruit and vegetable intake, it only included 10 items and was developed over 25 years ago, hence it is unlikely to reflect contemporary diets. Therefore, further research using a comprehensive, validated FFQ which assesses a larger variety of fruit and vegetables items is needed to evaluate these findings in an Australian population group where mean national fruit and vegetable intakes differ, as well as potential sun exposure.

The aim of this cross-sectional study amongst young (18–30 years) Australian women is to evaluate associations between (i) fruit and vegetable intake; (ii) dietary carotenoid intake and skin colour (CIE L*a*b). Secondly, the study explored the relationships between fruit, vegetable, dietary carotenoid intakes and skin reflectance in order to verify whether carotenoid pigments are responsible for any observed changes in skin colour, as changes in skin colour, for instance skin yellowness, can also be affected by melanin (Stamatas, Mudzka, Kollias, & Beer, 2004).
3.3 Methods
3.3.1 Study population
This cross-sectional study was conducted at the University of Newcastle, Australia, between October 2012 and June 2013, which was predominantly in Spring, Summer and Autumn. Women aged ≥18 years (n=176) were recruited using flyers posted on University noticeboards, advertisements on the University’s Facebook page, and media releases (Appendix 1). In addition, participants were recruited from the University’s School of Nursing Research Awareness Program with students given credit towards their course for research participation. The study was approved by the Human Research Ethics Committee at the University of Newcastle (H-2012–0217) (Appendix 2). An online information statement was provided to those interested, and implied consent was assumed for those who proceeded to the online survey (Appendix 3). Written informed consent was obtained prior to physical data collection in the laboratory (Appendix 4).

For the current analysis, women aged 18–30 years were eligible for inclusion (n = 112). Young women were selected as a group potentially motivated by appearance (LaRose et al., 2013; Traill et al., 2012). Current smokers (n=6) were excluded due to the impact of smoking on antioxidant status, and systemic carotenoid levels (Assalle, Maffei, Ndreu, & Mercuri, 2009). Non-Caucasian women (n=9) were excluded, to reduce heterogeneity of skin lightness, as the sample was predominantly Caucasian. Those who had a fake tan (n=6) at the time of the assessment were also ineligible due to non-dietary impacts on skin pigmentation which is likely to affect skin lightness (L*) and yellowness (b*) (Stamatas et al., 2004). In addition, no participants were excluded for skin conditions (e.g., high bilirubin, vitiligo). The final sample consisted of 91 women who completed the online survey and had completed physical assessments.

3.3.2 Measurements
Data was from participants via an online survey followed by in-person assessments, which were conducted at the University of Newcastle by Kristine Pezdirc using standardised operating procedures and protocols for the measurements. Participants
were advised before their appointment to not wear make-up to their assessment. Cleansing wipes were provided for use and time was allowed for skin to dry prior to skin spectrophotometer measures being taken. In addition they were asked to refrain from any physical activity two hours prior to their appointment.

### 3.3.3 Skin colour and skin spectral reflectance

Skin colour and spectral reflectance (excluding specular reflectance component) were measured using a CM700D spectrophotometer (Konica Minolta, Osaka, Japan) with an 8mm diameter aperture, 2-degree observer angle and illuminant D65. The spectrophotometer was white point calibrated at each measurement session. Skin colour (CIE L*a*b values, where positive L*, a* and b* values represent lightness, redness and yellowness respectively) was recorded for each participant at nine body locations on the left-hand side of the body unless stated otherwise. The nine body locations included the forehead (glabella), left and right cheek (orbitale), shoulder (acromiale), inner arm (radiale), outer arm (medial humeral epicondyle), palm (thenar muscle), waist (iliocristale) and foot sole (planta). The measurements were repeated three times at each site and the average recorded. All researchers taking measures were trained in standardise use of the spectrophotometer (by Ross Whitehead). Body locations were selected according to anatomical landmarks using the ISAK International standards for anthropometric assessment (Marfell-Jones, Olds, Steward, & LLindsay Carter, 2007). Inter-rater reliability for the nine body locations sites was assessed between five research assistants involved in the measurements using the standardised procedure. For all body locations and between experimenters across L*a* b* values the standard deviation (SD) was 0.9. The within experimenter standard deviation (SD) across the three measurements at each location was low at 0.61 across L*a*b* values.

Overall (all nine body locations), sun exposed (left and right cheeks, forehead and outer arm) and unexposed (inner arm, shoulder, hip, palm and sole of foot) L*a*b values were calculated by averageing the three measurements at each location, these were then summed and the average of those values calculated. Skin spectral reflectance (overall body locations), which measures the percentage of light reflected by skin, was
measured at each site at wavelengths between 360–740 nm (10 nm intervals). Reflectance at each wavelength was divided by summed reflectance across all measured wavelengths to adjust for overall skin lightness (Stephen et al., 2011). Spectral absorption coefficient values were obtained for a subset of common carotenoids including α-carotene, β-carotene and lycopene (excluding lutein/zeaxanthin) based on those available from Miller et al. (Miller, 1937). These coefficients were used to examine the involvement of carotenoid pigments in any observed association between skin colour and diet.

3.3.4 Fruit, vegetable and carotenoid intake

Dietary intake was measured using the Australian Eating Survey 2010, a validated 120-item semi-quantitative FFQ that assesses dietary intake over the previous six months. The FFQ has been tested for reliability and relative validity against three-day weighed food records and is accurate for ranking nutrient intakes in Australian adults (Collins et al., 2014). Recently, the FFQ has been validated for fruit and vegetable intake against plasma carotenoid concentrations (Burrows et al., 2015). Significant positive correlations were found between carotenoid intakes assessed by the FFQ and plasma carotenoids for α-carotene (52%, p<0.001) β-carotene (47%, p<0.003) and Lutein/zeaxanthin (26%, p=0.041) (Burrows et al., 2015). Analysis of the mean daily nutrient intakes (including total energy intake) were performed using the latest databases available at that time, Australian AusNut 1999 (All foods), revision 17, and AusFoods (brands) Revision 5 accessed through FoodWorks version 3.02.581 (Xyris Software, Brisbane, Queensland, Australia). A standard portion size was used for each food item, determined using standard serving sizes (for example, one slice of bread) or unpublished data purchased from the Australian Bureau of Statistics data on the latest available national nutrition data at that time.

Twenty-one questions related to the intake of vegetables and 11 to fruit. Total servings of fruit and vegetables were calculated by summing the weight of relevant food items estimated by the FFQ divided by the standard serving size dictated by the AGTHE (fruit serving 150 g, vegetable serving 75 g) (Australian Government Department of Health, 2013). The FFQ included questions on seasonal fruit. Consumption of seasonal
fruits such as peach, mango, paw-paw, pineapple, melon, grapes and berries were calculated using participants’ estimation of consumption frequency when they are in season. This was adjusted by the number of months each year the fruit is available. Fruit juice was not included as part of fruit intake.

Daily carotenoid intakes for the mostly commonly consumed in the human diet α-carotene, β-carotene, lycopene and combined lutein-zeaxanthin, were calculated from FFQ fruit and vegetable responses using the US Department of Agriculture National Cancer Institute carotenoids food composition database (Chug-Ahuja et al., 1993).

### 3.3.5 Other measures

Self-reported data for, dietary supplement use (yes or no, an open response for type of supplements, if yes), and cigarette smoking (current, past or non-smokers) were collected. Height was measured using a portable BSM370 stadiometer correct to 0.1 cm using the stretch stature method. Weight was measured using the Inbody720 Body Composition Analyzer (Biospace Co., Ltd., Seoul, Korea). BMI was calculated using the standard equation (weight kg/height m²). Two measures were collected for both weight and height and averaged at the same point and the same researcher.

### 3.3.6 Statistical analysis

Data analysis was undertaken using Stata (Version 11.0; StataCorp, College Station, TX, USA). A significance level was set at p < 0.05. Normally distributed variables are presented as means (± SD) and skewed variables are presented as medians with IQRs. Multiple linear regression was used to investigate the association between fruit, vegetable intakes (servings per day) and dietary carotenoid intakes and overall, unexposed and sun exposed skin colour redness (a*) and yellowness (b*). Variables that were not normally distributed were transformed including BMI (1/square), fruit, vegetable, combined fruit and vegetable servings, dietary β-carotene, lutein, lycopene intakes (square root) and total energy and fat intakes (log).

The models were adjusted for: age, as changes in skin colour are often associated with ageing (Boelsma et al., 2001); skin lightness (L*), as melanin affects both skin yellowness and lightness (L*) (Stamatas et al., 2004); BMI and total energy intake/day
as people with higher BMI and total energy intake generally consume less fruit and vegetables (Ghalaeh, Gholi, Bank, & Azadbakht, 2012) and this is likely to be associated with lower skin yellowness (Burrows, Warren, Colycas, Garg, & Collins, 2009); supplementation use as a potential source of β-carotene or other antioxidants (Stephen et al., 2011) and total fat intake (g) as carotenoids are fat soluble and bioavailability is affected by dietary fat (Castenmiller & West, 1998; van het Hof et al., 2000). As fruit and vegetable intakes are related to each other and impact on skin colour the model has also been adjusted for either fruit or vegetable intake depending on the exposure variable in the analysis. Similarly dietary carotenoid intakes have been adjusted by the sum of the other carotenoids.

Following and extending the methods used by Stephen et al. (Stephen et al., 2011) and Whitehead et al. (Whitehead, Re, et al., 2012), partial correlations were used to investigate the relationships between fruit, vegetable or combined fruit and vegetable intake and overall skin reflectance at 10 nm intervals between wavelengths 400–540 nm (controlling for, age, fruit/vegetable servings, other carotenoids, skin lightness (L*), BMI, total daily energy, fat intake and supplement use). This was repeated for dietary carotenoid intake (α-carotene, β-carotene, lycopene and lutein). Spearman correlations were then used to assess whether the strength of these partial correlation values per wavelength was associated with the absorption spectra of common dietary carotenoids (see Figure 3.1). Carotenoids characteristically absorb light in the 400–540 nm region of the spectrum and reflect back longer (yellow) wavelengths, hence we expect to see negative correlations between spectral reflectance at these wavelengths and estimates of dietary carotenoid intake.
Figure 3.1 Spearman correlation coefficients between skin reflectance values and fruit, vegetable and combined fruit and vegetable consumption (servings per day) plotted with absorption spectra of common carotenoids.
3.4 Results

3.4.1 Study population

Of the ninety-one women included in the analyses, 86 had measurements taken on all nine sites, with the remaining five missing the hip measurement (due to the type of clothing worn during assessment). Characteristics of the sample are summarised in Table 3.1. The median (Interquartile Range (IQR) age was 22.1 (19.8–26.1) years; BMI 22.9 (20.9–24.9) kg/m² and percent body fat 27.9 (23.7–35.9). Thirty eight percent (n=35) were taking dietary supplements with 14.3% of these participants (n= 5) taking supplements containing carotenoids and 51.4% taking a multivitamin that contained various antioxidants including vitamin A, C, E and zinc. Other supplements included fish oil (n= 5), iron (n=4) and vitamin B complex (n=5). Daily fruit, vegetables, dietary carotenoid, total daily energy intakes and fat intakes are summarised in Table 3.1. Median (IQR) fruit and vegetable intakes were 1.8 (1.0–2.7) and 3.8 (2.7–5.2) servings/day respectively, with β-carotene the most common dietary carotenoid, 6872.4 μg/day (4462.6–8918.6) (Table 3.1). Only 16.5% (n=15) of this sample of women met current Australian recommendations of consuming at least two servings of fruit and five servings of vegetables a day (Australian Government Department of Health, 2013). Combined fruit and vegetable intake (servings) was significantly lower (p=0.03) for women (n=20) who were overweight or obese. Green coloured vegetables and other fruits were those that were consumed in the greatest amounts with Median (IQR) intakes of 1.3 (0.8–1.7) and 0.8 (0.5–1.2) servings/day. Whilst some of these fruit and vegetables may contain large amounts of a dominant carotenoid, most contain a number of different carotenoids.
Table 3.1 Characteristics of women (n=91) participating in a cross-sectional evaluation of fruit, vegetable and dietary carotenoid intake and skin colour and reflectance

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<td>Taking Supplements Yes n (%)</td>
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<td>Weight (kg) median (IQR)</td>
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<tr>
<td>BMI (kg/m²) median (IQR)</td>
<td>22.9 (20.9–24.9)</td>
</tr>
<tr>
<td>Fruit intake (servings/day) median (IQR)</td>
<td>1.8 (1.0–2.7)</td>
</tr>
<tr>
<td>Citrus fruit a (servings/day) median (IQR)</td>
<td>0.1 (0.1–0.4)</td>
</tr>
<tr>
<td>Berries b (servings/day) median (IQR)</td>
<td>0.1 (0.1–0.3)</td>
</tr>
<tr>
<td>Core c fruits (servings/day) median (IQR)</td>
<td>0.7 (0.2–1.2)</td>
</tr>
<tr>
<td>Other fruits d (servings/day) median (IQR)</td>
<td>0.8 (0.5–1.2)</td>
</tr>
<tr>
<td>Vegetable intake (servings/day) median (IQR)</td>
<td>3.8 (2.7–5.2)</td>
</tr>
<tr>
<td>Orange/yellow e coloured vegetables (servings/day) median (IQR)</td>
<td>0.8 (0.5–1.1)</td>
</tr>
<tr>
<td>Red f coloured vegetables (servings/day) median (IQR)</td>
<td>0.6 (0.2–0.9)</td>
</tr>
<tr>
<td>Green g coloured vegetables (servings/day) median (IQR)</td>
<td>1.3 (0.8–1.7)</td>
</tr>
<tr>
<td>White h coloured vegetables (servings/day) median (IQR)</td>
<td>0.8 (0.4–1.3)</td>
</tr>
<tr>
<td>Total fruit and vegetable intake (servings/day) median (IQR)</td>
<td>5.9 (4.1–7.4)</td>
</tr>
<tr>
<td>α-carotene (µg/day) median (IQR)</td>
<td>1988.6 (1220.2–2611.6)</td>
</tr>
<tr>
<td>β-carotene (µg/day) median (IQR)</td>
<td>6872.4 (4462.6–8918.6)</td>
</tr>
<tr>
<td>Lutein zeaxanthin (µg/day) median (IQR)</td>
<td>2276.8 (1523.6–2895.1)</td>
</tr>
<tr>
<td>Lycopene (µg/day) median (IQR)</td>
<td>5054.8 (2975.1–7488.5)</td>
</tr>
<tr>
<td>Total energy (kilojoules/day) median (IQR)</td>
<td>7921 (6538–9801)</td>
</tr>
<tr>
<td>Total fats (grams/day) median (IQR)</td>
<td>66.9 (53.9–84.0)</td>
</tr>
<tr>
<td>Overall L* mean ± SD</td>
<td>65.2 ± 2.1</td>
</tr>
<tr>
<td>Overall a* mean ± SD</td>
<td>9.3 ± 1.2</td>
</tr>
<tr>
<td>Overall b* mean ± SD</td>
<td>16.3 ± 2.1</td>
</tr>
<tr>
<td>Unexposed L* mean ± SD</td>
<td>66.5 ± 2.2</td>
</tr>
<tr>
<td>Unexposed a* mean ± SD</td>
<td>7.3 ± 1.2</td>
</tr>
<tr>
<td>Unexposed b* mean ± SD</td>
<td>16.3 ± 2.2</td>
</tr>
<tr>
<td>Exposed L* mean ± SD</td>
<td>63.5± 2.3</td>
</tr>
<tr>
<td>Exposed a* mean ± SD</td>
<td>11.8 ±1.4</td>
</tr>
<tr>
<td>Exposed b* mean ± SD</td>
<td>16.3 ± 2.1</td>
</tr>
</tbody>
</table>

a Orange, mandarin, grapefruit; b grapes, strawberries, blueberries; c apples, pears; d banana, stone fruits, mango, pineapple, and melons; e pumpkin, sweet potato, carrots and corn; f tomato and capsicum; g green beans, spinach, broccoli, peas and lettuce; h potato, cauliflower, mushrooms and onion.
3.4.2 Associations between fruit, vegetable, dietary carotenoid intakes and skin colour

Higher daily fruit, vegetable and combined fruit and vegetable intakes were associated with increased overall, unexposed and exposed skin redness and yellowness values except for vegetable intake and skin a* (Table 3.2). After adjusting for age, fruit/vegetable servings, skin lightness (L*), BMI, total daily energy, fat intake and supplement use, the associations remained significant for combined fruit and vegetable intakes (β=1.0, p=0.004), and unexposed skin yellowness values; and for combined fruit and vegetable intakes (β=0.3, p=0.049) and unexposed skin redness values. Combined fruit and vegetable intake (β=0.8, p=0.017) were significantly associated with overall skin yellowness values.

For dietary carotenoid intakes, only lutein/zeaxanthin was significantly associated with overall skin yellowness (β=0.03, p=0.010) values after adjustment for confounders (refer to supplementary data Table 3.4). Intakes of lutein/zeaxanthin (β=0.03, p=0.042) and lycopene (β= −0.25, p=0.023) were significantly associated with unexposed skin yellowness values.
Table 3.2 Multiple regression analyses between fruit and vegetable intake and skin colour (CIE L’*a*’b’*).

<table>
<thead>
<tr>
<th></th>
<th>Overall Redness (a*) n = 86</th>
<th>Overall Yellowness (b*) n = 86</th>
<th>Unexposed Redness (a*) n = 86</th>
<th>Unexposed Yellowness (b*) n = 86</th>
<th>Exposed Redness (a*) n = 91</th>
<th>Exposed Yellowness (b*) n = 91</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Unadjusted</strong></td>
<td><strong>Adjusted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \beta ) Coefficient ± SE</td>
<td>95% Confidence Interval</td>
<td>p value</td>
<td>( \beta ) Coefficient ± SE</td>
<td>95% Confidence Interval</td>
<td>p value</td>
</tr>
<tr>
<td>Fruit (servings/day)</td>
<td>0.8 ± 0.3 (0.3, 1.3)</td>
<td>0.002</td>
<td>0.02 ± 0.2 (−0.4, 0.4)</td>
<td>0.918</td>
<td>0.8 ± 0.3 (0.3, 1.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>Vegetable (servings/day)</td>
<td>0.6 ± 0.3 (0.1, 1.1)</td>
<td>0.017</td>
<td>0.2 ± 0.2 (−0.1, 0.6)</td>
<td>0.162</td>
<td>0.8 ± 0.3 (0.3, 1.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>Combined Fruit and vegetables (servings/day)</td>
<td>0.7 ± 0.2 (0.2, 1.1)</td>
<td>0.003</td>
<td>0.2 ± 0.1 (−0.1, 0.5)</td>
<td>0.167</td>
<td>0.7 ± 0.2 (0.3, 1.2)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.4 (0.9, 2.6)</td>
<td>&lt;0.001</td>
<td>0.9 ± 0.4 (−0.1, 1.8)</td>
<td>0.065</td>
<td>1.9 ± 0.5 (1.0, 2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.4 (0.6, 2.3)</td>
<td>0.002</td>
<td>0.4 ± 0.4 (−0.4, 1.1)</td>
<td>0.297</td>
<td>1.8 ± 0.5 (0.9, 2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.5 ± 0.4 (0.8, 2.2)</td>
<td>&lt;0.001</td>
<td>0.8 ± 0.3 (0.1, 1.4)</td>
<td>0.017 *</td>
<td>1.8 ± 0.4 (1.0, 2.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.8 ± 0.3 (0.3, 1.3)</td>
<td>0.005</td>
<td>0.2 ± 0.2 (−0.3, 0.6)</td>
<td>0.406</td>
<td>0.8 ± 0.3 (0.2, 1.4)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>0.8 ± 0.3 (0.3, 1.3)</td>
<td>0.004</td>
<td>0.3 ± 0.2 (−0.1, 0.6)</td>
<td>0.135</td>
<td>0.4 ± 0.3 (−0.2, 1.0)</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>0.7 ± 0.2 (0.3, 1.2)</td>
<td>0.001</td>
<td>0.3 ± 0.2 (0.007, 0.6)</td>
<td>0.049 *</td>
<td>0.5 ± 0.3 (0.1, 1.0)</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>1.9 ± 0.5 (1.0, 2.9)</td>
<td>&lt;0.001</td>
<td>0.9 ± 0.5 (−0.04, 1.9)</td>
<td>0.060</td>
<td>1.5 ± 0.4 (0.7, 2.4)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.5 (0.9, 2.7)</td>
<td>&lt;0.001</td>
<td>0.6 ± 0.5 (−0.2, 1.5)</td>
<td>0.120</td>
<td>1.1 ± 0.4 (0.2, 1.9)</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.4 (1.0, 2.5)</td>
<td>&lt;0.001</td>
<td>1.0 ± 0.4 (0.3, 1.7)</td>
<td>0.004 *</td>
<td>1.2 ± 0.4 (0.5, 1.9)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Adjusted for fruit/vegetable servings L’, supplementation use, BMI, total energy intake, fat intake and age; Unexposed: Total of inner arm, shoulder, hip, palm and sole of foot; Exposed: Total of face (left and right cheeks, forehead) and outer arm; Significant \( p < 0.05 \).
### 3.4.3 Relationships between fruit, vegetable, dietary carotenoid intakes and skin reflectance

Table 3.3 and Figure 3.1 demonstrate that the partial correlations between fruit intake and overall skin spectral reflectance across wavelengths 400–540 nm is significantly and negatively correlated with the absorption spectra of α-carotene ($r = -0.89$, $p < 0.001$), β-carotene ($r = -0.97$, $p < 0.001$), lycopene ($r = -0.69$, $p = 0.0047$) and the mean absorption of these three common carotenoids ($r = -0.94$, $p < 0.001$). The relationship between vegetable intake and skin reflectance across wavelengths 400–540 nm was significantly negatively correlated with the absorption spectra of lycopene ($r = -0.80$, $p = 0.0004$). The relationship between combined fruit and vegetable intake and skin reflectance was significantly negatively correlated with the absorption spectra of β-carotene ($r = -0.58$, $p = 0.0236$), lycopene ($r = -0.90$, $p < 0.001$) and mean carotenoid ($r = -0.65$, $p = 0.0086$). The correlation coefficients between dietary intakes of β-carotene ($r = -0.69$, $p = 0.0045$) and lycopene ($r = 0.82$, $p = 0.0002$) with their respective absorption spectra were significant.

### Table 3.3 Relationship between partial correlation coefficients between fruit, vegetable and dietary carotenoid intake, overall skin reflectance and absorption spectra of common carotenoids as measured by spectrophotometry.

<table>
<thead>
<tr>
<th></th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>Lycopene</th>
<th>Mean Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\rho$</td>
<td>$p$ value</td>
<td>$\rho$</td>
<td>$p$ value</td>
</tr>
<tr>
<td>Fruit (servings/day)</td>
<td>-0.89 *</td>
<td>&lt;0.001</td>
<td>-0.97 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vegetable (servings/day)</td>
<td>-0.08</td>
<td>0.7641</td>
<td>-0.31</td>
<td>0.2603</td>
</tr>
<tr>
<td>Combined Fruit and vegetables (servings/day)</td>
<td>-0.37</td>
<td>0.1734</td>
<td>-0.58 *</td>
<td>0.2336</td>
</tr>
<tr>
<td>α-carotene intake (µg/day)</td>
<td>-0.31</td>
<td>0.2576</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-carotene intake (µg/day)</td>
<td></td>
<td></td>
<td>-0.69</td>
<td>0.0045</td>
</tr>
<tr>
<td>Lycopene intake (µg/day)</td>
<td>0.82 *</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant $p < 0.05$. Spearman correlations were used to assess whether the strength of these partial correlation values was associated with the absorption spectra of common dietary carotenoids.
Table 3.4 Multiple regression analyses between dietary carotenoid intake and skin colour (CIE L*a*b*)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted *</th>
<th></th>
<th>Unadjusted</th>
<th>Adjusted *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β Coefficient ± SE</td>
<td>95% Confidence Interval</td>
<td>p value</td>
<td>β Coefficient ± SE</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td><em><em>Overall Redness (a</em>) n = 86</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene intake (μg/day)</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
<td>0.101</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
</tr>
<tr>
<td>β carotene intake (μg/day)</td>
<td>0.02 ± 0.01</td>
<td>(0.00, 0.03)</td>
<td>0.015</td>
<td>–0.001 ± 0.01</td>
<td>(–0.02, 0.02)</td>
</tr>
<tr>
<td>Lycopene intake (μg/day)</td>
<td>0.00 ± 0.01</td>
<td>(–0.01, 0.02)</td>
<td>0.665</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.01)</td>
</tr>
<tr>
<td>Lutein zeaxanthin intake (μg/day)</td>
<td>0.02 ± 0.01</td>
<td>(0.01, 0.04)</td>
<td>0.010</td>
<td>0.00 ± 0.01</td>
<td>(–0.01, 0.14)</td>
</tr>
<tr>
<td><em><em>Overall Yellowness (b</em>) n = 86</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene intake (μg/day)</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
<td>0.089</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
</tr>
<tr>
<td>β carotene intake (μg/day)</td>
<td>0.03 ± 0.01</td>
<td>(0.01, 0.05)</td>
<td>0.009</td>
<td>0.01 ± 0.02</td>
<td>(–0.03, 0.04)</td>
</tr>
<tr>
<td>Lycopene intake (μg/day)</td>
<td>–0.03 ± 0.01</td>
<td>(–0.05, –0.01)</td>
<td>0.011</td>
<td>–0.02 ± 0.01</td>
<td>(–0.04, 0.00)</td>
</tr>
<tr>
<td>Lutein zeaxanthin intake (μg/day)</td>
<td>0.06 ± 0.01</td>
<td>(0.03, 0.09)</td>
<td>0.000</td>
<td>0.03 ± 0.15</td>
<td>(0.00, 0.06)</td>
</tr>
<tr>
<td><em><em>Unexposed Redness (a</em>) n = 86</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene intake (μg/day)</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
<td>0.063</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
</tr>
<tr>
<td>β carotene intake (μg/day)</td>
<td>0.02 ± 0.00</td>
<td>(0.01, 0.03)</td>
<td>0.003</td>
<td>0.01 ± 0.01</td>
<td>(–0.01, 0.03)</td>
</tr>
<tr>
<td>Lycopene intake (μg/day)</td>
<td>0.00 ± 0.01</td>
<td>(–0.01, 0.02)</td>
<td>0.720</td>
<td>0.01 ± 0.00</td>
<td>(–0.00, 0.01)</td>
</tr>
<tr>
<td>Lutein zeaxanthin intake (μg/day)</td>
<td>0.03 ± 0.00</td>
<td>(0.01, 0.05)</td>
<td>0.000</td>
<td>0.01 ± 0.01</td>
<td>(–0.01, 0.02)</td>
</tr>
<tr>
<td><em><em>Unexposed Yellowness (b</em>) n = 86</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene intake (μg/day)</td>
<td>0.00 ± 0.00</td>
<td>(0.00, 0.00)</td>
<td>0.025</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
</tr>
<tr>
<td>β carotene intake (μg/day)</td>
<td>0.03 ± 0.00</td>
<td>(0.02, 0.06)</td>
<td>0.001</td>
<td>0.02 ± 0.02</td>
<td>(–0.25, 0.58)</td>
</tr>
<tr>
<td>Lycopene intake (μg/day)</td>
<td>–0.03 ± 0.01</td>
<td>(–0.05, –0.00)</td>
<td>0.019</td>
<td>–0.25 ± 0.01</td>
<td>(–0.05, –0.00)</td>
</tr>
<tr>
<td>Lutein zeaxanthin intake (μg/day)</td>
<td>0.07 ± 0.01</td>
<td>(0.04, –0.10)</td>
<td>0.000</td>
<td>0.03 ± 0.02</td>
<td>(0.00, 0.07)</td>
</tr>
<tr>
<td><em><em>Exposed Redness (a</em>) n = 91</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene intake (μg/day)</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
<td>0.282</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
</tr>
<tr>
<td>β carotene intake (μg/day)</td>
<td>0.01 ± 0.00</td>
<td>(0.00, 0.03)</td>
<td>0.144</td>
<td>–0.01 ± 0.01</td>
<td>(–0.31, 0.01)</td>
</tr>
<tr>
<td>Lycopene intake (μg/day)</td>
<td>0.00 ± 0.01</td>
<td>(–0.01, 0.02)</td>
<td>0.745</td>
<td>0.00 ± 0.01</td>
<td>(–0.01, 0.01)</td>
</tr>
<tr>
<td>Lutein zeaxanthin intake (μg/day)</td>
<td>0.01 ± 0.00</td>
<td>(–0.01, 0.03)</td>
<td>0.258</td>
<td>–0.00 ± 0.01</td>
<td>(–0.02, 0.01)</td>
</tr>
<tr>
<td><em><em>Exposed Yellowness (b</em>) n = 91</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene intake (μg/day)</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
<td>0.333</td>
<td>0.00 ± 0.00</td>
<td>(–0.00, 0.00)</td>
</tr>
<tr>
<td>β carotene intake (μg/day)</td>
<td>0.02 ± 0.01</td>
<td>(–0.00, 0.04)</td>
<td>0.068</td>
<td>0.00 ± 0.02</td>
<td>(–0.04, 0.04)</td>
</tr>
<tr>
<td>Lycopene intake (μg/day)</td>
<td>0.03 ± 0.01</td>
<td>(–0.05, –0.01)</td>
<td>0.012</td>
<td>–0.01 ± 0.01</td>
<td>(–0.03, 0.01)</td>
</tr>
<tr>
<td>Lutein zeaxanthin intake (μg/day)</td>
<td>0.05 ± 0.01</td>
<td>(0.02, 0.01)</td>
<td>0.001</td>
<td>0.03 ± 0.02</td>
<td>(–0.01, 0.06)</td>
</tr>
</tbody>
</table>

* Adjusted for total other carotenoid intake, L*, supplementation use, BMI, total energy intake, fat intake and age; ^ Unexposed: Total of inner arm, shoulder, hip, palm and sole of foot; ^ Exposed: Total of face (left and right cheeks, forehead) and outer arm
3.5 Discussion

The current study demonstrates that higher fruit and vegetable and dietary carotenoid intakes in young Australian women are associated with skin colour, especially skin yellowness. In the adjusted regression models, for every additional serving of combined fruit and vegetables per day there was an increase of 0.8 units in overall skin yellowness and 1.0 units in unexposed skin yellowness. A Delta E (\(\Delta E\)) of 1.37 to 1.55 in skin colour change which is a very small difference (\(\Delta E\) is defined as the difference between colours in the CIE L*a*b* colour space) is perceived as a visible increase in the appearance of skin as healthy and attractive (Whitehead, Re, et al., 2012).

As anticipated, negative significant correlations were found between fruit and vegetable intakes and skin reflectance at wavelengths associated with light absorption by carotenoids. For fruit consumption, these relationships were strongest at wavelengths associated with high light absorption by common dietary carotenoids (\(\alpha\)-carotene, \(\beta\)-carotene and lycopene). For vegetable intake it was only demonstrated for the absorption spectra of lycopene. In addition, both dietary lycopene and \(\beta\)-carotene were significantly correlated with their absorption spectra. This provides further evidence that human skin colouration is related to fruit and vegetable consumption, with this relationship strongest at the specific wavelengths associated with greater light absorption by carotenoid pigments.

Our findings support evidence from previous studies reporting that higher fruit and vegetable consumption was associated with higher skin yellowness (Stephen et al., 2011; Whitehead, Re, et al., 2012). Using a 63-item FFQ, 10 of which related to fruit and vegetables, Whitehead et al. (Whitehead, Re, et al., 2012) examined change in fruit and vegetable intake over a six-week period. They found a modest increase in consumption was associated with a significant increase in skin yellowness and redness in both exposed and unexposed skin. In the current cross-sectional study, after adjusting for fruit/vegetable servings, BMI, dietary supplement use, skin lightness, total energy, fat intake and age which were not accounted for (except skin lightness) in previous studies, only the relationship between combined fruit and vegetables and skin yellowness in unexposed skin remained statistically significant. This suggests that
the relationship between diet and skin colour is strongest at unexposed areas of the skin. This may be because sun exposed skin might be less likely to show dietary effects because carotenoids are locally expended by UV exposure (Alaluf et al., 2002). These findings however need to be confirmed in larger prospective cohort studies using objective measures of carotenoid intakes such as plasma carotenoids and reflectance spectrophotometry.

This is the first study to explore the association between dietary lutein/zeaxanthin and skin colour. We found significant associations between lutein/zeaxanthin intake and overall, unexposed and exposed skin yellowness after adjusting for confounders. This could possibly be because the relative bioavailability of lutein in particular, if consuming a variety of vegetables, is much greater than that of β-carotene (van het Hof et al., 2000). However there was no significant association between vegetable intake and skin yellowness. In addition previous research has shown that lutein and zeaxanthin accumulate in macula of the retina rather than in the skin (Scarmo et al., 2010). No data could be reported on the relationship between the absorption spectrum of lutein and diet because dietary lutein/zeaxanthin intake has been calculated together.

Unlike the findings from Whitehead et al. (Whitehead, Re, et al., 2012), the only significant relationships between combined fruit and vegetable intakes with skin redness were observed in unexposed areas. Lycopene, a pigment that is found in tomatoes and other fruits and vegetables may contribute to skin redness, just as β-carotene impacts skin yellowness (Whitehead, Re, et al., 2012). Hence one possibility for the difference in findings is the differing food lists in each respective FFQ used, with the FFQ in the current study potentially failing to capture all sources of dietary lycopene, or that lycopene intakes were low, hence the lack of significant relationship between fruit and/or vegetable intake and skin redness.

Strengths of the current study include the objective measurement of skin colour, which was based on standardised anthropometric sites (Marfell-Jones et al., 2007). Assessors were trained prior to the study starting and inter-reliability was tested amongst
researchers and multiple measures performed on both sun exposed and unexposed sites for each participant. The main limitation is the cross-sectional design of the current study. Hence only associations between intake and skin colour can be evaluated. While the relationship was adjusted for potential confounders it is possible that the residual confounding due to other factors associated with fruit and vegetable consumption could account for some of the relationship seen. Other potential limitations include the use of the FFQ to measure carotenoid intake rather than using the objective measure of plasma carotenoids. However this FFQ has been shown to provide a valid estimate of fruit and vegetable intake in comparison to plasma carotenoid concentrations (Burrows et al., 2015). The USDA database was used to calculate dietary carotenoids. This database is limited with respect to the foods that contain carotenoids although it is the most updated and reliable database available. In addition the FFQ does not include questions on how the food was prepared (e.g., with or without fat, raw or heated) as these factors highly influence the bioavailability of carotenoids (van het Hof et al., 2000). The impact of the food processing method on bioavailability of carotenoids could not be ascertained for all food items, hence results should be interpreted with caution as the bias could impact the relationship in either direction. Fruit juices were excluded from the analysis as the questions in the FFQ ask about the frequency of consumption of fruit based drinks (e.g., orange juice or poppers). These are considered as sweetened beverages and are categorised in the FFQ as non-core food/drinks. This could potentially have underestimated carotenoid intakes hence biasing the results. The FFQ reports intake over the previous six months and carotenoids are absorbed into the bloodstream after three to four days (van het Hof et al., 2000). These limitations may explain why correlations between dietary carotenoid intakes and their respective absorption spectra were not found. Only Caucasian women were included due to very small numbers from other ethnicities being recruited, which limits the external validity to this population group only. In addition, the reported fruit and vegetable intake for this group of women was high compared to the Australian population (Australian Bureau of Statistics, 2012), which could have been due to FFQ overestimation or that those participating were more health conscious compared to the general population. This also impacts external
validity, as we cannot compare these results to women who have a lower intake of fruit and vegetables. In addition, The FFQ has been validated in the adult population for nutrient intakes (Collins et al., 2014) (which includes β-carotene) and has been validated for fruit and vegetable intake (Burrows et al., 2015). There was no data collected on physical activity and hence any potential effects of exercise on skin colour cannot be controlled for. There is evidence to suggest that people who consume a healthy diet eat more fruit and vegetables and engage in physical activity (Stephen et al., 2011).

3.6 Study implications

Evidence suggests that young women are motivated to change their behaviour by appearance rather than health (LaRose et al., 2013; Traill et al., 2012). The results of the current study provide support that higher consumption of fruit and vegetables is associated with higher skin yellowness. Previous evidence has suggested that this has a beneficial effect on both perceived and actual appearance. Studies by Whitehead et al. (Whitehead, Re, et al., 2012) and Stephen et al. (Stephen et al., 2011) have shown that individuals find the yellow colouration of skin healthier and more attractive than tanned skin (Lefevre & Perrett, 2015; Whitehead, Ozakinci, et al., 2012). Therefore future interventions could focus on improving young women’s skin colour to improve their perceived health and appearance, to motivate them to increase their fruit and vegetable intake.

The findings of the current study suggest that further research is required to determine if spectrophotometer assessment has the potential for use as a biomarker for fruit and vegetable intake. Plasma carotenoids are a biological marker of fruit and vegetable intake, however analysis is expensive and labour intensive, whilst dermal spectrophotometry is non-invasive, easy to use, and generates instant results. Skin carotenoids measured by RRS have been validated against plasma carotenoids and indicate that lycopene, β-carotene and total carotenoids are significantly correlated with their respective plasma concentrations (Jahns et al., 2014; Scarmo et al., 2012). Future research should examine the relationship between repeated measurement of
skin colour using the spectrophotometer and long-term carotenoid status in comparison with plasma carotenoid concentrations.

### 3.7 Conclusions

In conclusion, the current study provides evidence that fruit, vegetable and dietary carotenoid intake has an impact on skin colour, in particular skin yellowness. Further research across a range of ethnicities other than Caucasians and including males is warranted to evaluate these relationships further. Future research evaluating whether carotenoid enrichment in skin, as measured by spectrophotometry, can be detected as a result of interventions that promote greater intakes of fruit and vegetable is warranted. Further work is also required to determine whether the observed effects could be used as a tool to motivate young women to change their dietary behaviour.
Chapter 4  Randomised cross-over trial

This article is currently under review


The work presented in the manuscript will be presented at The Annual Scientific Meeting Nutrition Society of Australia and New Zealand in December 2014, Wellington, New Zealand (Poster presentation).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 22).
4.1 Abstract

**Background** Consumption of dietary carotenoids from fruit and vegetables leads to accumulation in human skin, altering skin yellowness. The impact of the quantity of fruit and vegetables consumed on skin yellowness and plasma carotenoid concentration has not been examined previously.

**Objective** To compare the impact of consuming high carotenoid containing fruit and vegetables (HCFV) (β-carotene of 176, 425 μg/week) versus low carotenoid fruit and vegetables (LCFV) (2,073 μg /week) on skin yellowness and plasma carotenoid concentrations, over 4 weeks.

**Design/Intervention** A single blind randomised controlled cross-over trial from October 2013 to March 2014. Thirty women were randomised to receive 7-daily servings of HCFV or LCFV for 4 weeks. Following a 2 week wash-out period they followed the alternate intervention.

**Main outcome measures** Skin colour (CIE L*a*b*) was assessed by reflectance spectroscopy in both sun exposed and non-exposed skin areas. Fasting plasma carotenoids were determined by HPLC, pre- and post each intervention period.

**Statistical analysis performed** Linear mixed models were used to determine the HCFV and LCFV response on skin colour and plasma carotenoids, adjusting for intervention order, time and interaction between baseline differences and time.

**Results** There were no significant differences in mean daily fruit (p=0.42) and vegetable (p=0.17) intakes between HCFV and LCFV groups. Dietary α-carotene, β-carotene, lutein and cryptoxanthin intakes were significantly different between the two groups (p<0.01). Following HCFV there was a significantly greater increase in skin yellowness (b*) in both sun-exposed (p<0.001) and unexposed areas, (p<0.001), with no change in skin lightness (L*) or redness (a*). Significantly higher plasma α-carotene (p=0.004), β-carotene (p=0.001) and lutein (p=0.028) concentrations were found following the HCFV intervention. Skin yellowness correlated with α-carotene and β-carotene.

**Conclusions** Skin yellowness (b*) and fasting plasma carotenoid concentrations were significantly higher following HCFV than LCFV over 4 weeks.
4.2 Introduction

Carotenoids are a group of red, yellow and orange fat soluble pigments that are found primarily in fruit and vegetables (Alaluf et al., 2002). The most common dietary carotenoids are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin. β-carotene is one of the most studied carotenoids (Burrows et al., 2015) due to reported health effects and reduced risk of some diseases (Scarmo et al., 2010). β-carotene is highly abundant in brightly coloured fruit and vegetables including carrots, pumpkin and sweet potato (Ermakov & Gellermann, 2015).

Once fruit and vegetables are consumed, dietary carotenoids are absorbed via the intestines then transported through the bloodstream to various target tissues including layers of the skin (Alaluf et al., 2002; Mayne et al., 2010). Circulating carotenoid levels can be measured by biochemical methods in blood samples, while non-invasive optical methods such as reflectance spectroscopy can be used to detect carotenoids present in human skin (Ermakov & Gellermann, 2015). Reflectance spectroscopy involves measuring skin colour using CIE L*a*b* colour space (where L* represents skin lightness and positive values of a* and b* represent degrees of redness and yellowness respectively) (Alaluf et al., 2002).

The accumulation of dietary carotenoids in the human skin contributes to skin colour, particularly yellowness (b*) (Alaluf et al., 2002; Mayne et al., 2010). This yellow colouration has been reported to be perceived as more healthy and attractive by young adults than colouration from tanning (Lefevre & Perrett, 2015; Whitehead, Ozakinci, et al., 2012). Several studies have examined the association between fruit and vegetable intake and skin colour using spectrophotometry. Individuals who reported higher fruit and vegetable intake using a validated food frequency questionnaire had a skin tone that had a higher b* value (indicating increased skin yellowness) (Pezdirc et al., 2015b; Stephen et al., 2011). Increased fruit and vegetable intake over a 6-week period was also found to be associated with increased skin yellowness (b*) and redness (a*) (Whitehead, Re, et al., 2012).
Recently these associations were confirmed in a RCT conducted by Tan et al (Tan, Graf, Mitra, & Stephen, 2015), in which participants consumed a daily fruit and vegetable drink for 6-weeks. The study found significant increases of facial skin yellowness (b*) and redness (a*) in those participants in the intervention group (Tan et al., 2015).

Although evidence suggests there is an association between fruit and vegetable consumption and skin yellowness and redness, the impact of the quantity of fruit and vegetables on skin colour with intake consistent with dietary guidelines has not been previously examined. Furthermore, the relationship between changes in skin colouration and plasma carotenoid concentrations has not been evaluated.

The primary aim of this randomised controlled cross-over trial was to determine whether, over a 4 week period, consumption of fruit and vegetables high in β-carotene (HCFV) (β-carotene of 176, 425 μg/week) compared to low β-carotene fruit and vegetables (LCFV) (β-carotene of 2,073 μg/week) can lead to a difference in i) skin yellowness as measured by reflectance spectroscopy (CIE L*a*b*) and ii) plasma carotenoid concentration levels. A secondary aim was to examine the relationship between the change in skin colouration and the change in plasma carotenoids following the intervention. We hypothesise that consumption of the HCFV diet will increase skin yellowness (b*) and plasma carotenoid levels, in particular β–carotene. We also hypothesise that changes in plasma concentrations will be associated with changes in skin yellowness.

4.3 Methods
4.3.1 Study design
This study was a randomised controlled 2 × 2 cross-over trial with a 2 -week washout period, with participants blinded to group allocation. Participants were randomly assigned to either a HCFV (β-carotene 176, 425 μg/week) or LCFV (β-carotene 2,073 μg/week) intervention during the first 4 weeks (weeks 1-4). The following 2-week period (weeks 5 and 6) was a washout period based on the estimated half-life of β–carotene of 14 days (Rock et al., 1992; Yeum et al., 1996). During the second 4-week period (weeks 7-10) participants followed the alternate intervention. This study was
conducted at the University of Newcastle. The study protocol was approved by the University of Newcastle Human Research Ethics committee (H-2012-0338) (Appendix 5). The trial is registered at www.isrctn.com (ISRCTN8629745) (Appendix 6).

4.3.2 Participants

Study participants were recruited between October 2013 and March 2014, via flyers (Appendix 7) posted on the University of Newcastle’s noticeboards and through the University’s School of Nursing Research Awareness Program, where students were advised they would receive course credit points for research participation. Eligible participants were non-smoking females aged 18-30 years, with a BMI >18.5 kg/m2, who had low fruit and vegetable consumption (consumed vegetables with evening meals <3-4 per week and < 5-6 pieces of fruit per week). Young women were chosen as they have amongst the lowest adult intakes and as a group they are potentially more motivated by appearance (LaRose et al., 2013; Traill et al., 2012). Exclusion criteria included current eating disorder, pregnancy, lactation, diagnosis of liver, renal, gastrointestinal tract or cardiovascular disease, type 2 diabetes, hypertension, hypotension or with any special dietary requirements (e.g. Coeliac, FODMAPS, low fibre diet). Participants were required to abstain from using tanning/lotion/sprays and sunbathing for the 11-week study period due to the impact on skin pigmentation and the likelihood of affecting skin lightness (L*) and yellowness (b*) (Stamatas et al., 2004). They also had to be available to attend the University laboratory on 4 occasions for measurements. To improve compliance to each of the intervention regimes, boxes of fruit and vegetables were provided for the duration of both intervention periods. Participants were asked to collect their weekly fruit and vegetables boxes containing all serves of fruit and vegetables for the week on eight occasions.

Of the 154 participants who completed the online eligibility screen (Appendix 8), 50.6% (n=78) were eligible. Thirty one consented to participate and were enrolled, with written informed consent obtained (Appendix 9, 10). Participants received monetary compensation ($25 gift voucher) for their time and travel associated with data collection. Post randomisation one participants (randomly allocated to commence with the low carotenoid box) withdrew as they became pregnant. Per protocol, data analysis
was conducted using data from the 30 participants who completed both arms of the study.

4.3.3 Randomisation

Participants who were eligible were provided with a written consent form (Appendix 9). Upon written consent participants were randomly assigned to one of the two groups (HCFV or LCFV). The allocation sequence was generated by a computer-based random number producing algorithm in block lengths of five. The randomisation sequence was generated by an investigator not involved in the allocation of participants. Another investigator involved in enrolment assigned the fruit and vegetable boxes, organised the fruit and vegetables and conducted the data collection.

4.3.4 Sample size

An estimate sample size of 24 participants was required to detect a difference of 0.5 in skin yellowness (b*), assuming SD= 0.76, at $\alpha=0.05$ with 90% power. To allow for 20% dropouts, n=30 women were recruited.

4.3.5 High and low carotenoid fruit and vegetables

Participants received a weekly fruit and vegetable box for each 4-week intervention period. Each box contained a total of two servings of fruit and five servings of vegetables per day (49 servings in total) and included fresh, canned and frozen products. Fruits and vegetables were defined as having high carotenoid content if their $\beta$-carotene content was $>50\mu g/100g$ serve and low carotenoid content if $<50\mu g/100g$. Table 4.1 summarises the fruit and vegetables in each group and their respective $\beta$-carotene content. FoodWorks version 7.0.3016 (Xyris Software, Brisbane Qld Australia) was used to calculate carotenoid values based on the USDA National Nutrient database SR24 (Holden et al., 1999).
Table 4.1 Dietary β-Carotene values of the HCFV and LCFV provided to participants each week in a study to examine the effects on skin yellowness and plasmas carotenoid concentrations.

<table>
<thead>
<tr>
<th>Item</th>
<th>HCFV</th>
<th>Grams (g)</th>
<th>β-carotene (μg)</th>
<th>Item</th>
<th>LCFV</th>
<th>Grams (g)</th>
<th>β-carotene (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bag of carrots</td>
<td></td>
<td>1000</td>
<td>82850</td>
<td>1 eggplant</td>
<td></td>
<td>500</td>
<td>195</td>
</tr>
<tr>
<td>Sweet potato (medium)</td>
<td></td>
<td>250</td>
<td>21272</td>
<td>20 mushroom cups</td>
<td></td>
<td>200</td>
<td>18</td>
</tr>
<tr>
<td>Half pumpkin</td>
<td></td>
<td>1200</td>
<td>62000</td>
<td>7 pears (medium)</td>
<td></td>
<td>1400</td>
<td>140</td>
</tr>
<tr>
<td>1 Red capsicum</td>
<td></td>
<td>180</td>
<td>508</td>
<td>7 apples (red) (medium)</td>
<td></td>
<td>1050</td>
<td>63</td>
</tr>
<tr>
<td>1 whole cos lettuce</td>
<td></td>
<td>250</td>
<td>3025</td>
<td>1 cauliflower</td>
<td></td>
<td>1000</td>
<td>130</td>
</tr>
<tr>
<td>1 x tomato</td>
<td></td>
<td>110</td>
<td>165</td>
<td>1 onion (medium)</td>
<td></td>
<td>200</td>
<td>2</td>
</tr>
<tr>
<td>1 head broccali</td>
<td></td>
<td>300</td>
<td>828</td>
<td>½ white cabbage</td>
<td></td>
<td>1000</td>
<td>60</td>
</tr>
<tr>
<td>1 x cucumber</td>
<td></td>
<td>150</td>
<td>375</td>
<td>3 potatoes (medium)</td>
<td></td>
<td>300</td>
<td>3</td>
</tr>
<tr>
<td>1 x zucchini</td>
<td></td>
<td>210</td>
<td>168</td>
<td>1 parsnip</td>
<td></td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>5 x bananas (medium)</td>
<td></td>
<td>550</td>
<td>143</td>
<td>1/2 bunch celery</td>
<td></td>
<td>500</td>
<td>1350</td>
</tr>
<tr>
<td>5 x oranges (medium)</td>
<td></td>
<td>640</td>
<td>461</td>
<td>1 x tinned brown lentils</td>
<td></td>
<td>400</td>
<td>20</td>
</tr>
<tr>
<td>Frozen peas and corn</td>
<td></td>
<td>500</td>
<td>3185</td>
<td>1 x chickpeas tinned</td>
<td></td>
<td>400</td>
<td>92</td>
</tr>
<tr>
<td>1 x Tinned tomatoes</td>
<td></td>
<td>400</td>
<td>1312</td>
<td>1 tin beetroot</td>
<td></td>
<td>225</td>
<td>0</td>
</tr>
<tr>
<td>Frozen Mixed Berries</td>
<td></td>
<td>500</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>176, 425</td>
<td></td>
<td>2,073</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* HCFV=high carotenoid fruit and vegetables  
*b* LCFV= low carotenoid fruit and vegetables

Participants were encouraged to consume the fruit and vegetables and were given written instructions for each of the boxes (Appendix 12 & 13), which included suggestions on serving, preparation and cooking of raw or cooked vegetables to facilitate an increased consumption. The recipe ideas were based on the vegetables provided in the box. Participants were also provided with a list of fruit and vegetables and were told to avoid eating these when they were allocated to the LCFV group, in order to maintain the integrity of the study. Before commencing the study the participants were informed that the aim was to examine whether eating more fruit and vegetables could lead to a change in their skin colour. However they were unaware that the different groups contained different carotenoid concentrations, and therefore were blinded to intervention allocation. During the wash-out period the participants were instructed to resume their usual intake of fruit and vegetables, prior to re-commencing the intervention.
4.3.6 Outcome measures

Skin colour, fasting blood samples, usual fruit and vegetable intake, quality of life, weight and height measurements were obtained at the beginning and end of each intervention period. All measurements were completed by trained assessors. Participants were instructed to avoid wearing makeup and refrain from any exercise for each of the study visits.

4.3.6.1 Skin colour measurements

Skin colour was measured using a CM700D spectrophotometer (Konica Minolta, Osaka Japan) with an 8mm diameter aperture, 2-degree observer angle and illuminant D65. The spectrophotometer was white point calibrated for each participant at each measurement session. Skin colour (CIE L*a*b* values, where positive L*, a* and b* values represent lightness, redness and yellowness respectively) was recorded for each participant at nine body locations on the left-hand side of the body unless stated otherwise. The nine body locations included the forehead, left & right cheek, shoulder, inner arm, outer arm, palm, waist and foot sole. The measurements were repeated three times at each site and the average recorded. Body locations were selected according to anatomical landmarks using the ISAK International standards for anthropometric assessment (Marfell-Jones et al., 2007). Overall (nine body locations), sun exposed (left and right cheeks, forehead and outer arm) and unexposed (inner arm, shoulder, hip, palm and sole of foot) L*a*b* values were calculated by averaging the three measurements at each location.

4.3.6.2 Blood collection and plasma carotenoid analysis

Phlebotomists collected blood samples in EDTA-coated tubes after an overnight fast at an accredited pathology service (National Association of Testing Authorities, Australia). Plasma was separated from red blood cells by centrifugation (3,600rpm, for 15 min at 4 °C) and remaining samples were frozen within 2 hr at −80 °C. Samples were thawed and high performance liquid chromatography (HPLC) methodology was used to determine plasma carotenoid concentration of β-carotene, lycopene, α-carotene, β-cryptoxanthin and lutein/zeaxanthin as previously described (Wood et al., 2012). All extractions were carried out in a darkened laboratory under red light. To extract the
carotenoids from the plasma, ethanol was added followed by ethyl acetate containing an internal standard (canthaxanthin). The solution was then vortexed and centrifuged (3,000rpm, for 5 min at 4 °C) and the supernatant collected. This process was repeated three times, adding ethyl acetate twice, then hexane to the pellet. Milli-Q water was then added to pooled supernatant and the mixture was vortexed and centrifuged. The supernatant was decanted and placed on a nitrogen evaporator until completely evaporated. The dried extract was reconstituted in dichloromethane:methanol (1:2 vol/vol). The supernatant was decanted, the solvents evaporated with nitrogen and the sample reconstituted in dichloromethane:methanol (1:2 v/v). Chromatography was performed on an Agilent 1200 HPLC system using Hypersil ODS column (100mm X 2.1mm X 5um). Carotenoids were analysed using a mobile phase of acetonitrile: dichloromethane: methanol 0.05 % ammonium acetate (85:10:5v/v) at a flow rate of 0.3mL/min, using a diode array detector (450 nm).

4.3.7 Other measurements
Demographic data including age, ethnicity, nutritional supplementation use and skin type were measured via an online survey prior to commencing the study (Appendix 11).

Participants self-reported their fruit and vegetable intake using the Australian Eating Survey, a 120–item semi-quantitative FFQ. The FFQ has been validated for fruit and vegetable intake in comparison to plasma carotenoid concentrations in the population group studied (Burrows et al., 2015; Collins et al., 2014). Twenty-two questions related to the intake of vegetables and 11 to fruit. Total servings of fruit and vegetables were calculated by summing the weight of relevant food items estimated by the FFQ, divided by the standard serving size indicated by the Australian Guide to Healthy Eating (fruit serving 150g, vegetable serving 75g) (Australian Government Department of Health, 2013). The FFQ included questions on seasonal fruit with intakes calculated using participant consumption frequency when these items were in season, adjusted by the number of months each year the fruit is available. Daily carotenoid intakes of α-carotene, β-carotene, lycopene and combined lutein-zeaxanthin, were calculated from the FFQ fruit and vegetable responses using the US Department of Agriculture
National Cancer Institute carotenoids food composition database (Chug-Ahuja et al., 1993). Compliance with consumption of the fruit and vegetables provided each week was confirmed by a self-reported acceptability questionnaire (Appendix 14) at the end of each 4-week intervention period. The questionnaire was based on overall consumption of fruit and vegetables and the difficulty of consuming and cooking the type and quantity of fruit and vegetables provided.

Quality of life was assessed using the paper-based SF-12, version 2.0 (QualityMetric Incorporated, Lincoln, RI, USA), a multipurpose, generic, short-form health survey consisting of an 8 sub scales to measure functional health and well-being (Ware, Kosinski, Turner-Bowker, & Gandek, 2002).

Weight was measured using the Inbody720 Body Composition Analyzer (Biospace Co., Ltd., Seoul, Korea). BMI was calculated using the standard equation (weight kg/height m²). Height was measured using a portable BSM370 stadiometer correct to 0.1 cm using the stretch stature method. The average of two measures was used for both weight and height.

### 4.3.8 Statistical analysis

This current study employed a cross-over design and participants served as their own control. The statistical programs SAS (Version 9.4 SAS Institute, Cary, NC, USA) and Stata (Version 11.0; StataCorp, College Station, TX, USA) were used for the data analysis. For all statistical analyses, p<0.05 was considered significant. Normally distributed variables are presented as means ± SD otherwise medians (interquartile ranges) are reported. A paired t-test was used to evaluate whether there were any intervention carry-over effects between the baselines values of all outcome variables. Linear mixed models were used for each HCFV and LCFV outcome response adjusting for the difference in baseline responses (Mehrotra, 2014). The models were then further adjusted for HCFV and LCFV order, time and the interaction between baseline difference and time. The within person intervention effect was assessed using a t-test with the Kenward –Roger method of estimating denominator degrees of freedom (Mehrotra, 2014). Unstructured variance-covariance structure was used to model the
within-person correlations and restricted maximum likelihood was used for parameter estimation. Intention-to-treat analysis was used for the missing fasting blood sample. Spearman correlations were used to test the correlation between change in skin colour and change in plasma concentrations.

### 4.4 Results

Demographic and baseline characteristics of the participants [n=30 females, median (IQR) age 22.0 (4.2) years, BMI 23.4 (9.7) kg/m²] are summarised in Table 4.2. Participants remained weight stable throughout the study (p=0.94). There was no significant difference in any of the eight SF-12 subscales for quality-of-life between the intervention groups during the study.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>White (Australia, American/British) 25 (83.4)</td>
<td></td>
</tr>
<tr>
<td>Asian (Chinese) 4 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Other 1 (3.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Currently taking Supplements n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Age 22.0 (4.2)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg) 64.8 (22.4)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²) 23.4 (9.7)</td>
<td></td>
</tr>
<tr>
<td>Body fat % 29.2 (16.6)</td>
<td></td>
</tr>
<tr>
<td><strong>SF12 scores</strong></td>
<td></td>
</tr>
<tr>
<td>Physical functioning 100 (25)</td>
<td></td>
</tr>
<tr>
<td>Role physical 87.5 (25)</td>
<td></td>
</tr>
<tr>
<td>Bodily pain 100 (25)</td>
<td></td>
</tr>
<tr>
<td>General health 60 (25)</td>
<td></td>
</tr>
<tr>
<td>Vitality 50 (50)</td>
<td></td>
</tr>
<tr>
<td>Social functioning 75 (25)</td>
<td></td>
</tr>
<tr>
<td>Role emotional 87.5 (25)</td>
<td></td>
</tr>
<tr>
<td>Mental health 75 (25)</td>
<td></td>
</tr>
<tr>
<td><strong>Dietary intake</strong></td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kJ/day) 7914.5 ± 2192.2</td>
<td></td>
</tr>
<tr>
<td>Total fat intake (g/day) 70.8 ± 23.3</td>
<td></td>
</tr>
<tr>
<td>Fruit intake (servings/day) 1.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Vegetable intake (servings/day) 3.2 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Total Fruit and vegetable intake (servings/day) 4.4 ± 2.0</td>
<td>1458.0 ± 697.3</td>
</tr>
<tr>
<td>α-carotene (μg/day) 5237.4 ± 2783.2</td>
<td></td>
</tr>
<tr>
<td>β-carotene (μg/day) 323.0 ± 323.9</td>
<td></td>
</tr>
<tr>
<td>Lutein (μg/day) 1699.1 ± 1161.9</td>
<td></td>
</tr>
<tr>
<td>Lycopene (μg/day) 4625.5 ± 2610.7</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma carotenoids</strong></td>
<td></td>
</tr>
<tr>
<td>α-carotene (μmol/l) 0.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>β-carotene (μmol/l) 0.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Lutein (μmol/l) 0.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Lycopene (μmol/l) 0.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>β-cryptoxanthin (μmol/l) 0.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td><em><em>Skin Colour (CIE L<em>a</em>b</em>)</em>*</td>
<td></td>
</tr>
<tr>
<td>Overall L* 64.5 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Overall a* 9.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Overall b* 16.9 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Unexposed L* 66.0 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Unexposed a* 7.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Unexposed b* 16.9 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Exposed L* 62.7 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Exposed a* 11.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Exposed b* 16.8 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD or medians (interquartile range 75-25), HCFV=high carotenoid fruit and vegetables, LCFV=low carotenoid fruit and vegetables, Unexposed=Total of Inner arm, shoulder, hip, palm and sole of foot, Exposed=Total of face (left and right cheeks, forehead) and outer arm.
4.4.1 Outcome measures

4.4.1.1 Skin colour

The mean (± SD) overall, unexposed and exposed skin colour L*a*b* at baseline and the end of each intervention period are summarised in Table 4.3. After adjusting for baseline skin colour L*a*b* differences, intervention order and time, the HCFV intervention resulted in significantly higher skin yellowness (b*) overall (0.6, p<0.001) and in the unexposed (0.70, p<0.001) and exposed (0.5, p<0.001) skin sites compared to the LCFV intervention. There was no significant impact on overall skin lightness (L*) or redness (a*) values. There was no evidence of a carryover effect on overall skin colour or plasma carotenoid concentrations.

4.4.1.2 Plasma carotenoids

Changes in plasma carotenoid concentrations are summarised in Table 4.4. There were significantly higher plasma concentrations of α-carotene (0.09, p=0.004), β-carotene (0.49, p=0.001) and lutein (0.07, p=0.028) following the HCFV intervention compared to the LCFV intervention, with no significant change in lycopene or β-cryptoxanthin plasma concentrations.

4.4.1.3 Correlations between skin colour and plasma carotenoids

Correlations between change in skin colour and change in plasma carotenoid concentrations are summarised in Table 4.5. Weak positive correlations were observed between overall (r=0.35), facial (r=0.35), unexposed(r=0.31) and exposed (r=0.28) skin colour yellowness (b*) and plasma β-carotene concentrations. There were weak positive correlations for overall (r=0.29) and unexposed (r=0.27) skin colour yellowness (b*) and α-carotene. No correlations were seen between skin lightness (L*) and redness (a*) and plasma carotenoid concentrations.

4.4.1.4 Fruit, vegetable and dietary carotenoid intake

The intakes of fruit, vegetable and dietary carotenoids are summarised in Table 4.6. The mean intake of fruit and vegetables at follow-up were 2.2 and 4.6 servings/day for the HCFV and 2.4 and 4.2 servings/day for the LCFV group. Both groups met their fruit intake of 2 serves per day and were close to the target vegetable intake of 5 serves
per day. There was no difference in the number of participants who reported that they consumed all 49 serves per week of fruit and vegetables between the HCFV (n=7) and the LCFV (n=6) group (p=0.10).

There were no significant differences reported in total fruit or vegetable intakes between the HCFV or LCFV interventions groups, and intakes remained the same after adjusting for differences in baseline fruit and vegetable intake, time, intervention order and interaction. During the HCFV intervention α-carotene (p=0.001), β-carotene (p=0.0004), cryptoxanthin (p<0.0001) and lutein/zeaxanthin (p=0.031) dietary intakes were significantly higher compared to the LCFV intervention, with no difference in lycopene intakes (p=0.44).
Table 4.3 Skin color results at baseline and after study participants consumed each HCFV and LCFV for 4 weeks

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Baseline</th>
<th>End of intervention</th>
<th>Baseline</th>
<th>End of intervention</th>
<th>Estimate</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCFV</td>
<td>LCFV</td>
<td>HCFV</td>
<td>LCFV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall L*</td>
<td>64.66 ± 2.86</td>
<td>64.62 ± 2.41</td>
<td>64.68 ± 2.35</td>
<td>64.74 ± 2.37</td>
<td>-0.12</td>
<td>(-0.44-0.19)</td>
<td>0.439</td>
</tr>
<tr>
<td>Overall a*</td>
<td>9.17 ± 1.33</td>
<td>9.33 ± 1.16</td>
<td>9.37 ± 1.08</td>
<td>9.25 ± 1.18</td>
<td>0.18</td>
<td>(-0.08-0.45)</td>
<td>0.171</td>
</tr>
<tr>
<td>Overall b*</td>
<td>16.77 ± 2.35</td>
<td>17.29 ± 2.40</td>
<td>17.01 ± 2.33</td>
<td>16.67 ± 2.46</td>
<td>0.60</td>
<td>(0.36-0.84)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Facial L*</td>
<td>63.65 ± 2.74</td>
<td>63.54 ± 2.20</td>
<td>63.60 ± 2.22</td>
<td>63.63 ± 2.28</td>
<td>-0.14</td>
<td>(-0.52-0.23)</td>
<td>0.442</td>
</tr>
<tr>
<td>Facial a*</td>
<td>12.13 ± 1.58</td>
<td>12.29 ± 1.38</td>
<td>12.34 ± 1.52</td>
<td>12.24 ± 1.79</td>
<td>0.15</td>
<td>(-0.30-0.59)</td>
<td>0.506</td>
</tr>
<tr>
<td>Facial b*</td>
<td>15.21 ± 2.36</td>
<td>15.78 ± 2.35</td>
<td>15.43 ± 2.44</td>
<td>15.03 ± 2.55</td>
<td>0.69</td>
<td>(0.35-1.03)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Unexposed L*</td>
<td>66.11 ± 2.85</td>
<td>66.13 ± 2.57</td>
<td>66.09 ± 2.48</td>
<td>66.19 ± 2.50</td>
<td>-0.06</td>
<td>(-0.43-0.32)</td>
<td>0.767</td>
</tr>
<tr>
<td>Unexposed a*</td>
<td>7.23 ± 1.33</td>
<td>7.38 ± 1.25</td>
<td>7.41 ± 1.10</td>
<td>7.31 ± 1.09</td>
<td>0.13</td>
<td>(-0.19-0.45)</td>
<td>0.406</td>
</tr>
<tr>
<td>Unexposed b*</td>
<td>16.91 ± 2.45</td>
<td>17.50 ± 2.54</td>
<td>17.17 ± 2.39</td>
<td>16.85 ± 2.52</td>
<td>0.67</td>
<td>(0.38-0.96)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Exposed L*</td>
<td>62.85 ± 3.06</td>
<td>62.74 ± 2.48</td>
<td>62.91 ± 2.44</td>
<td>62.93 ± 2.43</td>
<td>-0.19</td>
<td>(-0.57-0.18)</td>
<td>0.293</td>
</tr>
<tr>
<td>Exposed a*</td>
<td>11.60 ± 1.53</td>
<td>11.76 ± 1.28</td>
<td>11.82 ± 1.34</td>
<td>11.67 ± 1.54</td>
<td>0.18</td>
<td>(-0.15-0.51)</td>
<td>0.262</td>
</tr>
<tr>
<td>Exposed b*</td>
<td>16.59 ± 2.42</td>
<td>17.03 ± 2.39</td>
<td>16.81 ± 2.50</td>
<td>16.45 ± 2.53</td>
<td>0.55</td>
<td>(0.23-0.87)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Note:**
- **HCFV** = high carotenoid fruit and vegetables
- **LCFV** = low carotenoid fruit and vegetables
- Adjusting for differences in baselines, time, intervention order and interaction between baseline difference and time
- **Facial** = Total of left and right cheeks and forehead
- **Unexposed** = Total of Inner arm, shoulder, hip, palm and sole of foot
- **Exposed** = Total of face (left and right cheeks, forehead) and outer arm

CIE L*a*b* = L* represents skin lightness, positive values of a* and b* represents degrees of redness and yellowness.
Table 4.4 Plasma carotenoids concentrations results at baseline and after study participants consumed each HCFV and LCFV for 4 weeks

<table>
<thead>
<tr>
<th></th>
<th>HCFV Baseline (n=29)</th>
<th>HCFV End of intervention (n=29)</th>
<th>LCFV Baseline (n=29)</th>
<th>LCFV End of intervention (n=29)</th>
<th>Intervention effect</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-Carotene</td>
<td>0.09 ± 0.15</td>
<td>0.17 ± 0.24</td>
<td>0.09 ± 0.07</td>
<td>0.10 ± 0.16</td>
<td>0.09</td>
<td>(0.03-0.14)</td>
<td>0.004</td>
</tr>
<tr>
<td>Beta-Carotene</td>
<td>0.27 ± 0.30</td>
<td>0.67 ± 1.18</td>
<td>0.31 ± 0.27</td>
<td>0.29 ± 0.43</td>
<td>0.50</td>
<td>(0.21-0.77)</td>
<td>0.001</td>
</tr>
<tr>
<td>Beta-Cryptoxanthin</td>
<td>0.13 ± 0.15</td>
<td>0.15 ± 0.21</td>
<td>0.12 ± 0.13</td>
<td>0.15 ± 0.25</td>
<td>-0.01</td>
<td>(-0.05-0.04)</td>
<td>0.813</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.36 ± 0.39</td>
<td>0.37 ± 0.36</td>
<td>0.32 ± 0.30</td>
<td>0.29 ± 0.29</td>
<td>0.07</td>
<td>(0.01-0.13)</td>
<td>0.028</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.44 ± 0.56</td>
<td>0.58 ± 0.83</td>
<td>0.49 ± 0.67</td>
<td>0.46 ± 0.84</td>
<td>0.13</td>
<td>(-0.07-0.34)</td>
<td>0.192</td>
</tr>
</tbody>
</table>

\(^a\) HCFV=high carotenoid fruit and vegetables

\(^b\) LCFV= low carotenoid fruit and vegetables

\(^c\) Adjusting for differences in baselines, time, intervention order and interaction between baseline difference and time
Table 4.5 Correlations between change in skin color (CIE L'*a'*b'*) and plasma carotenoids concentrations (μmol/l)

<table>
<thead>
<tr>
<th></th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-cryptoxanthin</th>
<th>Lutein</th>
<th>Lycopene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall L</td>
<td>-0.03</td>
<td>-0.06</td>
<td>0.03</td>
<td>-0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Overall a</td>
<td>0.05</td>
<td>0.10</td>
<td>0.09</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>Overall b</td>
<td>0.29*</td>
<td>0.35**</td>
<td>0.05</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>Facial L</td>
<td>-0.04</td>
<td>-0.00</td>
<td>-0.02</td>
<td>-0.15</td>
<td>-0.03</td>
</tr>
<tr>
<td>Facial a</td>
<td>0.00</td>
<td>0.00</td>
<td>0.11</td>
<td>0.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Facial b</td>
<td>0.26</td>
<td>0.35**</td>
<td>0.07</td>
<td>0.01</td>
<td>0.21</td>
</tr>
<tr>
<td>Unexposed L</td>
<td>-0.13</td>
<td>-0.16</td>
<td>-0.01</td>
<td>-0.06</td>
<td>-0.02</td>
</tr>
<tr>
<td>Unexposed a</td>
<td>0.06</td>
<td>0.15</td>
<td>0.15</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>Unexposed b</td>
<td>0.27*</td>
<td>0.31*</td>
<td>0.09</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Exposed L</td>
<td>0.02</td>
<td>-0.04</td>
<td>-0.00</td>
<td>-0.14</td>
<td>-0.04</td>
</tr>
<tr>
<td>Exposed a</td>
<td>0.05</td>
<td>0.11</td>
<td>0.13</td>
<td>0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>Exposed b</td>
<td>0.25</td>
<td>0.28*</td>
<td>0.05</td>
<td>-0.02</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*p<0.05, ** p<0.001
Table 4.6 Fruit, vegetable and dietary carotenoid intake at baseline and after study participants consumed each HCFV and LCFV for 4 weeks

<table>
<thead>
<tr>
<th></th>
<th>HCFV(^a) (n=30)</th>
<th>LCFV(^b) (n=30)</th>
<th>Estimate(^c)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End of intervention</td>
<td>Baseline</td>
<td>End of intervention</td>
<td></td>
</tr>
<tr>
<td>Fruit (servings)</td>
<td>1.57 ±1.04</td>
<td>2.16 ± 1.24</td>
<td>1.48 ± 0.92</td>
<td>2.40 (1.41)</td>
<td>-0.20</td>
</tr>
<tr>
<td>Vegetable (servings)</td>
<td>3.53 ±1.64</td>
<td>4.56 ±1.61</td>
<td>3.37 ±1.58</td>
<td>4.16 (1.51)</td>
<td>0.38</td>
</tr>
<tr>
<td>α-Carotene (ug/day)</td>
<td>1593.3 ± 686.72</td>
<td>2733.1 ± 1553.2</td>
<td>1831.0 ±1275.9</td>
<td>1376.5 ±823.80</td>
<td>1307.13</td>
</tr>
<tr>
<td>β-Carotene (ug/day)</td>
<td>5671.0 ± 2792.8</td>
<td>9008.1 ±4263.1</td>
<td>6145.8 ±3726.5</td>
<td>5106.9 ±3018.5</td>
<td>3808.76</td>
</tr>
<tr>
<td>Cryptoxanthin (ug/day)</td>
<td>343.81 ±323.48</td>
<td>588.91 ±390.40</td>
<td>348.30 ±338.34</td>
<td>283.47 ±290.50</td>
<td>305.10</td>
</tr>
<tr>
<td>Lutein/zeaxanthin (ug/day)</td>
<td>1811.8 ± 1191.2</td>
<td>2402.0 ± 1192.1</td>
<td>1763.4 ±1013.0</td>
<td>1851.2 ±1140.0</td>
<td>540.10</td>
</tr>
<tr>
<td>Lycopene (ug/day)</td>
<td>4917. ± 2212.8</td>
<td>4280.0 ± 2625.0</td>
<td>4409.7 ±2581.9</td>
<td>3948.7 ±2249.2</td>
<td>352.21</td>
</tr>
</tbody>
</table>

\(^a\) HCFV=high carotenoid fruit and vegetables  
\(^b\) LCFV= low carotenoid fruit and vegetables  
\(^c\) Adjusting for differences in baselines, time, intervention order and interaction between baseline difference and time
4.5 Discussion

The primary aim of this randomised controlled cross-over trial was to compare the differences in impact on skin yellowness (b*) and plasma carotenoid concentration over 4 weeks, following consumption of equivalent amounts of fruit and vegetables that were either high in β-carotene (HCFV) or of low (LCFV) β-carotene content. Significant increases were detected in overall, unexposed, exposed and facial skin colour yellowness (b*) following consumption of the HCFV, as well as higher plasma concentrations of α, β-carotene and lutein after 4 weeks. To our knowledge the current study is one of few to show skin yellowness change by providing fruit and vegetables with specified carotenoid content (Tan et al., 2015).

The primary hypothesis was supported, with significant positive correlations found between change in skin yellowness (b*) and plasma carotenoid concentrations. Weak positive correlations were found between change in skin yellowness (b*) and change in plasma α-carotene and β-carotene. No correlations were found for plasma lutein.

Findings from the current study indicate that even after a short period of consuming fruits and vegetables high in β-carotene, there is a measurable increase in skin yellowness (b*). The specific fruits and vegetables selected for the HCFV intervention arm were rich sources of β-carotene, a yellow-orange pigment, and this led to greater skin yellowness. Previous research has shown that higher skin yellowness is associated with higher total fruit and vegetable intakes and that higher skin yellowness is perceived by young adults as appearing more healthy and attractive (Lefevre & Perrett, 2015; Whitehead, Ozakinci, et al., 2012; Whitehead, Re, et al., 2012). This, together with results of the current study suggest that consuming a variety of HCFV, rather than LCFV, could potentially lead to a skin colour that is perceived to be healthier and more attractive.

Findings from the current study support previous research demonstrating that higher skin yellowness is associated with higher fruit and vegetable intakes (Pezdirc et al., 2015b; Tan et al., 2015; Whitehead, Re, et al., 2012). However, unlike other studies (Tan...
et al., 2015; Whitehead, Re, et al., 2012) the current intervention had no impact on skin redness ($a^*$), which has previously been shown to be associated with higher dietary lycopene intake (Stephen et al., 2011; Tan et al., 2015). Lycopene is found in red coloured fruit and vegetables, such as tomatoes and watermelon. We did not find an increase in skin redness following the HCFV, despite an estimated lycopene content of 11,669μg/week from the HCFV tomato-based products. This lack of effect is likely to be explained by the lack of change in dietary lycopene intake or plasma lycopene concentrations. The majority of fruit and vegetables provided in HCFV were higher in β-carotene, as opposed to lycopene specifically. Lycopene is also found in high concentrations in non-fruit and vegetables sources including pizza, pastas and tomato ketchup. Another possibility is that dietary intake of lycopene-rich foods in the background diet decreased from pre to post in both intervention groups, due to the consumption of alternative fruits and vegetables in the boxes provided. Lastly other factors other than diet can affect skin redness including oxygenation of the blood (Stephen et al., 2011).

As anticipated, plasma concentrations of α, β-carotene and lutein were higher following consumption of fruit and vegetables from the HCFV group compared to the LCFV group. These findings confirm that the quantity of dietary carotenoids provided from fruits and vegetables, in particular of β-carotene, was different between the HCFV and LCFV. The current results also indicate that if the goal is to optimise plasma carotenoid levels, then the type of fruit and vegetables consumed is also important.

Both groups reported an increased overall intake in daily fruit and vegetable servings compared to baseline with no significant difference between HCFV and the LCFV. This was an unexpected result, as we had anticipated that fruit and vegetable intakes and compliance would be significantly higher during the HCFV arm due to the variety of brightly coloured commonly consumed fruit and vegetables provided (Scarmo et al., 2010).
Strengths of the current study include the randomised cross-over design and the use of plasma carotenoids as biomarkers to supplement dietary carotenoids. Skin colour was measured objectively by trained researchers using standardised anthropometric sites. All fruit and vegetables were supplied weekly during both intervention periods to optimise compliance. Participants in the current study served as their own control, which reduced error variance and the potential for observation bias. Limitations of the current study include the small relatively homogenous sample of predominantly Caucasian females. The use of a cross-over study design can be seen as a limitation due to potential carry-over effects, however, our statistical analysis confirmed that the washout period that we used was adequate to avoid carry-over. It was not possible to blind all study researchers involved in data collection, however we used predominantly objective outcome measures to reduce bias. While weekly leftover fruit and vegetables from each participant were not weighed, plasma carotenoid biomarkers were measured on all 4 occasions as an indicator of participant adherence.

4.6 Conclusions

In summary, the current study demonstrates that consumption of HCFV for 4 weeks leads to a significant increase in skin yellowness and plasma carotenoid concentrations compared to LCFV in young women. While these results need to be confirmed in a larger more diverse population sample they suggest that skin yellowness can be increased by consuming a variety of fruit and vegetables high in carotenoids.
Chapter 5  Perception of health study

This article is currently submitted


The work presented in the manuscript was also presented at The International Society for Behavioural Nutrition and Physical Activity in May 2014, San Diego, CA USA (Poster Presentation).

The work presented in the manuscript was completed in collaboration with the co-authors (Appendix 23).
5.1 Abstract

Human skin colour is influenced by three pigments: haemoglobin, carotenoids and melanin. Carotenoids are abundant in fruits and vegetables, and when consumed accumulate in all layers of the skin, predominantly imparting yellowness (b*). This study investigated the effect of the manipulation of carotenoid-based skin colour, relative to the skin colour conferred by melanin on the perceptions of health amongst a group of Australian adults. Fifty-seven participants (n=4 male; mean age 27.9±7.5 years) completed three computer-based experiments on 50 trial faces. In the first two experiments, face image colour was manipulated along one or two independent single carotenoid or melanin axes on each trial to "make the face appear as healthy as possible". In the third trial, face colour was manipulated on both the carotenoid and melanin axes simultaneously. For the single axis, participants significantly increased melanin colouration and added carotenoid colouration to facial images that were initially low in skin yellowness (b*). When carotenoid and melanin axes were simultaneously manipulated, carotenoid colouration was raised (ΔE 3.15 (SE ± 0.19) and melanin colouration was lowered ΔE -1.04 (SE ± 0.1). Young Australian adults perceive facial skin colouration, associated with both carotenoid intake from fruit and vegetables and melanin due to sun exposure as, conveying the appearance of health in young adults. However carotenoid colouration was perceived more important to perception of overall health.
5.2 Introduction

Human skin colour is determined by three major pigments. Haemoglobin contributes to red colouration, melanin which darkens and yellows the skin colour and carotenoids which contribute predominantly to skin yellowness (Alaluf et al., 2002). Carotenoids are lipid soluble, red, orange and yellow pigments, abundant in many fruits and vegetables. Following fruit and vegetable consumption, these pigments accumulate in all layers of human skin (Alaluf et al., 2002; Mayne et al., 2010).

Fruit and vegetable consumption is related to the skin yellowness (b*) axis of the CIE L*a*b* colour space, where L* represents skin lightness and positive values of a* and b* represents degrees of redness and yellowness respectively (Pezdirc et al., 2015b; Stephen et al., 2011). Self-reported increases in fruit and vegetable intake over a six-week period have shown to be associated with a measurable increase in skin yellowness (b*) and redness (a*) (Whitehead, Re, et al., 2012).

Skin colour due to carotenoid content has been shown to affect young adults’ perceptions of an individual’s overall appearance of health (Stephen et al., 2011; Stephen et al., 2009; Whitehead, Ozakinci, et al., 2012). Recent studies using colour calibrated Caucasian facial images have shown that greater yellowness (b*) and lightness (L*) is perceived by Scottish adults as conveying a more healthier (Stephen et al., 2011; Stephen et al., 2009; Whitehead, Ozakinci, et al., 2012) and attractive appearance (Lefevre & Perrett, 2015). These results suggest that humans may express a preference for skin colour associated with higher intakes of fruit and vegetables (carotenoids), rather than skin colour associated with sun exposure (melanin) (Stephen et al., 2011; Stephen et al., 2009; Whitehead, Ozakinci, et al., 2012). Although this relationship has been investigated in other ethnicities including black South Africans and Asians (Stephen et al., 2011) it has not been investigated in an Australian population where sun exposure is common.

In Australia, young adults perceive sun tanned skin, to be healthy and attractive compared to untanned white skin (Hamilton et al., 2012). In addition, young Australian adults have one of the lowest intakes of fruit and vegetables compared to
other adult life stages with only 40.6% those aged 19-30 years meeting the guidelines of two servings of fruit and 2.9% meeting five servings of vegetables per day (Australian Bureau of Statistics, 2014).

Therefore, the aim of the current study is to investigate the perceptions related to health associated with carotenoid-based skin colour, relative to the skin colour conferred by melanin in a group of young Australian adults. It is hypothesised that participants will increase both melanin and carotenoid colouration of facial skin to enhance their perception of a healthy appearance. When colour manipulation is applied in isolation (i.e. two independent, single axes) perceptions of healthiness will be associated with an increased carotenoid colouration and increased melanin colouration. When manipulation of both colours is simultaneously applied (i.e. two-dimensional colour space) perceptions of health will reflect an increased carotenoid colouration and removal of melanin colouration.

5.3 Methods

5.3.1 Participants

A convenience sample (n=60) was recruited from April-September 2013 from the University of Newcastle campus through advertisements on notice boards (Appendix 15) and via a research program in the School of Nursing and Midwifery. Eligible participants were ≥18 years of age, proficient in English, self-reported normal colour vision and able to attend the university campus to complete the computer-based tests. All participants received a coffee voucher to compensate for their time and those from the School of Nursing and Midwifery research program were eligible to receive five course credit points. The study was approved by the University of Newcastle Human Research Ethics Committee (H-2012-0405) (Appendix 16). All participants provided informed consent (Appendix 17, 18).

Self-reported demographic data (e.g. age, gender, ethnicity), skin colouration type and quality (Fitzpatrick.T.B, 1988), height, weight, general health and fruit and vegetable intake was obtained for each participant (Appendix 19).
5.3.2 Trial face images

A detailed description of the photography methods has been reported elsewhere (Stephen et al., 2011; Stephen et al., 2009; Whitehead, Re, et al., 2012). Standardised photographs of 50 Caucasian adults (25 male: 25 female, mean age 20.5 range 18–27, SD ±1.75 years) were taken in a grey-coloured booth under standardised lighting conditions, with colour calibration conducted using a Gretag Macbeth Mini ColourChecker (Pantone). Hair was held back off the face and clothing covered with a grey coloured board to prevent colour reflection.

5.3.3 Melanin and carotenoid axis values

Existing skin colour measurements (CIE L*a*b* colour space) previously obtained from spectrophotometer readings from human skin were used to derive the melanin and carotenoid skin colour axis values (Stephen et al., 2011; Whitehead, Ozakinci, et al., 2012). Carotenoid values were determined based on the relationship between fruit and vegetable intake and change in skin colour, measured by the mean change in facial colour per additional portion of fruit and vegetables consumed (Stephen et al., 2011; Whitehead, Ozakinci, et al., 2012). This was scaled to a range between -40 to +40 portions of fruit and vegetables per day over a six week period to form the high carotenoid mask at L*=-4.12; a*=5.16 b*=15.96 and the low carotenoid mask at L*=4.12; a*=-5.16 b*=-15.96 (Whitehead, Ozakinci, et al., 2012). Melanin values were derived by measuring the difference between a sun-exposed region of the outer arm and unexposed region of the shoulder (Stephen et al., 2011; Whitehead, Ozakinci, et al., 2012). The high melanin mask was set at L*=-10.40; a*=-2.42 b*=14.20 and the low melanin mask at L*=10.40; a*=2.42 b*=-14.20. The melanin masks thus gave the approximately the same colour difference across the manipulation range (ΔE=17.8) as the carotenoid colour masks (ΔE=17.2).

Colour masks for each of the 50 trial face images were developed in MATLAB (using methods described in (Stephen et al., 2011; Whitehead, Ozakinci, et al., 2012) and reflected changes in single carotenoid (b*) and melanin (L*) one-dimensional axes. Mean CIEL*a*b* values across skin pixels for each image were calculated using MATLAB to define initial face colour. Thirteen image masks for each single axis were
developed and applied to each trial face. The matrix of image masks represented equal increments in colour change (from high to low colour) along a continuum, with the endpoints reflecting maximal colour change (increase and decrease) and the midpoint no change (i.e. original image).

Two-dimensional facial masks were generated by transforming each of the 13 single melanin axis frames along the carotenoid axis (13 frames), consisting of a total of 169 images (13x13).

5.3.4 Colour manipulation experiments

Each participant completed three computer-based experiments on 50 trial faces “to make the face appear as healthy as possible”. In the first two experiments participants independently manipulated the trial face colour along each single axis for carotenoid or melanin by moving the mouse horizontally to select the face frame image with the colour that they perceived as making each face appear the healthiest. In the third experiment, participants manipulated the skin colour along the two-dimensional carotenoid and melanin axes simultaneously, which allowed them to adjust both carotenoid colouration and melanin skin colouration using both horizontal and vertical movements of the mouse. In all three experiments trial faces appeared in random order using the same 50 faces, meaning that participants manipulated the colour for each face three times.

The computer tests were completed in a darkened room with no natural or artificial light, using a laptop with a 17.3 in LCD screen (Dell Inspiron 17R- 5721, Dell Inc., USA). To minimise potential variability introduced by the height and angle of the computer screens, the laptops were placed on laptop stands and the angle of screen set at ~90° from the base of the stand (i.e. perpendicular). The computer LCD screens were calibrated weekly using Spyder4 Pro (Datacolour, USA).

5.3.5 Data analysis

Data analysis was undertaken using Stata (Version 11.0; StataCorp, College Station, TX, USA). A significance level was set at 5%. Univariate ANOVA was used to compare the average colour change in melanin and carotenoid amongst the female and male face
trials. One sample t-tests were used to compare overall colour change which was expressed as ΔE (Euclidian distance is a standard measure of colour change in the CIE L*a*b* colour space using the formula $\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$). Linear regression was used to test the associations between starting yellowness (b*) of facial trials and melanin and carotenoid preferences.

5.4 Results

5.4.1 Participants

Sixty participants completed the set with all trial faces. For three participants an unexpected error with the computer program occurred during testing, which resulted in them not viewing the full set of trial faces. These participants were excluded leaving 57 (n=4 males) for analysis. The mean (±SD) age of participants was 27.9 ± 7.5 years with a BMI of 25.6 ± 7.7 kg/m². Seventy-two percent (n=41) reported their ethnicity as “White Australian” and 43.9% (n=25) reported their skin type as “burn easily” and skin quality as “average”. Self-reported fruit and vegetable intakes were 1.9 ± 1.1 and 2.5 ± 1.3 servings per day respectively, and 66.7% of participants reported not eating adequate fruit and vegetables compared to national recommendations.

5.4.2 Single axis experiment - melanin

The mean overall colour change preferred in the male face trials was ΔE 0.31 (SE ± 0.08) and for female faces ΔE 0.36 (SE ± 0.10). There was no main effect of the face trials by sex (p=0.72).

Figure 5.1 demonstrates an overall weak effect for melanin colour to be increased by participants. The amount of colour added was significantly associated with initial skin yellowness (b*) of the trial faces. Participants increased melanin colouration more for those faces which initially were low in yellowness (b*) ($\beta=-0.10$, t=-3.83, p<0.001 $R^2 =0.23$).
5.4.3 Single axis experiment - carotenoids

The mean colour change identified in the male face trials was ΔE 2.41 (SE ± 0.26), equivalent to 5.6 ± 0.6 additional servings of fruit and vegetables per day. In female faces it was ΔE 3.20 (SE ± 0.20), which was equivalent to 7.4 ± 0.6 additional daily servings of fruit and vegetables. When comparing colour change applied to male and female faces there was a main effect of sex (F=4.56, p=0.038), with more yellowness (b*) added to female faces. There was a significant preference for increased carotenoid values in both female (t24=12.30, p<0.0005) and male (t23=8.93, p<0.005) faces.

Figure 5.2 illustrates a strong overall preference for higher carotenoid colouration. Participants added carotenoid colouration more to those faces which were initially low in yellowness (b*) (β=-0.386, t=-5.245, p<0.001, R²=0.36).
5.4.4 Double axis experiment

One male trial image was excluded from the analysis due to accidental omission, with another male trial viewed twice, therefore the second result from the double axis experiment for that face was excluded leaving n=49 trial faces for analysis. The average carotenoid axis colour change was ΔE 3.15 (SE ± 0.19) and for melanin axis ΔE -1.04 (SE ± 0.1). The average colour change was ΔL* -0.15 (SE ± 0.04), Δa* 1.09 (SE ± 0.06; Δb* 2.09 (SE ± 0.15).

When participants were able to simultaneously manipulate both the level of melanin and the carotenoid colouration, they removed significantly more melanin (t48=-13.51, p<0.001) and added significantly more carotenoid (t48=16.82, p<0.001) compared to the single axis conditions. The overall colour change indicated a preference for higher facial skin yellowness (Δb* =+2.09± 0.15, t48 =14.05, p<0.001) and slightly lowered lightness (ΔL* =-0.15±0.04, t48 =-3.45, p=0.001).
Figure 5.3 shows that when both melanin and carotenoid colouration were able to be manipulated simultaneously there was a significantly higher change in carotenoid colouration ($\Delta E = 3.15$) relative to melanin ($\Delta E = -1.04$).

![Figure 5.3 Colour change applied along a carotenoid axis (fruit and vegetables) and melanin skin axis to original face images](image)

**5.5 Discussion**

The current study demonstrates that perceptions of the appearance of health of a group of facial images of young adults, by Australian adults is related to skin colouration contributed by higher carotenoid content (skin yellowness) and decreased melanin content (skin tanning). When the two colours were manipulated independently in the single axis experiments, in both conditions adults evaluating the facial images significantly increased melanin colouration and increased skin carotenoid colouration. When both carotenoid and melanin colouration were manipulated simultaneously in the facial images, participants chose to increase carotenoid colouration and remove melanin colouration in order to make the faces appear the most healthy. While both pigments contribute to a rise in the appearance of skin yellowness, higher melanin
induces a reduction in overall skin lightness. Of interest was that participants increased both carotenoid and melanin colour more in the facial images that were initially low in skin yellowness (b*). This suggests that skin colouration that is of low yellowness is viewed by other adults as appearing less healthy compared to higher levels of yellowness.

Our findings from the single carotenoid and melanin axes manipulations are consistent with previous studies conducted with Caucasian adults in Scotland (Stephen et al., 2011; Whitehead, Ozakinci, et al., 2012). In those studies, participant’s added relatively higher amounts of carotenoid based colour and smaller amounts of melanin to face images (Stephen et al., 2011). When Scottish participants simultaneously manipulated both the colour axes the results diverged from the Australian participants. Scottish participants like Australian participants increased carotenoid colouration more than melanin. However, Australian participants removed melanin colouration whereas Scottish participants significantly increased melanin based colour (Stephen et al., 2011; Whitehead, Ozakinci, et al., 2012). This suggests that Scottish adults associate a higher level of skin melanin pigmentation as conferring a healthy appearance compared to Australian adults. This finding could potentially be due to cultural differences in relation to tanning between the two groups and an increased awareness of the associated dangers of prolonged sun exposure likely to be greater in Australia due to increased risk of skin cancer compared to Scotland (Hamilton et al., 2012).

In the current study we found that carotenoid colouration was preferred more in female faces compared to male faces. These findings have also recently been reported from a study with a group of Scottish adults who have examined attractiveness in facial images (Lefevre & Perrett, 2015). There were no sex difference in preferences for melanin colouration however preferences for melanin were significantly higher in male faces for attractiveness compared to female faces (Lefevre & Perrett, 2015).

This current study has several limitations. The majority (93%) of participants in the current study were females thus limiting the ability to generalise the findings to Australian men. In studies conducted in Scottish participants they had a range of 38-
45% males participating. A further limitation may relate to the effects of using different facial identities or computer monitors as previous studies used cathode ray tube monitors, which may explain some of these differences. However, to minimise any potential effect of the display device, the monitors were regularly colour calibrated and setup in a standardised way with the screen viewed at a standardised angle.

5.6 Conclusion

In summary, Australians perceive facial skin colouration associated with both carotenoid intake from fruit and vegetables and melanin due to sun exposure as conveying the appearance of health in young adults. However, colouration due to carotenoids was preferred over melanin as contribution to the appearance of health in young adult facial images. Further research using Australian facial images and rating performed by male participants is warranted in order to evaluate these relationships in the population more broadly.
Chapter 6  Discussion and recommendations for future research and practice

6.1 Overview

This chapter summarises the key findings of the body of research undertaken and places them in the context of current literature. The overall findings from this thesis are then examined in relation to the original hypotheses. The strengths and limitations of this body of research are presented in (Section 6.4). This chapter closes with making future recommendations (Section 6.5) and concluding remarks (Section 6.6).

6.2 Summary of findings and discussion

6.2.1 Association between dietary intake and skin colour appearance

One of the first aims of this thesis was to examine established associations between dietary intake and actual appearance, which was addressed by the systematic review in Chapter 2. Nine observational studies (4 Cross-sectional, 1 prospective cohort study, 4 cases studies) were included in the review. Overall, most studies found statistically significant associations between dietary intakes, in particular fruit and vegetables and skin colour yellowness (b*) (n=6 studies) and measures of appearance (skin colour and skin aging). Due to the limited number of studies and different methodologies used to assess appearance, the review concluded that more studies were required to further examine these relationships, particular using sound study designs and validated methods to measure food intake and appearance.

A cross-sectional study (Chapter 3) was designed based on the limitation of the validation methods to assess dietary intake identified in the systematic review. The primary aim of the cross-sectional study was to evaluate potential associations between fruit, vegetable and dietary carotenoid intakes and skin colour in a sample of young women. A subsidiary/secondary aim of the study was to examine the relationships between fruit, vegetable and dietary carotenoid intakes and skin colour measured by reflectance spectrophotometry in order to verify whether carotenoid pigments are responsible for the observations in skin colour. Skin colour (CIE L*a*b colour space...
values) and spectral reflectance (nm) was measured using reflectance spectroscopy where the spectrophotometer shines a light of specific wavelengths using a filter and measures the intensity of the light reflected back from the skin. A validated 120-item semi-quantitative FFQ (Australian Eating Survey) (Collins et al., 2014) was used to assess fruit, vegetable and dietary carotenoid intake. This FFQ has been validated for fruit and vegetable intake against plasma carotenoid concentrations in adults demonstrating strong correlation between fruit and vegetables and plasma α-carotene, β-carotene and lutein/zeaxanthin carotenoids (Burrows et al., 2015).

The results indicated that women who self-reported higher fruit and vegetable intakes had significantly higher skin yellowness (b*) values for both overall and unexposed skin sites. For each additional serving of fruit and vegetable per day there was an increase of 0.8 units of overall skin yellowness and 1.0 units in unexposed skin yellowness. Statistically significant negative correlations were found between total fruit and vegetable intakes and skin reflectance at wavelengths associated with light absorption by carotenoids across the wavelength range (400–540 nm) corresponding to the peaks for β-carotene, lycopene and total mean carotenoids. For fruit consumption alone, these relationships were strongest at wavelengths associated with light absorption peaks due to common dietary carotenoids (α-carotene, β-carotene and lycopene). For vegetable intake alone this was only demonstrated for the absorption spectra corresponding to that of lycopene.

These results further support the findings from the previous studies (n=2) identified in the systematic review that also reported that higher fruit and vegetable consumption was associated with higher skin yellowness (b*) (Stephen et al., 2011; Whitehead, Re, et al., 2012). However, our findings did not support the previous studies that identified a relationship between overall skin redness (a*) and total fruit and vegetable intakes. Their findings suggested that lycopene, a pigment found in fruit and vegetables such as tomatoes, may contribute to skin redness (a*). A possible reason for this difference is that the list of fruit and vegetable items in each FFQ could have differed and also the FFQ used in this study may not have captured all sources of dietary lycopene.
6.2.2 The effect of dietary intake on skin colour appearance

A secondary aim of the systematic review (addressed in Chapter 2) was to examine the evidence on the effectiveness of dietary interventions on perceived or actual appearance. Appearance was defined as either an individual’s perception of physical appearance or an objective measure of physical appearance such as skin colour, tone or body shape. Included studies had to report dietary intake from either whole foods or dietary supplement use or both.

A total of 18 experimental studies (17 RCT’s, 1 pre-post study) that examined the impact of a nutrition intervention (whole foods (n=1), nutrient supplements (n=15)) on appearance were included. The fifteen studies that had evaluated the effect due to dietary supplements used a range of active ingredients including polyphenols, omega 3 fatty acids, vitamins E and C, carotenoids, green tea, squalene and red ginseng. The majority of the studies showed significant improvements in at least one outcome relating to skin health and appearance (facial wrinkling, skin roughness, elasticity and skin colour) following consumption of the dietary supplements. However the lack of homogeneity across all the studies made it impossible to determine which active ingredients or dosage related most strongly to these improvements or for which skin or appearance related outcome.

There was only one study that examined the effects of dietary intake using whole foods (Micozzi et al., 1988). This RCT evaluated plasma carotenoid levels and skin colour following consumption of an equal dose of β-carotene supplements or vegetables high in β-carotene over 12 weeks (30 mg per day). This study was conducted over 25 years ago, was in a small sample of men (n=30) and had a moderate risk of bias.

Consequently, the review concluded that there was insufficient evidence on the actual impact of whole food intake on appearance related outcomes and further studies were required to examine the impact, in particular on skin colour appearance, using validated tools in well-designed high-quality RCTs.
Therefore, the RCT cross-over trial (Chapter 4) was designed to further examine the impact of dietary intake on appearance with a specific focus on high and low carotenoid fruit and vegetables. At the time of the systematic review, no studies had examined the impact of providing participants with the recommended daily servings of fruit and vegetables, according to the national recommendations, on skin colour change. The primary aim of this study was to determine whether consuming the same total quantity of fruit and vegetables that were either high in β-carotene versus low in β-carotene was associated with a difference in skin yellowness (b*) and in plasma carotenoid concentrations over a four week intervention period.

Results from this study revealed that consuming high β-carotene fruit and vegetables resulted in significantly higher skin yellowness (b*). These findings support previous research, including the previously described cross-sectional study, demonstrating that higher skin yellowness is associated with higher fruit and vegetable intakes (Pezdirc et al., 2015b; Tan et al., 2015; Whitehead, Re, et al., 2012). However, unlike other studies (Tan et al., 2015; Whitehead, Re, et al., 2012), our results found no impact on skin redness (a*), which has previously been shown to be associated with higher dietary lycopene intake. (Stephen et al., 2011; Tan et al., 2015).

Higher plasma α-carotene, β-carotene and lutein concentrations were found after consuming the high β-carotene fruit and vegetables compared to the low β-carotene intervention period. When the study went on further to examine the relationship between the change in skin coloration and the change in plasma carotenoids, significant positive correlations were found between change in plasma α-carotene and β-carotene and change in skin yellowness (b*). This study is one of the first studies to demonstrate the impact of fruit and vegetable intake on skin colour, in particular skin yellowness, over a relative short period of time by providing participants with fruit and vegetables with a specific carotenoid content.

6.2.3 Does fruit and vegetable intake influence perceived facial health

The systematic review identified no studies that focused on dietary intake and perceived appearance. Therefore the final component of this research project was to
examine whether skin colouration attributed to fruit and vegetable consumption influences our perception of health. Previous research has examined the impact of carotenoid skin colouration from fruit and vegetables compared to melanin colouration due to sun exposure/tanning, on the perception of health (Lefevre & Perrett, 2015; Stephen et al., 2011; Stephen et al., 2009; Whitehead, Ozakinci, et al., 2012). These studies were conducted in a group of Scottish adults where they evaluated a series of facial images. It was found that carotenoid colouration is perceived by young adults as appearing more healthy and attractive than skin coloration attributed to melanin (Lefevre & Perrett, 2015; Stephen et al., 2011; Stephen et al., 2009; Whitehead, Ozakinci, et al., 2012).

This appearance evaluation research had only been conducted in Scottish participants, where sun exposure is less common compared to Australia. In addition, sun tanned skin has been reported as being perceived to be healthy and attractive to young Australian adults compared to untanned white skin (Hamilton et al., 2012). Therefore this study was conducted to evaluate the perception of the appearance of health and attractiveness amongst a group of Australian adults, using the same series of facial images as was used in the study of Scottish adults (Chapter 5).

The overall results showed that Australians adults perceived facial skin coloration indicative of higher carotenoid content and decreased melanin, attributed to sun exposure, as appearing healthier when evaluating facial images of young adults. These findings are mostly consistent with the previous studies in Scottish participants as they both increased carotenoid coloration. However, unlike Australian participants, the Scottish participants also significantly increased melanin colouration. This suggests that the Scottish participants perceive melanin colouration due to sun tanning as associated with a more healthy appearance, compared to Australian participants.

Although the results have shown that skin yellowness is perceived to be healthier among young Australian adults it is not evident if this is limited to Caucasians only as the study only used Caucasian facial images. Furthermore it would important to
determine if young adults perceive skin yellowness to be attractive, as this component was also not evaluated in the facial images used in the study.

This study has demonstrated that in a group of Australian adults carotenoid colouration from fruit and vegetables on facial images is perceived to be healthier compared to sun exposure. However further research using Australian facial images with diverse ethnicities and assessments performed by male participants is required in order to evaluate these relationships in the broader population.

6.3 Findings from this thesis in relation to the research hypotheses

The findings of the research in this thesis are considered in response to the pre-determined hypotheses below.

1. A healthy diet is associated with appearance of health.

The findings from the systematic review does not support the hypothesis as there was limited number of studies, using different methodologies with poor study design. In addition only one study examined the effects of dietary intake of actual food.

2. Young women who reported higher fruit and vegetable intake will have greater skin yellowness (CIE b* values).

This hypothesis was supported as women who reported higher total fruit and vegetable intake had significantly higher overall skin yellowness values b* (β=0.8, p=0.017).

3. The consumption of the high carotenoid fruit and vegetables will increase skin colour, in particular skin yellowness (b*) and plasma carotenoid levels, more than the low carotenoid fruit and vegetables.
This hypothesis was supported as the high carotenoid fruit and vegetables intervention resulted in significantly higher skin colour yellowness (b*) for overall (0.6, p<0.001), unexposed (0.70, p <0.001) and exposed (0.5, p<0.001) sites compared to the low carotenoid fruit and vegetables.

This hypothesis was supported as there were significantly higher plasma concentrations of α-carotene (0.09, p=0.004), β-carotene (0.49, p=0.001) and lutein (0.07, p=0.028) during the high carotenoid fruit and vegetables intervention period.

4. Changes in plasma concentrations will be correlated with changes in skin yellowness (b*)

This hypothesis was supported as there were positive correlations between changes in overall (r=0.35), facial (r=0.35), unexposed(r=0.31) and exposed (r=0.28) skin sites for skin colour yellowness (b*) and plasma β-carotene concentrations.

In addition there were positive correlations between change in overall (r=0.29) and unexposed (r=0.27) sites for skin colour yellowness (b*) and α-carotene plasma concentrations.

5. Australian adults’ perception of health on facial images will be associated with a change in skin colour that is associated with higher carotenoid and melanin colouration.

This hypothesis was rejected for the double axis experiment as participants increased carotenoid colouration, however reduced the melanin colouration. The original hypothesis was based on the results found from research conducted in Scottish adults. This difference suggests that the Scottish attribute skin colour due to melanin colouration from sun tanning as conferring a healthy appearance compared to Australian adults.
6.4 Strengths and limitations of the research project

There are specific strengths and limitations that need to be acknowledged for each study.

6.4.1 Systematic review

The review has a number of strengths. The systematic review was novel as it was the first review internationally that sought to evaluate the body of evidence related to the relationship between dietary intake and overall appearance of health and attractiveness. This review examined the relationship between both dietary intake from food and dietary supplements on actual or perceived appearance. The conduct and reporting of this systematic review adhered to the PRISMA statement and a comprehensive search strategy was used across seven databases. Detailed data extraction was undertaken and the studies were critically appraised using a standardised quality assessment tool to identify risk of bias. The review had several limitations. The review only included studies up to August 2012, since then there has been one other study (Tan et al., 2015), which has been referred to and discussed in Chapter 4. In addition some publications may have been missed especially those published in other languages other than English.

6.4.2 Cross-sectional study

The cross-sectional study had several strengths. Skin colour was measured objectively using a spectrophotometer and the measurement sites were based on internationally accepted standardised anthropometric sites (Marfell-Jones et al., 2007). Inter-rater reliability of skin colour measurements on both exposed and unexposed body locations was tested amongst researchers prior to commencing data collection (Pezdirc et al., 2015b). However, this study also had a number of limitations that need to be recognised. A FFQ was used to measure dietary carotenoid intake rather than measurement of plasma carotenoids. However the FFQ has been shown to provide a valid estimate of fruit and vegetable intakes in comparison to plasma α, β-carotene and lutein concentrations in adults (Burrows et al., 2015). The USDA database was used to calculate estimated dietary carotenoid intakes (Holden et al., 1999), although it is the
most up-to-date database and is reliable, it is limited in regards to the number of foods for which carotenoid values are reported. Thus dietary carotenoid intake may be underestimated. Despite this, the study found significant associations and hence these methods could be used in future studies. Only Caucasian women were included in the analysis and hence findings may not be generalisable to other ethnicities and for males.

6.4.3 RCT cross-over trial

The trial had several strengths. It employed a high methodological quality RCT cross-over design, with each subject serving as their own control. A high retention rate was achieved for the 11 week duration (94%). Plasma carotenoid concentrations were used as an independent biomarker of intake, in addition to estimated dietary intakes of both fruit and vegetables and dietary carotenoids derived from the FFQ responses. To optimise participant compliance, all fruit and vegetables were supplied weekly, during both interventions periods. However, limitations include the small and relatively homogenous sample of predominantly Caucasian females. The sample size was calculated based on the expected change in skin colour from previous research. However, the previous research was in Caucasian populations, whereas our study did not exclude participants based on ethnicity. It was not possible to blind all study researchers involved in data collection. To minimise the potential effect from lack of study blinding, objective outcome measures (such as fasting plasma carotenoids and skin colour measured by spectrophotometer) were predominantly used to reduce the risk of bias.

6.4.4 Perception of health study

One main strength of the perception of health study was that the methods used had been previously developed and tested by researchers from St Andrews University in Scotland. However, this study had several limitations. Firstly, the colour value ranges attributed to fruit and vegetable consumption that were used for the facial image assessments represented extreme increases and decreases in intake corresponding to ±40 portions per day. Although this is not realistic in terms of usual intake this was necessary in order to evaluate potential extremes in terms of how participants may manipulate skin colour attributed to health. Secondly, the sample was predominately
female limiting the ability to generalise the findings to Australian men, and hence limiting the external validity of the study in terms of the whole population. All facial images used in the study were Caucasian therefore the lack of ethnic diversity limits the generalisability of the findings. In the future this study needs to be repeated in males and also in population samples of more ethnic diversity.

6.5 Implications and recommendations for future research

The implications and future recommendations below are based on the research findings from this thesis.

1. This thesis has demonstrated a strong relationship between fruit, vegetable and dietary carotenoid intakes and skin colour in young Australian females. Further research is warranted to confirm findings in a range of ethnicities, males and older adults.

2. Positive correlations between skin yellowness and plasma concentrations were found from the consumption of high carotenoid fruit and vegetables. Further research is required to assess the validity and reproducibility of repeated measurement of skin colour using the spectrophotometer against changes in plasma carotenoids and dietary carotenoid intake. Research should be conducted in diverse population samples and age groups. This will determine whether the spectrophotometer assessment can be used as a non-invasive biomarker of fruit and vegetable intake and change in intake. If the spectrophotometer can be shown to be a potential biomarker this would greatly assist in nutritional interventions and in a clinical setting assessing nutrition related health outcomes for the following reasons:

   - Currently, plasma carotenoids, after accounting for confounding variables, are the best biological marker of fruit and vegetable intake (Burrows et al., 2015). The gold standard method by which to measure plasma carotenoids is by HPLC, however this methods is expensive and carries a high analytic burden
and well as the participant burden and cost in terms of collection of a blood samples and time taken to obtain the results.

- There are numerous methods in use for measuring fruit and vegetable intake however the most frequently used to measure compliance of interventions are 24-hour recalls, weighed food record and FFQ. All these measures have limitations, as there is potential for measurement error and bias related to recording or recall across all self-reported methods. Each method can take considerable time to complete and hence increase participant burden. All carry a substantial researcher burden in terms of analysis and reporting. The spectrophotometer is a non invasive objective measure and does not have the same issues as these methods, in addition it reduces participant burden.

3. The findings of this study indicate that young Australian adults perceive carotenoid skin colouration attributed to fruit and vegetable consumption as appearing more healthy relative to that conferred by melanin colouration. Further studies need to be conducted using facial images of Australians with diverse ethnicities to confirm these findings and also to determine if there is variability based on ethnicity. In addition, asking participants to evaluate their perception related to healthiness of their own facial images and appearance is warranted.

6.5.1 Appearance based interventions

The findings from this thesis have strengthened the evidence regarding the relationship between fruit and vegetable intake and skin colour appearance and how we perceive health. The results from this study and the recommendations made in this thesis could be used to design an appearance based intervention. In the literature review (Chapter 1) it was identified that young women have one of the lowest fruit and vegetable intakes compared to other adults. Interventions promoting an increase in fruit and vegetable intakes in adults have shown some success. However, there have only been a few interventions that have solely focused on young adults, especially women. Current evidence suggests that young women may be motivated to change their behaviours to change their, appearance rather than being motivated by
improving their health outcomes. Therefore a behavioural intervention that focuses on appearance, as a motivator to improve fruit and vegetable intake may be effective but requires further testing.

6.6 Concluding remarks

The research conducted as part of this thesis has further strengthened the relationship between dietary intake and appearance, in particular with fruit and vegetable intake and skin colour. This novel research has demonstrated that skin colour yellowness can be increased by consuming a variety of fruits and vegetables high in carotenoids. It has also provided some evidence that Australian adults perceive carotenoid skin colouration from fruit and vegetables to be healthier than melanin colouration. Further research is needed to confirm these findings in larger broader population in order to potentially develop an appearance based interventions.
References


World Health Organisation. (2014). Global Strategy on Diet, Physical Activtiy and Health: Promoting fruit and vegetable consumption around the world: WHO.

Appendices
Appendix 1: Cross-sectional study flyer

VOLUNTEERS NEEDED
Are you 18 years and over?

Would you like to participate in a research study to evaluate whether your fruit and vegetable intake is related to your skin colour?

Details
- We are conducting a study to find out whether the amount of fruit and vegetables you consume has an influence on your skin colour appearance.
- This is done by asking you to fill in an online survey and to then make an appointment to come to our research lab in the Hunter Building on the Callaghan campus to complete a Food Frequency Questionnaire on your usual food intake and have some measurements taken of your body composition, blood pressure and skin colour on one occasion.
- You will receive feedback on your measurements. In addition you will be invited to return to the lab on another occasion if you would like to, to undergo the assessments a second time.

Who can volunteer?
We need approximately 300 adults aged 18 years and over to volunteer:
- You must be able to travel to and from the University on one occasion for assessments and have access to the internet to complete the online survey.

This project has been approved by The University of Newcastle Human Research Ethics Committee, Approval Number H-2012-0217. Chief investigator Professor Clare Collins

CONTACT
If you are interested or for further information please go to:
https://www.surveymonkey.com/s/795JTLX

or contact:
Kristine Plezirc
T 4621 7374
Email: fruitvegskincolour@newcastle.edu.au
Appendix 2: Ethics approval for Cross-sectional study

HUMAN RESEARCH ETHICS COMMITTEE

Notification of Expedited Approval

To Chief Investigator or Project Supervisor: Professor Clare Collins
Cc Co-investigators / Research Students: Doctor Melinda Neve
                                           Mr Ross Whitehead
                                           Professor David Perrett
                                           Dr Gozde Ozakinci
                                           Miss Kristine Pezdirc

Re Protocol: Is there a link between Fruit and Vegetable intake and skin colour?

Date: 08-Aug-2012
Reference No: H-2012-0217
Date of Initial Approval: 07-Aug-2012

Thank you for your Response to Conditional Approval submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under Expedited review by the Chair/Deputy Chair.

I am pleased to advise that the decision on your submission is Approved effective 07-Aug-2012.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research, 2007, and the requirements within this University relating to human research.

Approval will remain valid subject to the submission, and satisfactory assessment, of annual progress reports. If the approval of an External HREC has been "noted" the approval period is as determined by that HREC.

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal Certificate of Approval will be available upon request. Your approval number is H-2012-0217.

If the research requires the use of an Information Statement, ensure this number is inserted at the relevant point in the Complaints paragraph prior to distribution to potential participants. You may then proceed with the research.

Conditions of Approval

This approval has been granted subject to you complying with the requirements for Monitoring of Progress, Reporting of Adverse Events, and Variations to the Approved Protocol as detailed below.
PLEASE NOTE:
In the case where the HREC has “noted” the approval of an External HREC, progress reports and reports of adverse events are to be submitted to the External HREC only. In the case of Variations to the approved protocol, or a Renewal of approval, you will apply to the External HREC for approval in the first instance and then Register that approval with the University’s HREC.

- Monitoring of Progress

Other than above, the University is obliged to monitor the progress of research projects involving human participants to ensure that they are conducted according to the protocol as approved by the HREC. A progress report is required on an annual basis. Continuation of your HREC approval for this project is conditional upon receipt, and satisfactory assessment, of annual progress reports. You will be advised when a report is due.

- Reporting of Adverse Events

1. It is the responsibility of the person first named on this Approval Advice to report adverse events.
2. Adverse events, however minor, must be recorded by the investigator as observed by the investigator or as volunteered by a participant in the research. Full details are to be documented, whether or not the investigator, or his/her deputies, consider the event to be related to the research substance or procedure.
3. Serious or unforeseen adverse events that occur during the research or within six (6) months of completion of the research, must be reported by the person first named on the Approval Advice to the (HREC) by way of the Adverse Event Report form within 72 hours of the occurrence of the event or the investigator receiving advice of the event.
4. Serious adverse events are defined as:
   - Causing death, life threatening or serious disability.
   - Causing or prolonging hospitalisation.
   - Overdoses, cancers, congenital abnormalities, tissue damage, whether or not they are judged to be caused by the investigational agent or procedure.
   - Causing psycho-social and/or financial harm. This covers everything from perceived invasion of privacy, breach of confidentiality, or the diminution of social reputation, to the creation of psychological fears and trauma.
   - Any other event which might affect the continued ethical acceptability of the project.
5. Reports of adverse events must include:
   - Participant's study identification number;
   - date of birth;
   - date of entry into the study;
   - treatment arm (if applicable);
   - date of event;
   - details of event;
   - the investigator’s opinion as to whether the event is related to the research procedures; and
   - action taken in response to the event.
6. Adverse events which do not fall within the definition of serious or unexpected, including those reported from other sites involved in the research, are to be reported in detail at the time of the
annual progress report to the HREC.

- Variations to approved protocol

If you wish to change, or deviate from, the approved protocol, you will need to submit an Application for Variation to Approved Human Research. Variations may include, but are not limited to, changes or additions to investigators, study design, study population, number of participants, methods of recruitment, or participant information/consent documentation. Variations must be approved by the (HREC) before they are implemented except when Registering an approval of a variation from an external HREC which has been designated the lead HREC, in which case you may proceed as soon as you receive an acknowledgement of your Registration.

Linkage of ethics approval to a new Grant

HREC approvals cannot be assigned to a new grant or award (ie those that were not identified on the application for ethics approval) without confirmation of the approval from the Human Research Ethics Officer on behalf of the HREC.

Best wishes for a successful project.

Professor Allyson Holbrook
Chair, Human Research Ethics Committee

For communications and inquiries:
Human Research Ethics Administration
Research Services
Research Integrity Unit
HA148, Hunter Building
The University of Newcastle
Callaghan NSW 2308
T +61 2 492 18999
F +61 2 492 17164
Human-Ethics@newcastle.edu.au

Linked University of Newcastle administered funding:

<table>
<thead>
<tr>
<th>Funding body</th>
<th>Funding project title</th>
<th>First named investigator</th>
<th>Grant Ref</th>
</tr>
</thead>
</table>

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Appendix 3: Cross-sectional information statement

Appendix 2

Information Statement for the Research Project:
Is there a link between Fruit & Vegetable intake and Skin colour?
Version 7; dated 15/0/13

We would like to offer you the opportunity to participate in a research study which is being conducted by Professor Clare Collins, Dr Melinda Neve and Ms Kristine Pezdiric from the Priority Research Centre in Physical Activity and Nutrition at the University of Newcastle.

The research is part of Ms Kristine Pezdiric’s PhD studies at the University of Newcastle, supervised by Professor Clare Collins and Dr Melinda Neve from the Faculty of Health Sciences /Priority Research Centre in Physical Activity and Nutrition.

Why is the research being done?

The project aims to find out whether the amount of fruit and vegetables you consume can influence your actual skin colour appearance.

Who can take part?

We are seeking adults aged 18 years and over to participate in this research.

You can participate in this project if you are:

• Aged 18 years and over.
• Able to complete an online survey about you and your general health and a Food Frequency Questionnaire
• Able to travel to the Callaghan campus of the University of Newcastle on one occasion to have your weight, height, body composition, blood pressure and skin carotenoid and melanin measurements taken.

What choice do you have?

Participation in this research is entirely your choice. You will be only included in the project if you have given your informed consent online. Whether or not you decide to participate, your decision will not disadvantage you. If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data that identifies you.
What would you be asked to do?

If you agree to participate you will be asked to:

- Complete an online survey about you, your health, skin type and your perception of appearance.

You will also be asked to visit the University of Newcastle Nutrition & Dietetics Anthropometry lab on ONE occasion to:

- Complete a questionnaire about your usual food intake (Food Frequency Questionnaire).
- Have your weight and body composition measurements taken using a bioelectrical impedance analyser. This will determine your body composition in terms of muscle and fat tissues.
- Have your height taken using a portable stadiometer.
- Have your blood pressure taken.
- Have your skin carotenoid and melanin measurements taken of skin at your shoulders; arms, face, hand, foot sole and hip using a hand held device called a spectrophotometer.

In addition, participants will be invited after 4-6 weeks to return to the lab if they are interested to repeat the same measures to see if their fruit and vegetable intake has changed and how this relates to the other measures.

How much time will it take?

- Completing the online survey should take around 15 minutes.
- Attendance at the lab to have your height, weight, body composition, blood pressure, skin measurements and to complete the food frequency questionnaire will take approximately one hour.

What are the benefits and risks of participating?

There are no known risks in this study. You may experience some discomfort from getting your blood pressure taken. The spectrophotometer used to measure your skin carotenoids is an entirely safe and simple process. This involves pressing the device to the skin surface to measure the level of carotenoid and melanin compounds are in the skin. All other measures in the study such as body composition and the questionnaires use standard tools that have been widely used in research.

Some questions in the online survey are of a sensitive nature (e.g. Requesting information about your prescribed medication, acne and feelings about your personal appearance). As with all information collected, your answers to these questions will be kept completely confidential and your name will not be stored alongside your responses. If you experience any feelings that are overwhelming or distressing while answering these questions, please seek help from your doctor. You also have the option of contacting Lifeline on 13 11 44 or
the University Counselling service on 49215801. The counselling service is available only to students of the University of Newcastle.

You will receive standardized individual feedback on your individual results for your body composition, blood pressure, fruit and vegetable intake and skin carotenoids and melanin levels from the researchers.

You will receive 5 bonus course marks towards one of the following courses NURS1101/NURS1201/NURS2101/NURS2201/NURS3101/NURS3201/MID2101/MID2101/MID2201/MID3102.

How will your privacy be protected?

Your online survey responses will be directed to a password protected Survey Monkey account, which only the research team has access to. Please refer to the Survey Monkey Privacy and Security policy http://www.surveymonkey.com/mp/policy/security/.

Your appointment booking through Doodle link will be also directed to a password protected Doodle link account which only the research team will have access to. Please refer to the Doodle Privacy Policy http://www.doodle.com/about/policy.html

All data obtained from your appointment at the lab will be de-identified by replacing names with numerical codes. De-identified data will be retained for at least 5 years at the University of Newcastle by University staff. No individual will be identifiable in any reports arising from the study; only summarised data will be presented.

How will the information collected be used?

The results of the research will be reported and distributed via national and international conferences and peer reviewed publications. You will not be personally identified in any reports arising from the study. Once the study is completed you will receive a brief summary from the Chief Investigator.

What do you need to do to participate?

Please read this Information Statement carefully and be sure you understand its contents before you consent to participate. If there is anything you do not understand, or have any questions, contact the research team.

Further information

If you would like further information about the study please contact Professor Clare Collins initially on 4921 5646 or fruitnvegnskincolour@newcastle.edu.au

Complaints about this research

This project has been approved by the University’s Human Research Ethics Committee, Approval No.H-2012-0217________
Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au.

I have read the information statement and would like to participate in the study. If you select yes this will be your informed consent to participate in the study. In addition you will be required to complete a consent form at your attendance at the Nutrition and Dietetics Anthropometry lab.

☐ Yes

☐ No - thank you for your time, you can exit the survey now

**ONLINE ELIGIBILITY SCREENING**

The following question is to check if you are eligible to participate in our study.

Are you 18 years or over?

☐ Yes (continue to survey)

☐ No (your are ineligible for this study, thank you for your interest in the study)
ON-LINE SURVEY

This survey will cover information about you and your food intake. You will be asked questions about your age, education, income, employment, living arrangements, health, medications, skin and appearance. In addition we will ask you to complete a food frequency questionnaire.

The following questions are in regards to details about you.

1. What is your Gender? (Drop down box Male, Female)
   - Male
   - Female

2. What is your date of birth? DD/MM/YYYY (Drop down boxes)

3. Please indicate your ethnic group (please tick all that apply)
   - White Australian
   - White British
   - White other European
   - White American
   - Hispanic
   - Chinese
   - Japanese
   - South east Asian
   - Other Asian
   - Indian
   - Polynesian
   - Mixed
   - Other – please specify

4. What is your postcode ----
5. Who lives with you? (tick all that apply)

☐ Partner/Spouse
☐ Own children
☐ Parents
☐ Other adults
☐ I live alone

The following questions are in regards to details about your education, income and employment.

6. What is the highest qualification you have completed? (mark one only)

☐ No formal qualifications
☐ Year 10 or equivalent (eg School Certificate)
☐ Year 12 or equivalent (eg Higher School Certificate)
☐ Trade / Apprenticeship (eg Chef, Hairdresser)
☐ Certificate / Diploma (eg Child care, Technician)
☐ University degree
☐ Higher university degree (eg Grad Dip, Masters, PhD)

7. How do you manage on the income you have available?

☐ It is impossible
☐ It is difficult
☐ It is difficult some of the time
☐ It is not too bad
☐ It is easy
8. How would you describe your current employment status? (mark one only)

☐ Employed full-time
☐ Employed part-time
☐ Employed, but currently on leave
☐ Retired
☐ Unemployed and seeking work
☐ Unemployed, but not seeking work
☐ Don’t wish to answer

The following questions are in regards to your health and medications

9. What health conditions or problems have you been diagnosed or treated for
(Please tick as many boxes as required and for any other please specify in the space provided)

☐ None of these conditions
☐ High cholesterol
☐ High blood pressure
☐ Type 1 Diabetes
☐ Type 2 Diabetes
☐ Arthritis
☐ Hepatitis B or C
☐ Liver disease
☐ Acne
☐ Eczema/Psoriasis
☐ Dermatitis
☐ Glandular Fever
☐ High Bilirubin (Gilberts Syndrome)
☐ Loss of pigmentation of the skin (vitiligo)
10. Would you say your health in general is

☐ Excellent
☐ Very good
☐ Good
☐ Fair
☐ Poor

11. How many times a year do you get a cold or flu?

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10
☐ 11
☐ 12
☐ 12+
12. How many days does one of your average cold or flu bouts last?

- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9
- [ ] 10
- [ ] 11
- [ ] 12
- [ ] 13
- [ ] 14
- [ ] 15 + days

13. How many times did you take a course of antibiotics in the last year?

- [ ] 1
- [ ] 2
- [ ] 3
- [ ] 4
- [ ] 5
- [ ] 6
- [ ] 7
- [ ] 8
- [ ] 9
- [ ] 10
14. Have you been ill in the last week?
   - Yes
   - No

15. In the last week have you had acne?
   - No
   - Yes a little
   - Yes a lot
   - Yes very much

16. Are you currently taking any prescribed medications? (including the contraceptive pill) If yes, please list the medication and dose where possible.
   - No
   - Yes
      - Medications and doses (Drop down comments box)

17. Are you currently taking any supplements or multivitamins? If yes, please list the type and brand where possible.
   - No
   - Yes (Drop down comments box)

18. Please list any products that you regularly apply to your skin? (e.g. moisturizer, creams, sunscreens, masks or cosmetics treatments, foundation, medical creams)
19. Do you currently smoke tobacco products
   □ Yes, Daily
   □ Yes, At least once a week
   □ Yes, but Less often than once a week.
   □ No, Not at all (go to question 20)

20. Did you ever smoke? If so how long ago did you quit?

21. Have you ever smoked at least 100 cigarettes or a similar amount of tobacco in your life ?)
   □ Yes
   □ No
   □ Not sure

22. How soon after waking up do you smoke your first cigarette?
   □ Within 5 minutes
   □ 6-30 minutes
   □ 31-60 minutes
   □ 61+ minutes

23. how many cigarettes do you smoke in a day?
   □ 10 or less
   □ 11-20
   □ 21-30.
   □ 31+
The following questions are in regards to your skin type

24.

<table>
<thead>
<tr>
<th>What is the colour of your eyes?</th>
<th>Light, blue, grey, green</th>
<th>Blue, grey or green</th>
<th>Blue</th>
<th>Dark brown</th>
<th>Brownish black</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is the natural colour of your hair?</th>
<th>Sandy red/ginger</th>
<th>Blond</th>
<th>Chestnut/dark blond</th>
<th>Dark brown</th>
<th>black</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is the colour of your skin? (Non exposed areas)</th>
<th>Reddish</th>
<th>Very pale</th>
<th>Pale with beige tint</th>
<th>Light brown</th>
<th>Dark brown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you have freckles on unexposed areas?</th>
<th>Many</th>
<th>Several</th>
<th>Few</th>
<th>Incidental</th>
<th>none</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

161
<table>
<thead>
<tr>
<th>What happens when you stay in the sun too long?</th>
<th>Painful, redness, blistering, peeling</th>
<th>Blistering followed by peeling</th>
<th>Burn sometimes followed by peeling</th>
<th>Rare burns</th>
<th>Never had burns</th>
</tr>
</thead>
<tbody>
<tr>
<td>To what degree do you turn brown?</td>
<td>hardly or not at all</td>
<td>light tan colour</td>
<td>reasonable tan</td>
<td>tan very easy</td>
<td>turn dark brown quickly</td>
</tr>
<tr>
<td>Do you turn brown within several hours after sun exposure?</td>
<td>Never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>always</td>
</tr>
<tr>
<td>How does your face react to the sun?</td>
<td>Very sensitive</td>
<td>sensitive</td>
<td>normal</td>
<td>very resistant</td>
<td>never had a problem</td>
</tr>
</tbody>
</table>
When did you last expose your body to sun or artificial sunlamp/tanning lotion?

- 
- 
- 
- 
- 

Did you expose the area to be treated to the sun?

- 
- 
- 
- 
- 

27. Which of the following currently best describes you?

- Sunbathe as well as fake tan
- I only sunbathe
- I use a fake tan until I can get a natural tan
- I use a fake tan instead of sunbathing
- I only use a fake tan for special events e.g. weddings, dance competitions,
- I only use a fake tan in Summer
- I do not sunbathe or use fake tan

28. How long do you typically spend outdoors each day? Please select

- Less than 30 minutes
- 30-60 minutes
- 1- 2 hours
- 2- 4 hours
- 4-6 hours
- 6-8 hour
- 8+ hours
The following questions are in regards to how important things are to you

29.

<table>
<thead>
<tr>
<th>Really True for Me</th>
<th>Sort of True for Me</th>
<th>REMEMBER to check only ONE of the four boxes</th>
<th>Sort of True for Me</th>
<th>Really True for Me</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>Some people believe that having an attractive appearance is vitally important to them</td>
<td>BUT</td>
<td>Others believe that having an attractive appearance is not all that important in their lives</td>
</tr>
</tbody>
</table>

| 2.     |     | Some people do not feel it so important to them to spend a lot of time and effort maintaining an attractive appearance | BUT | Others think that it is vitally important to spend time and effort maintaining an attractive appearance |     |     |

The following questions are in regards to how you feel about your body

30.

<table>
<thead>
<tr>
<th>Really True for Me</th>
<th>Sort of True for Me</th>
<th>REMEMBER to check only ONE of the four boxes</th>
<th>Sort of True for Me</th>
<th>Really True for Me</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td></td>
<td>Some people feel that compared to most, they have an attractive appearance</td>
<td>BUT</td>
<td>Others feel that compared to most, their appearance is not quite so attractive</td>
</tr>
</tbody>
</table>
31. In order for us to contact you to confirm your appointment, please enter your contact details:

Name:
Phone:
Mailing address:
Email address:

32. Thank you for taking the time to complete the online survey. Would you be willing for us to contact you in the future to participate in further research and to also email you.

☐ Yes (proceed to the doodle link)
☐ No (proceed to the doodle link)

**DOODLE LINK**

Please click on to the following link to make an appointment for your measurements

(Doodle link with dates and available times)
Appendix 4: Cross-sectional consent form

Consent Form for the Research Project:
Is there a link between Fruit & Vegetable intake and Skin colour appearance?
Version 2; dated: 20/02/2013

Professor Clare Collins, Dr Melinda Neve and Ms Kristine Pezdirc.

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to provide any reason for withdrawing.

I consent to:
- Having my height, weight, body composition, blood pressure and skin colour reflectance measurements taken
- Completing a questionnaire about my usual food intake (Food Frequency Questionnaire)

I understand that all personal information will remain confidential to the researchers and that data collected from my participation will be used in journal publications and conference presentations and may be used in future projects.

My refusal to participate or withdraw from the study will not affect my relationship with the University of Newcastle. I have had the opportunity to have questions answered to my satisfaction.

Print Name: ____________________________

Contact Details: e-mail_________________ Phone_________________

Signature: ____________________________ Date: ________________

☐ Please tick this box if you are enrolled in any of the following courses: NURS1101/NURS1201/NURS2101/NURS2201/NURS3101/NURS3201/MID1201/MID1210/MID2201/MID3102 and want to receive bonus credit marks.
Appendix 5: Ethics approval for RCT cross-over trial

HUMAN RESEARCH ETHICS COMMITTEE

Notification of Expedited Approval

To Chief Investigator or Project Supervisor: Professor Clare Collins
Cc Co-investigators / Research Students: Professor David Perrett
                                      Dr Gozde Ozakinci
                                      Doctor Megan Rollo
                                      Miss Kristine Pezdiric
                                      Doctor Melinda Neve
                                      Mr Ross Whitehead

Re Protocol: Can an increase in fruit and vegetable intake improve / change your skin colour and appearance?

Date: 11-Oct-2012
Reference No: H-2012-0338
Date of Initial Approval: 11-Oct-2012

Thank you for your Response to Conditional Approval (minor amendments) submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under Expedited review by the Ethics Administrator.

I am pleased to advise that the decision on your submission is Approved effective 11-Oct-2012.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research, 2007, and the requirements within this University relating to human research.

Approval will remain valid subject to the submission, and satisfactory assessment, of annual progress reports. If the approval of an External HREC has been "noted" the approval period is as determined by that HREC.

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal Certificate of Approval will be available upon request. Your approval number is H-2012-0338.

If the research requires the use of an Information Statement, ensure this number is inserted at the relevant point in the Complaints paragraph prior to distribution to potential participants You may then proceed with the research.
Appendix 6: RCT cross-over trial registration

ISRCTN86297454 assigned to your trial (ref: CCT-NAPN-23828)

Sripadma Ganapathi <Sripadma.Ganapathi@controlled-trials.com>

Thu 21/11/2013 3:28 AM

To: Clare Collins <clare.collins@newcastle.edu.au>
cc: Melinda Hutchesson <melinda.hutchesson@newcastle.edu.au>; Kristine Pezdiric <kristine.pezdiric@uon.edu.au>

Dear Prof Collins,

I am pleased to inform you that the following ISRCTN has been assigned to your trial: Can an increase in fruit and vegetable intake improve/change your skin colour and appearance?

ISRCTN86297454: http://www.controlled-trials.com/ISRCTN86297454/

Thank you for registering your trial with the ISRCTN register.

To help us improve our services, please tell us about your ISRCTN registration experience in this short survey https://www.surveymonkey.com/s/9PT7Wv2. Thank you in advance.

When quoting the ISRCTN, please make sure that no space is inserted between the ISRCTN and the actual number. Please refer to http://www.controlled-trials.com/isrtn/sample_documentation.asp for further guidance notes about how to use the ISRCTN. Please also note that once a trial has been registered on the ISRCTN Register and publicly displayed on the website, the study will remain permanently on the register and cannot be deleted (as per our letter of agreement on http://www.controlled-trials.com/isrtn/isrtn.htm).

CCT’s sister company, BioMed Central, currently publishes over 250 peer-reviewed open access journals, including the journal Trials, which is dedicated to publishing protocols, results and other issues relevant to clinical trials. Selected BioMed Central journals offer a 20% discount on the article processing charge to protocol authors who have registered their trial with the ISRCTN register. Authors should request a waiver during the submission process and provide their ISRCTN trial ID.

UK only - The UK National Institute of Health Research (NIHR) has signed up for a Supporter Membership which helps NIHR funded researchers to publish their work in any of the BioMed Central Journals.

Regards,
Padmini

Sripadma Ganapathi
Database Editor
Current Controlled Trials
236 Gray’s Inn Road
London WC1X 8HB
United Kingdom
T: +44 (0)20 3892 2205
Appendix 7: RCT cross-over study flyer

CAN AN INCREASE IN FRUIT AND VEGETABLE INTAKE IMPROVE YOUR SKIN COLOUR AND APPEARANCE?

Details
We are conducting a study to find out whether an increase in the amount of fruit and vegetables you consume can change or improve the appearance of your actual skin.

Who can volunteer?
We need approximately 30 FEMALES aged 18 to 30 years to volunteer in a 11-week study who:

- Have a body mass index of >18.5kgm²
- Are able to travel to the Callaghan campus of the University of Newcastle on four occasions to have your weight, height, body composition, blood pressure, photographs, blood samples, skin carotenoid and melanin measurements taken
- Able to collect the weekly fruit and vegetable box each week from the Callaghan Campus

You will be provided with a FREE weekly fruit and vegetable box for 8 weeks to consume and a $25 gift voucher for compensation for your time and travel costs.

This project has been approved by The University of Newcastle Human Research Ethics Committee, Approval Number [H-2012-0338]. Chief investigator Professor Clare Collins.

CONTACT
If you are interested or for further information and to find out if you are eligible please contact:
Kristine Pezdic
T: 4921 7374
Email: fruitnvegskincolour@newcastle.edu.au
2. Recruiting by other methods:

Email

Thank you for interest in our study “Can an increase in fruit and vegetable intake improve/change your skin colour and appearance.

Please find attached an information statement containing detailed information about this study.

If after reading the information statement, you would like to participate, please complete the online eligibility screening tool available at https://www.surveymonkey.com/s/5D5LSMC.

If you are eligible to participate a member of the research team will send you a consent form.

Online Eligibility Screen

1. Gender
   - Female- Go to Question 2
   - Male- Not eligible to participate

2. How old are you? _______

   If participant <18 years or >30 years they will receive ineligible to participate message

   If 18 to 30 years Go to question 3

3. How many pieces of fruit do you eat? (Include all types)
   - None- Go to question 4
   - Less than 1 per week - Go to question 4
   - 1-2 per week- Go to question 4
   - 3-4 per week- Go to question 4
   - 5-6 per week- Go to question 4
   - Once per day - Not eligible to participate
   - 2-3 per day - Not eligible to participate
   - 4 or more per day Not eligible to participate

4. How many times a week do you eat vegetables with your meal at night? (not including hot chips)
5. Are you currently pregnant or breastfeeding?
   □ Yes - Not eligible to participate
   □ No - Go to question 5

5. How tall are you? _______ cm

6. How much do you currently weigh? _______ kg

Calculated BMI: ____________________

If participant BMI < 18.5: Not eligible to participate and will receive message

If participant BMI > 18.5 Go to question 7

7. Do you currently smoke cigarettes?
   □ Yes - Not eligible to participate
   □ No - Go to question 8

8. Do you have any of the following health conditions? Please select the most relevant option(s)
   □ Diabetes mellitus (Type 2) - Not eligible to participate
   □ High blood pressure - Not eligible to participate
   □ Low blood pressure - Not eligible to participate
   □ Liver disease - Not eligible to participate
   □ Kidney disease - Not eligible to participate
   □ Cardiovascular disease - Not eligible to participate
   □ Gastrointestinal tract disease - Not eligible to participate
   □ None of the above - Go to question 9

9. Have you experienced or been diagnosed or treated for disordered eating?
   □ Yes - Not eligible to participate
   □ No - Go to question 10
10. Are you currently on a special diet for e.g. Gluten free (Coeliac), low fibre or FODMAPS?
   
   ☐ Yes- Not eligible to participate
   ☐ No go to question 11

11. You are eligible to participate in the study.

   You are eligible to participate in the study we will now email you a consent form.
   Once you return your consent form, we will contact you to organise your sessions.

12. In order for us to email you a consent form please enter your contact details:

   Name:

   Email address:
Appendix 9: RCT cross-over consent form

Clare Collins
PhD, BSc, Dip Nutr&Diet, Dip Clin Epi, advAPD, FDAA
Professor in Nutrition and Dietetics
Priority Research Centre for Physical Activity and Nutrition
Room 310, Level 3 TC Building
University Drive, Callaghan
NSW 2308 Australia
Phone: +61 2 49215646
Fax: +61 2 49217503
Email: Clare.Collins@newcastle.edu.au

Consent Form for the Research Project:
Can an increase in fruit and vegetable intake improve/change your skin colour and appearance?
Version 5; dated: 06/11/13
Professor Clare Collins, Dr Melinda Hutchesson and Ms Kristine Pezdirc.

I agree to participate in the above research project and give my consent freely. I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained. I understand I can withdraw from the project at any time and do not have to provide any reason for withdrawing.

I consent to:
- Completing an online questionnaire prior to the assessment session on you (demographic), your health, skin type, and your perception on appearance
- Attend four assessment sessions at the Nutrition & Dietetics Anthropometry Lab at the University of Newcastle
- Have my height, weight, body composition (fat free mass/fat mass), blood pressure and skin colour reflectance measured
- Fasting overnight and having a blood test at each assessment session
- Complete questionnaires on up to four occasions about my eating habits (food frequency questionnaire)
- Complete an acceptability survey on two occasions
- Complete a survey on your appearance on two occasions
- Complete a quality of life questionnaire on four occasions
- Having a photograph taken of my face on four occasions
- Aim to consume the fruit and vegetables provided to me during the study
- Collecting the weekly fruit and vegetable box from the research team at the Callaghan campus
- Refrain from using any tanning lotions/creams or sunbathing for 11 weeks

I understand that all personal information will remain confidential to the researchers and that data collected from my participation will be used in journal publications and conference presentations and may be used in future projects. My refusal to participate or withdraw from the study or will not affect my relationship with the University of Newcastle. I have had the opportunity to have questions answered to my satisfaction.

Are you a student enrolled in
NURS1101/NURS1201/ NURS2101/NURS2201/NURS3101/NURS3202
MID1201/ MID1202/MID2201/MID3102

☐ Yes ☐ No
By signing below, I indicate my consent to participate in the research project conducted by Professor Clare Collins, Dr Melinda Hutchesson and Ms Kristine Pezdiric.

Print Name: ________________________________

Contact Details: e-mail____________________  Phone____________________

Signature: ________________________ Date: __________________

Please return the signed consent form by email fruitnvegnskincolour@newcastle.edu.au or post, Attention: Professor Clare Collins at the above address.
Information Statement for the Research Project:
Can an increase in fruit and vegetable intake improve/change your skin colour and appearance?

Version 5, dated 06/11/2013

We would like to offer you the opportunity to participate in a research study which is being conducted by Professor Clare Collins, Dr Melinda Hutchesson and Ms Kristine Pezdiric from the Priority Research Centre in Physical Activity and Nutrition at the University of Newcastle.

The research is part of Ms Kristine Pezdiric’s PhD studies at the University of Newcastle, supervised by Professor Clare Collins and Dr Melinda Hutchesson from School of Health Sciences/Faculty of Health/Priority Research Centre in Physical Activity and Nutrition.

This project is funded by the Priority Research Centre in Physical Activity and Nutrition.

Why is the research being done?

The project aims to find out whether an increase in the amount of fruit and vegetables you consume can change the appearance of your actual skin colour.

Who can take part?

You can participate in this project if you are:

- Female, aged 18 to 30 years
- Proficient in English
- Have a body mass index of >18.5kgm$^2$ (calculated by dividing your weight (kg) by your height (in metres) squared)
- Able to travel to the Callaghan campus of the University of Newcastle on four occasions to have your weight, height, body composition, blood pressure, blood samples, photographs and skin carotenoid and melanin measurements taken
- Are not currently smoking
- Have not currently experienced or been diagnosed or treated for disordered eating
- Are not currently pregnant or lactating
- Are not currently on a special diet for e.g. Coeliac, FODMAPS, low fibre diet
- Have not been diagnosed with Liver, Renal, Gastrointestinal tract or Cardiovascular disease
- Do not have a metabolic disorder: Type 2 diabetes, high or low blood pressure
What choice do you have?

Participation in this research is entirely your choice. You will be only included in the project if you have given your informed consent. Whether or not you decide to participate, your decision will not disadvantage you. If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data you have provided. This project will involve taking photographs of your face (see below for further details). If you provide consent for the photographs to be used for displays, conference presentations or publication, upon viewing the photographs in week 11 you will have the option of withdrawing the photos to be used.

What would you be asked to do?

The study will run for a period of 11 weeks. If you agree to participate you will be asked to participate in:

- 2 x 4 week periods (Week 1 to 4 and Week 7 to 10) in which you will be provided with a weekly box of fruit and vegetables that is equivalent to 7 serves a day and asked to consume the fruit and vegetables that are provided to you. In addition we will need you to collect the fruit and vegetables from the university each week.
- 4 x measurement sessions at the University of Newcastle Nutrition & Dietetics Anthropometry lab.
- Having a blood test at each assessment, in which we will require you to fast overnight.
- Completing an online questionnaire prior to the assessment session on you (demographic), your health, skin type, and your perception on appearance.

You will also be asked to refrain from using any tanning lotions /creams or sunbathing for 11 weeks.

Measurement sessions:

All assessments will take place in the Nutrition & Dietetics Anthropometry lab in the Hunter building on the Callaghan Campus at the University of Newcastle during the morning (see attached map). The session dates and times will be individually organised and will occur in weeks 1, 5, 7, and 11 from the time you start the study.
Table 1 outlines what you will be asked to do in those sessions:

<table>
<thead>
<tr>
<th>Table 1</th>
<th>1</th>
<th>2</th>
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<th>11</th>
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</thead>
<tbody>
<tr>
<td>Complete a questionnaire about your usual food intake (Food Frequency Questionnaire) and 24 hour recall</td>
<td>✓</td>
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<tr>
<td>Have your weight and body composition measurements taken using a bioelectrical impedance analyser. (This will determine your body composition in terms of muscle and fat tissues).</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Have your height taken with a portable stadiometer, (a device that measures your height)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Have your blood pressure taken</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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</tr>
<tr>
<td>Have your skin carotenoid and melanin measurements taken on the skin at your shoulders; arms, face, hand, foot sole and hip using a hand held device called a spectrophotometer that measures the wave length of the carotenoids naturally present in your skin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Complete a quality of life questionnaire, a short form of 12 questions about functional health, well being and physical and mental health.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Have a blood test for plasma carotenoids (Approximately 5ml of blood will be drawn at each assessment)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>Have a photograph of your face taken in weeks 1, 5, 7, and 11. You will be required to be wear no makeup for the photographs. The photos will be taken under standard lighting and background conditions of the face and front profile only. In week 11 you will be asked to view these images and asked some questions in relation to the four images.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Complete an acceptability survey (evaluation of the study)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Collect the weekly fruit and vegetable box.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

✓ Denotes when these measurements will be taken and when questionnaires will be completed.
How much time will it take?

- Attendance at the lab to have your height, weight, body composition, blood pressure, blood tests, photographs, skin measurements and to complete questionnaires will take approximately 2 to 2.5 hours.

What are the benefits and risks of participating?

Risks:

- As a result from blood collection, some individuals may feel light headed, faint or experience some bruising at the sight of the blood collection. Breakfast will be provided after measurements and blood collection has taken place.
- You may experience some discomfort from getting your blood pressure taken.
- The spectrophotometer used to measure your skin carotenoids is an entirely safe and simple process. This involves pressing the device to the skin surface to measure the level of carotenoid and melanin compounds that are in the skin.
- All other measures in the study such as body composition and the questionnaires use standard tools that have been widely used in research.
- Some questions in the online survey and questionnaires are of a sensitive nature (e.g. Requesting information about your prescribed medication, acne, feelings about your personal appearance and the questions in relation to the photographs). As with all information collected, your answers to these questions will be kept completely confidential and your name will not be stored alongside your responses. If you experience any feelings that are overwhelming or distressing while answering these questions, please seek help from your doctor. You also have the option of contacting Lifeline on 13 11 44 or the University Counselling service on 49215801. The counselling service is available only to students of the University of Newcastle.

Benefits:

- You will receive standardized individual feedback on your individual results for your body composition, blood pressure, fruit and vegetable intake and skin carotenoids and melanin levels from the researchers at the end of the study.
- You will also receive a $25 gift voucher for participating in the study to cover travel costs.
- You will receive a weekly complementary fruit and vegetable box for eight weeks

In addition, if you are enrolled in one of the following courses, you will receive 5 bonus course marks towards one of the following courses:
NURS1101/NURS1201/ NURS2101/NURS2201/NURS3101/NURS3202
MIDI1201/ MIDI2102/MIDI2201/MIDI3102

How will your privacy be protected?

At the lab you will be identified with a numerical code rather than your name. Data will be retained for at least 5 years at the University of Newcastle by University staff. The data collected will be stored on a password-protected computer used by the research team. The consent forms will be securely stored in a locked filing cabinet in the School of Health Sciences Research Higher Degree room (HA06). The blood samples will be stored until data collection for the study and analysis has been completed, after that they will be destroyed. Your blood samples will labelled with a participant ID code, not your name. The
photos will be taken by the research team with a digital camera in the lab. The photos will be downloaded and stored on a password protected computer on the research drive where only members of the research team will have access to. They will be stored for 5 years and then files will be destroyed.

How will the information collected be used?

The results of the research will be reported and distributed via national and international conferences and peer reviewed publications. You will not be personally identified in any reports arising from the study. The research team will gain additional consent from you if they intend to use the photographs of you for the purposes of display, thesis or publications. We will report participant responses to their self evaluation on preferred skin colour and healthiness from the photographs taken. The data collected will also contribute towards Kristine Pezdirc’s PhD thesis. Once the study is completed you will receive a brief summary of the results from research team.

What do you need to do to participate?

Please read this Information Statement carefully and be sure you understand its contents before you consent to participate. If there is anything you do not understand, or have any questions, contact the research team.
If you agree to participate, please complete the online eligibility survey available at https://www.surveymonkey.com/s/5D5LSMC. If you are eligible we will provide you with a consent form. Please complete the consent form and return by mail to the Chief Investigator at the above address.

Further information

If you would like further information about the study please contact Ms Kristine Pezdirc, the PhD candidate initially on 4921 7374 or email at frultnyegnskincolour@newcastle.edu.au.

Thank you for considering this invitation

Professor Clare Collins
School of Health Sciences

Complaints about this research
This project has been approved by the University’s Human Research Ethics Committee, Approval No. H-2012-0338. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au.
Appendix 11: RCT cross-over online survey

This survey will cover information about you and your food intake. You will be asked questions about your age, education, income, employment, living arrangements, health, medications, skin and appearance.

The following questions are in regards to details about you.

1. **What is your Gender? (Drop down box Male, Female)**
   - [ ] Male
   - [ ] Female

2. **What is your date of birth? DD/MM/YYYY (Drop down boxes)**

3. **Please indicate your ethnic group (please tick all that apply)**
   - [ ] White Australian
   - [ ] White British
   - [ ] White other European
   - [ ] White American
   - [ ] Hispanic
   - [ ] Chinese
   - [ ] Japanese
   - [ ] South east Asian
   - [ ] Other Asian
4. What is your postcode ----

5. Who lives with you? (tick all that apply)
   - Partner/Spouse
   - Own children
   - Parents
   - Other adults
   - I live alone

The following questions are in regards to details about your education, income and employment.

6. What is the highest qualification you have completed? (mark one only)
   - No formal qualifications
   - Year 10 or equivalent (eg School Certificate)
   - Year 12 or equivalent (eg Higher School Certificate)
   - Trade / Apprenticeship (eg Chef, Hairdresser)
   - Certificate / Diploma (eg Child care, Technician)
   - University degree
   - Higher university degree (eg Grad Dip, Masters, PhD)

7. How do you manage on the income you have available?
   - It is impossible
   - It is difficult
   - It is difficult some of the time
   - It is not too bad
☐ It is easy

8. How would you describe your current employment status? (mark one only)
   ☐ Employed full-time
   ☐ Employed part-time
   ☐ Employed, but currently on leave
   ☐ Retired
   ☐ Unemployed and seeking work
   ☐ Unemployed, but not seeking work
   ☐ Don’t wish to answer

9. Would you say your health in general is
   ☐ Excellent
   ☐ Very good
   ☐ Good
   ☐ Fair
   ☐ Poor

10. How many times a year do you get a cold or flu?
    ☐ 0
    ☐ 1
    ☐ 2
    ☐ 3
    ☐ 4
    ☐ 5
    ☐ 6
    ☐ 7
11. How many days does one of your average cold or flu bouts last?

☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10
☐ 11
☐ 12
☐ 13
☐ 14
☐ 15 + days

12. How many times did you take a course of antibiotics in the last year?

☐ 0
☐ 1
☐ 2
13. Have you been ill in the last week?
   - Yes
   - No

14. In the last week have you had acne?
   - No
   - Yes a little
   - Yes a lot
   - Yes very much

15. Are you currently taking any prescribed medications? (Including the contraceptive pill)  
    *If yes, please list the medication and dose where possible.*
   - No
   - Yes
Medications and doses *(Drop down comments box)*

16. Are you currently taking any supplements or multivitamins? If yes, please list the type and brand where possible.

☐ No

☐ Yes *(Drop down comments box)*

17. Which of the following products do you regularly apply to your skin?

☐ Moisturizer

☐ Creams

☐ Sunscreens

☐ Masks or cosmetic treatments

☐ Foundation

☐ Medical creams

☐ Tanning sprays/lotions

☐ None of the above

18. How much a month would you spend on the following: moisturizer, creams, sunscreens, masks or cosmetics treatments, foundation, medical creams, *tanning sprays/lotions*

☐ None

☐ $0-$50

☐ $50-$100

☐ $100-$200

☐ $200-$300

☐ > $300
The following questions are in regards to your skin type

19.

<table>
<thead>
<tr>
<th>What is the colour of your eyes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light, blue, grey, green</td>
</tr>
<tr>
<td>Blue, grey or green</td>
</tr>
<tr>
<td>Blue</td>
</tr>
<tr>
<td>Dark brown</td>
</tr>
<tr>
<td>Brownish black</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is the natural colour of your hair?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy red/ginger</td>
</tr>
<tr>
<td>Blond</td>
</tr>
<tr>
<td>Chestnut/dark blond</td>
</tr>
<tr>
<td>Dark brown</td>
</tr>
<tr>
<td>black</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is the colour of your skin? (Non exposed areas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reddish</td>
</tr>
<tr>
<td>Very pale</td>
</tr>
<tr>
<td>Pale with beige tint</td>
</tr>
<tr>
<td>Light brown</td>
</tr>
<tr>
<td>Dark brown</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you have freckles on unexposed areas?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many</td>
</tr>
<tr>
<td>Several</td>
</tr>
<tr>
<td>Few</td>
</tr>
<tr>
<td>Incidental</td>
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<tr>
<td>none</td>
</tr>
</tbody>
</table>
20.

<table>
<thead>
<tr>
<th>What happens when you stay in the sun too long?</th>
<th>Painful, redness, blistering, peeling</th>
<th>Blistering followed by peeling</th>
<th>Burn sometimes followed by peeling</th>
<th>Rare burns</th>
<th>Never had burns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>To what degree do you turn brown?</th>
<th>hardly or not at all</th>
<th>light tan colour</th>
<th>reasonable tan</th>
<th>tan very easy</th>
<th>turn dark brown quickly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you turn brown within several hours after sun exposure?</th>
<th>Never</th>
<th>seldom</th>
<th>sometimes</th>
<th>often</th>
<th>always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How does your face react to the sun?</th>
<th>Very sensitive</th>
<th>sensitive</th>
<th>normal</th>
<th>very resistant</th>
<th>never had a problem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
21. When did you last expose your body to sun or artificial sunlamp/tanning lotion?

- more than 3 months ago
- 2-3 months ago
- 1-2 months ago
- less than a month ago
- less than 2 weeks ago

22. Which of the following currently best describes you?

- Sunbathe as well as fake tan
- I only sunbathe
- I use a fake tan until I can get a natural tan
- I use a fake tan instead of sunbathing
- I only use a fake tan for special events e.g. weddings, dance competitions
- I only use a fake tan in Summer
- I do not sunbathe or use fake tan

23. How long do you typically spend outdoors each day? Please select

- Less than 30 minutes
- 30-60 minutes
- 1-2 hours
- 2-4 hours
- 4-6 hours
- 6-8 hours
- 8+ hours

*The following questions are in regards to how important things are to you*
24.

<table>
<thead>
<tr>
<th>Really True for Me</th>
<th>Sort of True for Me</th>
<th>REMEMBER to check only ONE of the four boxes</th>
<th>Sort of True for Me</th>
<th>Really True for Me</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>BUT</strong> Others believe that having an attractive appearance is not all that important in their lives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td>Some people believe that having an attractive appearance is vitally important to them</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BUT</strong> Others think that it is vitally important to spend time and effort maintaining an attractive appearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Some people do not feel it so important to them to spend a lot of time and effort maintaining an attractive appearance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following questions are in regards to how you feel about your body

25.

<table>
<thead>
<tr>
<th>Really True for Me</th>
<th>Sort of True for Me</th>
<th>REMEMBER to check only ONE of the four boxes</th>
<th>Sort of True for Me</th>
<th>Really True for Me</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>BUT</strong> Others feel that compared to most, their appearance is not quite so attractive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Some people feel that compared to most, they have an attractive appearance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thank you for taking the time to complete the online survey. We will be in touch to arrange a time that is suitable for your first assessment.
Appendix 12: RCT instructions box A

**Instructions on Fruit and Vegetable box A**

Here is your weekly fruit and vegetable box. You will receive the same quantities and types of fruit and vegetables for the next four weeks.

We encourage your to eat as much as you can from this box as it will provide you with the daily recommended amounts of 2 serves of fruit and 5 serves of vegetables a day.

If you want to buy more fruit and vegetables please select the same as the box or try and SELECT from the following:

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passionfruit</td>
<td>Squash</td>
</tr>
<tr>
<td>Guava</td>
<td>Onion, spring</td>
</tr>
<tr>
<td>Rockmelon, watermelon</td>
<td>Shallot</td>
</tr>
<tr>
<td>Apricot, fresh, raw</td>
<td>Pea</td>
</tr>
<tr>
<td>Blueberry</td>
<td>Bok Choy</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>Tomato, cherry</td>
</tr>
<tr>
<td>Banana</td>
<td>Tomato</td>
</tr>
<tr>
<td>Oranges</td>
<td>Mixed vegetables (peas &amp; corn)</td>
</tr>
<tr>
<td>Mango</td>
<td>Broccoli</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>Snowpeas</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>snowpea sprouts</td>
</tr>
<tr>
<td>Frozen berries</td>
<td>Cucumber</td>
</tr>
<tr>
<td>Nectarine</td>
<td>Zucchini</td>
</tr>
<tr>
<td>Peach</td>
<td>Beans</td>
</tr>
<tr>
<td></td>
<td>Capsicum, green/red</td>
</tr>
<tr>
<td></td>
<td>Brussels sprout</td>
</tr>
<tr>
<td></td>
<td>Asparagus</td>
</tr>
<tr>
<td></td>
<td>Kale</td>
</tr>
<tr>
<td></td>
<td>Carrots</td>
</tr>
<tr>
<td></td>
<td>Sweet potato</td>
</tr>
<tr>
<td></td>
<td>Pumpkin</td>
</tr>
<tr>
<td></td>
<td>Spinach</td>
</tr>
<tr>
<td></td>
<td>Sweet corn</td>
</tr>
<tr>
<td></td>
<td>Leek</td>
</tr>
</tbody>
</table>
If you can try and **AVOID** eating the following vegetables for the next four weeks:

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple</td>
<td>Lentils, chickpeas</td>
</tr>
<tr>
<td>Apples</td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Strawberries</td>
<td>Cabbage, red or white</td>
</tr>
<tr>
<td>Cherry</td>
<td>Potato</td>
</tr>
<tr>
<td>Grapes: red/green</td>
<td>Garlic</td>
</tr>
<tr>
<td>Pears</td>
<td>Beetroot</td>
</tr>
<tr>
<td>Lychee</td>
<td>Cabbage, white, raw</td>
</tr>
<tr>
<td>Coconut</td>
<td>Swede</td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td>Turnip</td>
</tr>
<tr>
<td>Mandarin</td>
<td>Swede, peeled, raw</td>
</tr>
<tr>
<td></td>
<td>Turnip, white, peeled, raw</td>
</tr>
<tr>
<td></td>
<td>alfalfa sprouts</td>
</tr>
<tr>
<td></td>
<td>Radish</td>
</tr>
<tr>
<td></td>
<td>Eggplant</td>
</tr>
<tr>
<td></td>
<td>Mushrooms</td>
</tr>
<tr>
<td></td>
<td>Cauliflower</td>
</tr>
<tr>
<td></td>
<td>Onion</td>
</tr>
<tr>
<td></td>
<td>Parsnip</td>
</tr>
<tr>
<td></td>
<td>Celery</td>
</tr>
</tbody>
</table>
Tips for Fruit and vegetables

Serving suggestions:
Some simple ways to serve fruits and vegetables include:

- Fruit and vegetable salads
- Vegetable or meat-and-vegetable stir-fries
- Raw fruit and vegetables
- Vegetable soups
- Snack pack, stewed or canned fruits or dried fruits.

Limit fruit juice, as it does not contain the same amount of nutrients as fresh fruit. It also contains a lot of sugars. These sugars are not necessarily good for your health, even though they are 'natural'. Instead, have a drink of water and a serve of fruit.

Preparation and cooking
Vegetables are often cooked, although some kinds are eaten raw. Cooking and processing can damage some nutrients and phytochemicals in plant foods.

Suggestions to get the best out of your fruit and vegetables include:

- Eat raw vegetables and fruits if possible.
- Try fruit or vegetables pureed into smoothies.
- Use a sharp knife to cut fresh fruits to avoid bruising.
- Cut off only the inedible parts of vegetables — sometimes the best nutrients are found in the skin, just below the skin or in the leaves.
- Use stir-fry, grill, microwave, bake or steam methods with non-stick cookware and mono-unsaturated oils.
- Do not overcook, to reduce nutrient loss.
- Serve meals with vegetable pestos, salsas, chutneys and vinegars in place of sour cream, butter and creamy sauces.
- Eat raw vegetables with dip such as hoummus.
- You can eat vegetables raw, grate them, stir fry, steam or bake them.
- Mix them together and add herbs, lemon, and other spices.
Some useful websites for snack and recipe ideas to increase your daily fruit and vegetable intake:

www.eatforhealth.gov.au/eating-well/healthy-recipes
www.taste.com.au

RECIPES

Here are some recipe ideas for using the vegetables in this box. You can also search for recipes:

Carrots, pumpkin, sweet potato, broccoli


If you have any questions please do not hesitate to contact the research team

Email: fruitnvegnskincolour@newcastle.edu.au
Phone: 4921 7374
Appendix 13: RCT instructions box B

Instructions on Fruit and Vegetable box B

Here is your weekly fruit and vegetable box. You will receive the same quantities and types of fruit and vegetables for the next four weeks.

We encourage your to eat as much as you can from this box as it will provide you with the daily recommended amounts of 2 serves of fruit and 5 serves of vegetables a day.

If you want to buy more fruit and vegetables please select the same as the box or try and SELECT from the following:

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple</td>
<td>Lentils, chickpeas</td>
</tr>
<tr>
<td>Apples</td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Strawberries</td>
<td>Cabbage, red or white</td>
</tr>
<tr>
<td>Cherry</td>
<td>Potato</td>
</tr>
<tr>
<td>Grapes: red/green</td>
<td>Garlic</td>
</tr>
<tr>
<td>Pears</td>
<td>Beetroot</td>
</tr>
<tr>
<td>Lychee</td>
<td>Cabbage, white, raw</td>
</tr>
<tr>
<td>Coconut</td>
<td>Swede</td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td>Turnip</td>
</tr>
<tr>
<td>Mandarins</td>
<td>Swede, peeled, raw</td>
</tr>
<tr>
<td></td>
<td>Turnip, white, peeled, raw</td>
</tr>
<tr>
<td></td>
<td>alfalfa sprouts</td>
</tr>
<tr>
<td></td>
<td>Radish</td>
</tr>
<tr>
<td></td>
<td>Eggplant</td>
</tr>
<tr>
<td></td>
<td>Mushrooms</td>
</tr>
<tr>
<td></td>
<td>Cauliflower</td>
</tr>
<tr>
<td></td>
<td>Onion</td>
</tr>
<tr>
<td></td>
<td>Parsnip</td>
</tr>
<tr>
<td></td>
<td>Celery</td>
</tr>
</tbody>
</table>
If you can try and **AVOID** eating the following vegetables for the next four weeks.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passionfruit</td>
<td>Squash</td>
</tr>
<tr>
<td>Guava</td>
<td>Onion, spring</td>
</tr>
<tr>
<td>Rockmelon, watermelon</td>
<td>Shallot</td>
</tr>
<tr>
<td>Apricot, fresh, raw</td>
<td>Pea</td>
</tr>
<tr>
<td>Blueberry</td>
<td>Bok Choy</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>Tomato, cherry</td>
</tr>
<tr>
<td>Banana</td>
<td>Tomato</td>
</tr>
<tr>
<td>Oranges</td>
<td>Mixed vegetables (peas &amp; corn)</td>
</tr>
<tr>
<td>Mango</td>
<td>Broccoli</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>Snowpeas</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>snowpea sprouts</td>
</tr>
<tr>
<td>Frozen berries</td>
<td>Cucumber</td>
</tr>
<tr>
<td>Nectarine</td>
<td>Zucchini</td>
</tr>
<tr>
<td>Peach</td>
<td>Beans</td>
</tr>
<tr>
<td></td>
<td>Capsicum, green/red</td>
</tr>
<tr>
<td></td>
<td>Brussels sprout</td>
</tr>
<tr>
<td></td>
<td>Asparagus</td>
</tr>
<tr>
<td></td>
<td>Kale</td>
</tr>
<tr>
<td></td>
<td>Carrots</td>
</tr>
<tr>
<td></td>
<td>Sweet potato</td>
</tr>
<tr>
<td></td>
<td>Pumpkin</td>
</tr>
<tr>
<td></td>
<td>Spinach</td>
</tr>
<tr>
<td></td>
<td>Sweet corn</td>
</tr>
</tbody>
</table>
Tips for Fruit and vegetables

Serving suggestions:
Some simple ways to serve fruits and vegetables include:

- Fruit and vegetable salads
- Vegetable or meat-and-vegetable stir-fries
- Raw fruit and vegetables
- Vegetable soups
- Snack pack, stewed or canned fruits or dried fruits.

Limit fruit juice, as it does not contain the same amount of nutrients as fresh fruit. It also contains a lot of sugars. These sugars are not necessarily good for your health, even though they are ‘natural’. Instead, have a drink of water and a serve of fruit.

Preparation and cooking
Vegetables are often cooked, although some kinds are eaten raw. Cooking and processing can damage some nutrients and phytochemicals in plant foods.

Suggestions to get the best out of your fruit and vegetables include:

- Eat raw vegetables and fruits if possible.
- Try fruit or vegetables pureed into smoothies.
- Use a sharp knife to cut fresh fruits to avoid bruising.
- Cut off only the inedible parts of vegetables – sometimes the best nutrients are found in the skin, just below the skin or in the leaves.
- Use stir-fry, grill, microwave, bake or steam methods with non-stick cookware and mono-unsaturated oils.
- Do not overcook, to reduce nutrient loss.
- Serve meals with vegetable pestos, salsas, chutneys and vinegars in place of sour cream, butter and creamy sauces.
- Eat raw vegetables with dip such as hommus.
- You can eat vegetables raw, grate them, stir fry, steam or bake them.
- Mix them together and add herbs, lemon, and other spices.
Some useful websites for snack and recipe ideas to increase your daily vegetable and fruit intake:

www.eatforhealth.gov.au/eating-well/healthy-recipes
www.taste.com.au

RECIPES

Here are some recipe ideas for using the vegetables in this box. You can also search for recipes:

Cauliflower
(use cauliflower in this recipe)

Lentils

Chickpeas

Eggplant
Cabbage

If you have any questions please do not hesitate to contact the research team

Email: fruitnvegnskincolour@newcastle.edu.au
Phone: 4921 7374
Appendix 14: RCT acceptability questionnaire

Can an increase in fruit and vegetable intake improve /change your skin colour and appearance?

Acceptability Survey
Version 1.0 dated dd/mm/year

Name: ____________________________

RESEARCHERS:

<table>
<thead>
<tr>
<th><strong>Professor Clare Collins</strong></th>
<th><strong>Dr Melinda Hutchesson</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Priority Research Centre in Physical Activity and Nutrition</td>
<td>Priority Research Centre in Physical Activity and Nutrition</td>
</tr>
<tr>
<td>Faculty of Health</td>
<td>Faculty of Health</td>
</tr>
<tr>
<td>Phone: (02) 4921 5646</td>
<td>Phone: (02) 4921 5405</td>
</tr>
<tr>
<td><a href="mailto:Clare.Collins@newcastle.edu.au">Clare.Collins@newcastle.edu.au</a></td>
<td><a href="mailto:Melinda.Neve@newcastle.edu.au">Melinda.Neve@newcastle.edu.au</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Ms Kristine Pezdirc</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Priority Research Centre for Physical Activity and Nutrition</td>
</tr>
<tr>
<td>Faculty of Health</td>
</tr>
<tr>
<td>Phone: (02) 49217374</td>
</tr>
<tr>
<td><a href="mailto:kristine.pezdirc@newcastle.edu.au">kristine.pezdirc@newcastle.edu.au</a></td>
</tr>
</tbody>
</table>

We would like to know what you thought of the study and would be grateful if you could complete the following questions.

Please answer every question you can as honestly as you can. If you are unsure about how to answer a question, mark the response for the closest answer to how you feel.
The following questions are about the fruit and vegetable box you were assigned over the last 4 weeks.

Please select one option for each question that applies best to you.

1. Please select the fruit and vegetable box that you were assigned for the last 4 weeks

<table>
<thead>
<tr>
<th></th>
<th>Box 1</th>
<th>Box 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Please select the fruit and vegetable box that you were assigned for the last 4 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Please rate the difficulty in consuming the quantity of fruit provided</td>
<td>very easy</td>
<td>2</td>
</tr>
<tr>
<td>3. Please rate the difficulty in consuming the quantity of vegetables provided</td>
<td>very easy</td>
<td>2</td>
</tr>
<tr>
<td>4. Please rate the difficulty in consuming the type of fruit provided</td>
<td>very easy</td>
<td>2</td>
</tr>
<tr>
<td>5. Please rate the difficulty in consuming the type of vegetables provided</td>
<td>very easy</td>
<td>2</td>
</tr>
<tr>
<td>6. How difficult was it to prepare and cook the vegetables</td>
<td>very easy</td>
<td>2</td>
</tr>
<tr>
<td>7. The fruits in the box were tasty/palatable</td>
<td>strongly disagree</td>
<td>2</td>
</tr>
<tr>
<td>8. The vegetables in the box were tasty/palatable</td>
<td>strongly disagree</td>
<td>2</td>
</tr>
<tr>
<td>7. Overall how much of the fruits and vegetables did you consume?</td>
<td>All</td>
<td>Some (Question 8)</td>
</tr>
<tr>
<td>8. What was the main reason that you did not consume all the fruit and vegetables (tick all that apply)</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
- Shared amongst the people I live with (family/ housemates)
- Do not like to eat the fruit provided
- Do not like to eat the vegetables provided
- Too busy/ inconvenience
- Dieting
- Fasting
- Another reason. Please specify
9. list any of the fruits that you enjoyed eating

10. list any fruits that you would of liked to have been included

11. list any of the vegetables you enjoyed eating

12. list any vegetables that you would of liked to have been included

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE. YOUR COOPERATION IS GREATLY APPRECIATED.
Appendix 15: Perception study flyer

Does the colour of our skin influence how healthy we appear?

Skin Colour and the Perception of Health Study

Details
The pigments present in our skin affect its’ colour. The aim of this study is to investigate the effect of changes in skin colour on perceived health. Participating in this study will involve manipulating the colour of images of human faces to make them look as healthy as possible.

Who can volunteer?
- Aged 18 years and above
- Able to travel to and from the University on one occasion.

You will be given a voucher for a free coffee at one of the university cafes following completion of the assessment session as compensation for your time (~45 minutes).

How do I find out more information?
Please contact Dr Megan Rollo on melton.megan@newcastle.edu.au for further information and to find out if you are eligible to enrol.

This project has been approved by the University’s Human Research Ethics Committee, Approval No. H-2012-0405. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au.
Appendix 16: Ethics approval for perception study

HUMAN RESEARCH ETHICS COMMITTEE

Notification of Expedited Approval

To Chief Investigator or Project Supervisor: Professor Clare Collins
Cc Co-investigators / Research Students: Mr Ross Whitehead
                                        Doctor Melinda Neve
                                        Professor David Perrett
                                        Miss Kristine Pezdiric
                                        Dr Gozde Ozakinci
                                        Doctor Megan Rollo

Re Protocol: Skin colour and the perception of health.
Date: 14-Dec-2012
Reference No: H-2012-0405
Date of Initial Approval: 14-Dec-2012

Thank you for your Response to Conditional Approval (minor amendments) submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under Expedited review by the Ethics Administrator.

I am pleased to advise that the decision on your submission is Approved effective 14-Dec-2012.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research, 2007, and the requirements within this University relating to human research.

Approval will remain valid subject to the submission, and satisfactory assessment, of annual progress reports. If the approval of an External HREC has been "noted" the approval period is as determined by that HREC.

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal Certificate of Approval will be available upon request. Your approval number is H-2012-0405.

If the research requires the use of an Information Statement, ensure this number is inserted at the relevant point in the Complaints paragraph prior to distribution to potential participants You may then proceed with the research.

Conditions of Approval

This approval has been granted subject to you complying with the requirements for Monitoring of Progress, Reporting of Adverse Events, and Variations to the Approved Protocol as detailed below.
Appendix 17: Perception study consent form

Consent Form for the Research Project:
Skin colour and the perception of health (H-2012-0405)
Professor Clare Collins, Dr Megan Rollo, Dr Melinda Neve and Ms Kristine Pezdirc

Document Version 6 dated [20/7/13]

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to:
- Completing an online questionnaire; and
- Completing a computer-based activity in a darkened room which involves manipulating the colour of images of human faces to make them look as healthy as possible.

I understand that my personal information will remain confidential to the researchers.

I have had the opportunity to have questions answered to my satisfaction.

I have read the information statement and would like to participate in the study.

☐ Yes  ☐ No

If you select yes this will be your informed consent to participate in the study and an appointment for you to attend a testing session will be organised. In addition, you will be required to sign your consent form at the start of the testing session, before you complete the online questionnaire and computer-based activity.

Are you a student enrolled in NURS1101/NURS1201/NURS2101/NURS2201/NURS3101/NURS3201/NURS3301/MID1201/MID2102/MID2202/MID3102 and wish to receive 5 bonus course marks towards your course?

☐ Yes  ☐ No

Please complete with your name, contact details and date and return to Dr Megan Rollo (megan.rollo@newcastle.edu.au).

Print Name: ____________________________________________

Contact Details (to arrange time and date for testing session):

Email address: __________________________________________

Phone number: __________________________________________

Signature: ___________________________ Date: ________________
Appendix 18: Perception study information statement

Information Statement for the Research Project:
Skin colour and the perception of health
Document Version 7 dated [30/7/13]

You are invited to participate in the research project identified above which is being conducted by Professor Clare Collins, Dr Megan Rollo, Dr Melinda Neve and Ms Kristine Pezzlirc from the School of Health Sciences and the Priority Research Centre in Physical Activity and Nutrition at the University of Newcastle.

Why is the research being done?
The aim of this study is to investigate the effect of changes in skin colour on perceived health among Australian adults.

Who can participate in the research?
You can participate in this project if you are:

- Aged 18 years and older;
- Proficient in English.
- Are able to travel to the University of Newcastle (Callaghan campus) on one occasion to complete a computer-based activity.

If you are colour-blind, unfortunately you cannot participate.

What choice do you have?
Participation in this research is entirely your choice. Only those people who give their informed consent will be included in the project. Whether or not you decide to participate, your decision will not disadvantage you.

What would you be asked to do?
If you agree to participate, you will be asked to:

- Complete an online questionnaire about your background (date of birth, gender, ethnicity), skin, general health and diet;
- Complete a computer-based activity involving manipulating the colour of images of human faces to make them look as healthy as possible. This activity will be undertaken in a darkened room.
Following completion of the testing session you will be provided with a complimentary coffee voucher to compensate you for your time.

**How much time will it take?**
Completion of both the questionnaire and computer-based activity should take approximately 45 minutes.

**What are the risks and benefits of participating?**
There are no known risks to you in participating in this research. Both the questionnaire and the computer-based activity should take less than 45 minutes to complete and takes place in a darkened room. You may experience some discomfort from sitting at the computer for a prolonged period. While participation in this study may not directly benefit you, it is likely that the findings will benefit the wider Australian community by improving our understanding of the relationship between skin colour and perceived health.

In addition, if you are enrolled in one of the following courses, you will receive 5 bonus course marks towards one of the following courses:
NURS1101/NURS1201/NURS2101/NURS2201/NURS3101/NURS3201

**How will your privacy be protected?**
Identifying information will be removed and replaced with a numerical code. All information collected by the researchers will be stored securely on a password protected server and only accessed by the researchers unless you consent otherwise, except as required by law. Data will be retained for at least 5 years at the University of Newcastle. If you are enrolled in one of the following courses (NURS1101/NURS1201/NURS2101/NURS2201/NURS3101/NURS3201/MIDI1201/MIDI2102/MIDI2202/MIDI3102) and would like to receive the 5 bonus course marks towards the course, only your name and course will be passed on the Research Awareness Experience Administrator.

**How will the information collected be used?**
The results of this study will be reported and disseminated at national and international conferences and scientific publications. You will not be identified in any reports arising from this study. You will receive a brief written summary of results from the research team at the conclusion of the study.

**What do you need to do to participate?**
Please read this Information Statement and be sure you understand its contents before you consent to participate. If there is anything you do not understand, or you have questions, contact the researcher.

If you would like to participate, please complete the attached Consent Form and return it to Dr Megan Rollo via email (megan.rollo@newcastle.edu.au).

You will then be contacted to arrange a time convenient to you to attend a testing session.

**Further information**
If you would like further information please contact Professor Clare Collins (clare.collins@newcastle.edu.au) or Dr Megan Rollo (megan.rollo@newcastle.edu.au) or phone 02 4985 4956.

Thank you for considering this invitation.

Professor Clare Collins
Professor in Nutrition and Dietetics
NHMRC CDA Research Fellow
Co-Director, Priority Research Centre in Physical Activity and Nutrition

Dr Megan Rollo
Lecturer in Nutrition and Dietetics.
Post-Doctoral Research Fellow, Priority Research Centre in Physical Activity and Nutrition.
School of Health Sciences
Complaints about this research
This project has been approved by the University’s Human Research Ethics Committee, Approval No. H-2012-0405.

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au.
Appendix 19: Perception study online survey

Skin colour and the perception of health
Questionnaire

Name: ______________________________

Date: __ / __ / __________

To protect your privacy this cover sheet will be removed and destroyed once you have been allocated a study number.

RESEARCHERS:

Prof Clare Collins
Faculty of Health
School of Health Sciences
Phone: (02) 4921 5646
Clare.Collins@newcastle.edu.au

Dr Megan Rollo
Faculty of Health
School of Health Sciences
Phone: (02) 4921 5649
Megan.Rollo@newcastle.edu.au

Dr Melinda Neve
Faculty of Health
School of Health Sciences
Phone: (02) 4921
Melinda.Neve@newcastle.edu.au

THE UNIVERSITY OF NEWCASTLE AUSTRALIA
ID: ___ ___ ___

1. What is your current age: ___ ___ years

2. Gender:
   - Female
   - Male

3. Do you have any form of colour-blindness?
   - Yes → unfortunately, you are not eligible to participate in this study.
   - No
   - Don’t Know → Please complete this online test: http://www.archimedes-lab.org/colorblindnesstest.html

4. What is your current weight: ___ ___ . ___ kg

5. What is your current height: ___ ___ . ___ cm

6. Please indicate your ethnic group:
   - White Australian
   - White British
   - White other European
   - White American
   - Hispanic
   - Chinese
   - Japanese
   - South east Asian
   - Other Asian
   - Indian
   - Polynesian
   - Mixed
   - Other – please specify: _______________________

7. What is the highest qualification you have completed? (mark one only)
   - No formal qualifications
   - Year 10 or equivalent (eg School Certificate)
   - Year 12 or equivalent (eg Higher School Certificate)
   - Trade / Apprenticeship (eg Chef, Hairdresser)
   - Certificate / Diploma (eg Child care, Technician)
   - University degree
   - Higher university degree (eg Grad Dip, Masters, PhD)
8. What is your post code: ________________

9. What do you consider to be your skin colouration type?
   - [ ] Only burns/pale
   - [ ] Burns easily/turns rarely to light bronze
   - [ ] Medium/can burn, tan to bronze
   - [ ] Turns easily to brown/burns rarely
   - [ ] Only turns to dark brown
   - [ ] Deep pigment, dark

10. How would you rate the quality of your own skin?

<table>
<thead>
<tr>
<th></th>
<th>Very bad</th>
<th>Bad</th>
<th>Below average</th>
<th>Average</th>
<th>Above average</th>
<th>Good</th>
<th>Very good</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11. How would you rate your own general health?
   - [ ] Excellent
   - [ ] Very good
   - [ ] Good
   - [ ] Fair
   - [ ] Poor

12. In the last week have you had acne?
   - [ ] No
   - [ ] Yes a little
   - [ ] Yes a lot
   - [ ] Yes very much

13. How often do you get bouts of cold/flu per year?
   - [ ] 0
   - [ ] 1
   - [ ] 2
   - [ ] 3
   - [ ] 4
   - [ ] 5
   - [ ] 6
   - [ ] 7
ID: ____ ____

14. How long do these cold/flu bouts last?

☐ 1 day
☐ 2 days
☐ 3 days
☐ 4 days
☐ 5 days
☐ 6 days
☐ 7 days
☐ 8 days
☐ 9 days
☐ 10 days
☐ 11 days
☐ 12 days
☐ 13 days
☐ 14 days
☐ 15 days
☐ 15+ days

15. How many serves of fruit (fresh, frozen, canned or cooked) (excluding fruit juice) do you typically consume per day? One serve equals 150g, one medium piece or two small pieces, 1 cup canned or tinned.

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10
☐ +10
16. How many serves of vegetables (not including potatoes) do you typically consume per day? One serve equals 75g, one cup of salad or raw vegetables or ½ cup cooked vegetables.

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10
☐ +10

17. Do you think you eat enough fruit and vegetables?

☐ Yes
☐ No
☐ Don't know

18. Do you currently smoke tobacco products

☐ Yes, daily
☐ Yes, at least once a week
☐ Yes, but less often than once a week.
☐ No, not at all (go to question 18)

19. Did you ever smoke?

☐ No
☐ Yes. How long ago did you quit? ___ ___ years and ___ ___ months

20. Have you ever smoked at least 100 cigarettes or a similar amount of tobacco in your life?

☐ Yes
☐ No
☐ Not sure

21. How soon after waking up do you smoke your first cigarette?

☐ Within 5 minutes
ID: __ __ __

☐ 6-30 minutes  
☐ 31-60 minutes  
☐ 61+ minutes

22. How many cigarettes do you smoke in a day?

☐ 10 or less  
☐ 11-20  
☐ 21-30  
☐ 31+
Appendix 20: Statement of contribution and collaboration for Chapter 2

I attest that The Research Higher Degree candidate Kristine Pezdirca contributed to the following paper:


For this paper Kristine Pezdirca (KP), her PhD supervisors (CC and MH) and the authors RW, GO and DP were responsible for the study design and development of the search strategy. KP conducted the literature review search, retrieval of articles, screening the studies, performed the quality appraisals and extracted data from all of the studies. CC and MH shared the screening, quality appraisal and data extraction duties of the second reviewer. KP drafted the initial paper, while all authors contributed to the interpretation of the results and drafts of the manuscript.

Kristine Pezdirca
Date: 03/11/15

Dr Melinda Hutchesson
Date: 03/11/15

Dr Ross Whitehead
Date: 06/11/15
Dr Gozde Ozakinci,
Date: 11/11/15

Professor David Perrett
Date: 11/11/15

Professor Clare Collins
Date: 03/11/15

Professor Robert Callister
Assistant Dean Research Training
Date: 12/11/15
Appendix 21: Statement of contribution and collaboration for Chapter 3

I attest that The Research Higher Degree candidate Kristine Pezdirc contributed to the following paper:


For this paper Kristine Pezdirc (KP), her PhD supervisors (CC and MH) and the authors RW, GO and DP were responsible for the study design. KP was responsible for the data collection. KP, RW and DP conducted the data analysis. KP drafted the initial paper, while all authors contributed to the interpretation of the results and drafts of the manuscript.

Kristine Pezdirc  
Date: 03/11/15

Dr Melinda Hutchesson  
Date: 03/11/15

Dr Ross Whitehead  
Date: 06/11/15

Dr Gozde Ozakinci,  
Date: 11/11/15
Professor David Perrett
Date: 11/11/15

Professor Clare Collins
Date: 03/11/15

Professor Robert Callister
Assistant Dean Research Training
Date: 12/11/15
Appendix 22: Statement of contribution and collaboration for Chapter 4

I attest that The Research Higher Degree candidate Kristine Pezdiric contributed to the following paper:


For this paper Kristine Pezdiric (KP) and her PhD supervisors (CC and MH) were responsible for the study design. KP was responsible for the data collection and the running of the RCT. KP, RW and LW were responsible for the analysis of plasma carotenoids. KP was responsible for the data analysis. KP drafted the initial paper, while all authors contributed to the interpretation of the results and drafts of the manuscript.

Kristine Pezdiric
Date: 03/11/15

Dr Melinda Hutchesson
Date: 03/11/15

Rebecca Williams
Date: 03/11/15

Dr Megan Rollo
Date: 03/11/15
Dr Tracy Burrows
Date: 03/11/15

Associate Professor Lisa Wood
Date: 03/11/15

Professor Clare Collins
Date: 03/11/15

Professor Robert Callister
Assistant Dean Research Training
Date: 12/11/15
Appendix 23: Statement of contribution and collaboration for Chapter 5

I attest that the Research Higher Degree candidate Kristine Pezdirc contributed to the following paper:


This study design was a replication of that conducted at St Andrews University by a research team led by Professor David Perrett. MR and KP were responsible for the data collection. MR, KP, RW and DP conducted the data analysis. KP drafted the initial paper, while all authors contributed to the interpretation of the results and drafts of the manuscript.

Kristine Pezdirc
Date: 03/11/15

Dr Megan Rollo
Date: 03/11/15

Dr Ross Whitehead
Date: 10/11/15

Dr Melinda Hutchesson
Date: 03/11/15
Dr Gozde Ozakinci,
Date: 11/11/15

Professor David Perrett
Date: 11/11/15

Professor Clare Collins
Date: 03/11/15

Professor Robert Callister
Assistant Dean Research Training
Date: 12/11/15