DIETARY EFFECTS ON SKIN COLOUR: APPEARANCE-BASED INCENTIVES TO IMPROVE FRUIT AND VEGETABLE CONSUMPTION

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Appearance-Based Incentives to Improve

Fruit and Vegetable Consumption

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Collaboration Statement

Throughout the experimental chapters (Chapters 5-10) in this body of work, the use of the personal pronoun "we" reflects the collaborative nature of experiments conducted in the Perception Lab. The use of "I" would betray the joint effort in running studies, however the design, analysis and discussion of experiments as detailed in the presented work is my own under the support of my supervisors.

This thesis is partly based on works submitted to and accepted for publication in peer-reviewed academic journals. These articles are identified at the beginning of each chapter in which they are featured. Co-authors are listed when they contributed intellectually to the presented work. Dr Dengke Xiao constructed the computer programmes used to display facial stimuli and record observers' perceptual preferences (Chapters 5-9). Dr David Hunter developed the computer programme used to manipulate facial skin colour (Chapters 5-9) and measure skin colour from facial photographs (Chapter 5). Dr Ian Stephen contributed to the development of the procedure used to colour-calibrate facial photographs (Chapters 5-9). Dr Stephen also contributed to revisions of the manuscript on which Chapters 2-4 are partially based (accepted for publication in the *American Journal of Public Health*). Daniel Re assisted my design, analysis and write-up of the psychophysical experiment conducted in section 7.4.

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Abstract

Poor diet precipitates significant social and economic burden, necessitating effective and economical dietary intervention strategies. Current population-level campaigns provide guidelines for living healthily and focus on the impact of lifestyle on chronic disease risk. Behavioural interventions which capitalise on individuals' existing cognitions are likely to be more effective. A programme of work is presented here which evaluates the feasibility and efficacy of an appearance-based dietary intervention approach. This project aims to improve fruit and vegetable consumption by illustrating the associated benefits to skin appearance.

The impact of fruit and vegetable consumption on skin colour is assessed (Chapter 6), corroborating previous between-subjects evidence which finds that dermal yellowness (CIE b*) is positively associated with fruit and vegetable intake. This work also discovers that modest within-subject dietary change is sufficient to perceptibly alter skin colour within six weeks (Chapter 7). Perceptual preferences are examined (Chapters 5 to 9), finding that optimally healthy skin colouration is that associated with increased fruit and vegetable consumption.

Two behavioural intervention trials are conducted (Chapters 6 and 9) to evaluate whether visualising the impact of fruit and vegetable consumption on skin colour motivates dietary improvement. Relative to control groups, participants receiving an appearance-based intervention (in which the above effects are illustrated and explained) reported improvements in diet, particularly when illustrations were performed upon images of one's own face. It may be valuable to disseminate such an intervention at a population level, though a number of further longitudinal studies are necessary to determine the wider effectiveness of this approach.

1. The Burden of Disease Attributable to Diet

and

Health Benefits of Fruit and Vegetable Intake

1.1. Summary

Lifestyle-precipitated chronic diseases contribute heavily to incidences of preventable morbidity and mortality worldwide. Poor diet places a particularly substantial burden on wellbeing. This chapter reviews past and present evidence linking diet to health outcomes and evaluates epidemiological methodology. Special attention is paid to diabetes, cancer and vascular diseases as the endpoints responsible for the greatest loss of healthy life-years. Fruit and vegetables are identified as a food group which is particularly valuable for health, as their consumption confers active benefits and may also preclude the intake of deleterious foodstuffs. The biology behind the observed associations will be discussed and attention will be paid to the mechanisms by which fruit and vegetables are associated with positive health outcomes.

1.2. The Impact of Lifestyle-Precipitated Disease

Advances in modern medicine have facilitated reductions in the risks posed by acute illnesses, but this blessing has an associated drawback. Non-communicable diseases are increasingly responsible for major threats to health, especially in industrialised nations (WHO, 2011a). Fifty-seven million people died worldwide in 2008, an estimated 36 million of these deaths were due to non-communicable chronic diseases (WHO, 2011a). This threat to longevity is precipitated largely by a small number of avoidable lifestyle-factors (WHO, 2011a). Alcohol consumption, tobacco use, inadequate exercise and unhealthy diet are held as central behavioural risk factors, collectively held responsible for 13.2 million deaths per year worldwide and over 18% of the world's global health burden (WHO, 2010), according to the number of disability-adjusted life-years (DALYs) lost.

The negative ramifications of these deleterious behaviours are widely felt; in addition to the consequences for personal wellbeing, poor population health is associated with an overburdening of healthcare systems, fiscal strain due to lost productivity and the necessity to support increasing numbers who are too unwell to support themselves (WHO, 2001). This issue is magnified by the demographic shift towards longer life expectancy which accompanies economic development (Thompson, 1929).

1.3. Diet and Chronic Disease

Diet has a unique influence on population health. As indicated by a wealth of epidemiological investigations, dietary intake is a key factor in the development and prevention of chronic diseases. Many of the problems precipitated by diet are attributable to one overarching factor; economic development facilitates the availability and reduces the cost of foods which are able to satisfy an evolved biological urge for calorie-density (Drewnowski & Popkin, 1997; S. K. Lee & Sobal, 2003; Leonard, Snodgrass, & Robertson, 2010). Whilst this desire for energy-dense foods is beneficial in the short-term, sustaining calorifically expensive brains and musculature, this preference often precludes the consumption of nutrient-rich foods (Drewnowski & Popkin, 1997), resulting in largely negative consequences for long-term health. As such, diet is firmly established as an important determinant of chronic disease in developed nations and this issue is expected to become increasingly prevalent in developing countries (WHO, 2004). Consequently, a global priority of the World Health Organisation (WHO, 2004) is to ameliorate the growing challenge posed by unhealthy diet.

Epidemiology is concerned with identifying the root causes of disease and is characterised by the employment of case-control and prospective cohort studies to investigate hypothesised determinants. Case-control studies examine differences in *previous* exposure to hypothesised risk-factors between individuals with and without a target disease (Beaglehole, Bonita, & Kjellström, 1993; Miettinen, 2011). Prospective-cohort designs conversely measure exposure to potential risk-factors *longitudinally* in large groups of individuals assumed to be disease-free. Differences in risk-factor exposure are then investigated as predictors of disease presence at follow-up (Beaglehole et al., 1993; Miettinen, 2011). Experimental evidence can also offer valuable insight into the causes of disease, and involves the purposeful manipulation of risk-factor exposure in both humans and animals to examine hypothesised effects (Beaglehole et al., 1993).

Numerous dietetic risk-factors have been explored with epidemiological methods, identifying potential links between specific dietary components and disease prevalence. For instance, the consumption of fat-rich food items is associated with cancer incidences. Significantly higher total fat intake has been found in individuals with malignant lung tumours, compared to matched controls (Wynder, Hebert, & Kabat, 1987; Xie, Lesaffre, & Kesteloot, 1991). This association has also been found in the absence of tobacco use (Alavanja, Brownson, & Benichou, 1996), which is itself a clear determinant of lung cancer (Boyle, 1997). Intake of animal-derived fats has been prospectively associated with colon (Willett, 1998), prostate (Giovannucci et al., 1993) and breast (Cho et al., 2003) cancers, suggesting a specific role of *saturated* fat. Indeed, weaker associations have been found with unsaturated and total fat intakes (Bairati, Meyer, Fradet, & Moore, 1998; Hursting, Thornquist, & Henderson, 1990). Consumption of hydrogenated fats, chiefly via red meats, dairy products and eggs (United States Department of Agriculture, 2005), has also been associated with cancers of the breast (Hankin, Zhao, Wilkens, & Kolonel, 1992; Hursting et al., 1990), prostate (Hursting et al., 1990), ovaries (Blank, Wentzensen, Murphy, Hollenbeck, & Park, 2012; Genkinger et al., 2006; Huncharek & Kupelnick, 2001), colon (Hursting et al., 1990) and lung (Hankin et al., 1992) to a greater extent than consumption of unsaturated fats. A number of further studies have, though, suggested that the link between saturated fat consumption and cancer risk is weak at best (Crowe et al., 2008; Holmes, Hunter, Colditz, Stampfer, & Hankinson, 1999; Sieri et al., 2008; Smith-Warner et al., 2001; Willett, 1998).

Saturated fat consumption has also been associated with the development of atherosclerosis (Merchant et al., 2008). This precipitates vascular diseases (Ross,

1986) which are among the leading causes of DALY losses worldwide (WHO, 2003). Instances of cardiovascular disorder (Hu & Willett, 2002), cerebrovascular disorder (Kesteloot, Sasaki, Xie, & Joossens, 1994) and peripheral vascular disorder (Katsouyanni et al., 1991) have been attributed to high saturated fat intake, however other epidemiological research has returned equivocal results in this area, with a number of both observational (Ravnskov, 1998; Siri-Tarino, Sun, Hu, & Krauss, 2010) and experimental (Ball et al., 1965; Burr et al., 1989; Howard et al., 2006) studies finding little or no association between saturated fat intake and vascular diseases. There have also been a number of studies suggesting a *protective* association between saturated fat consumption and vascular disease, for instance strokes have been revealed to be less common amongst those that consume greater amounts of saturated fat (Gillman et al., 1995; Sauvaget, Nagano, Allen, Grant, & Beral, 2003).

The contradictions above highlight the complex nature of dietary behaviour, and the importance of investigating interactions between key dietary components and health outcomes. Determining the independent influence of a particular dietary element is essential, but problematic, particularly in the case of fats, as this food component is responsible for a large percentage of daily caloric intake (Kennedy, Bowman, & Powell, 1999). Individuals that consume low amounts of fats, or reduce the amount of fats that they consume must fulfil their daily energy quota through other sources. Carbohydrate-rich foods are a viable alternate source of calories. The consumption of such foods, however, may also be positively associated with incidences of chronic diseases, perhaps to an even greater extent than saturated fats (Hu, 2010). Glycemic-load (a measure of immediately available energy from carbohydrates; Jenkins et al., 1981) has been positively associated with incidences of cancer (Augustin et al., 2001; George et al., 2009; Lajous, Boutron-Ruault, Fabre, Clavel-Chapelon, & Romieu, 2008; Silvera et al., 2005), type II diabetes (Dong, Zhang, Zhang, & Qin, 2011) and cardiovascular disorder (Beulens et al., 2007) across a number of case-control and prospective cohort studies. A number of further studies, though, have conversely indicated no strong relationship between glycemic-load and chronic disease (Larsson, Friberg, & Wolk, 2007; Neilsen, Olsen, Christensen, Overvad, & Tjonneland, 2005; Tavani et al., 2003; S. H. Yun, Kim, Nam, Kong, & Kim, 2010).

It is likely that epidemiological studies investigating the impact of saturated fat on chronic disease are confounded by the deleterious impact of increased carbohydrate consumption, and vice versa, meaning that independent links between these dietary components and health outcomes are hard to extricate. Supporting this hypothesis, a number of clinical trials have found no improvement in cardiovascular disease and cancer risk when experimentally replacing saturated fat with increased intake of carbohydrates (Ball et al., 1965; Burr et al., 1989; Howard et al., 2006; Hu, 2010; Jakobsen et al., 2009; Smith-Warner et al., 2001). Conversely, replacing dietary saturated fat with *unsaturated* fat yields a more positive impact on chronic disease outcomes and markers in humans (Cortese et al., 1983; Jakobsen et al., 2009; Mozaffarian, Micha, & Wallace, 2010; Tanasescu, Cho, Manson, & Hu, 2004; Turner, Le, & Brown, 1981) and non-human primates (Rudel, Johnson, Sawyer, Wilson, & Parks, 1995). Further, a prospective study of over 80,000 women found that combined fat intake was not associated with cardiovascular disease risk, yet increasing the ratio of unsaturated to saturated fats was found to have a protective effect (Hu et al., 1999; Hu et al., 1997). Jakobsen (2010) also revealed that replacing dietary saturated fats with low glycemic-index (slow energy release) carbohydrates was inversely, but non-significantly associated with myocardial infarction risk, whereas substitution of saturated fats for high glycemic-index carbohydrates was associated with significantly increased myocardial infarction risk. This, as the first prospective cohort study to explicitly examine the relative contributions of both fats and carbohydrates to cardiovascular disease prevalence suggests that the deleterious and confounding effect of carbohydrate intake is largely driven by the consumption of refined carbohydrates. This evidence suggests that salient recommendations to decrease total fat consumption at a population level (e.g., United States Department of Agriculture & United States Department of Health and Human Services, 1980, 1985; WHO, 1990), whilst having the best intentions, may have precipitated widespread *negative* health consequences by inadvertently causing increased consumption of carbohydrates (Marantz, 2010; Marantz, Bird, & Alderman, 2008).

Since the 1970s there have been significant, widespread increases in carbohydrate intake and total caloric consumption (Centers for Disease Control, 2005; Gaesser, 2007; Kearney, 2010; Maskarinec et al., 2006). This trend, coupled with increased sedentariness is associated with the rising prevalence of obesity and diabetes (Haslam & James, 2005; Mokdad et al., 2001; WHO, 2011a, 2011b) in economically developed countries. Excess calorie intake (consuming more calories than those expended via physical activity), total calorie intake and the consumption of high glycemic-index foods have been associated with overweight (Gross, Li, Ford, & Liu, 2004; J. O. Hill & Peters, 1998; Spiegelman & Flier, 2001; Yoshita et al., 2010). Experimental trials have also elucidated the relationship between caloric intake and obesity. For example, manipulations to decrease total calorie intake (and glycemic load) have reliably led to reduced body mass index in obese and diabetic individuals (Ebbeling, Leidig, Sinclair, Hangen, & Ludwig, 2003; Ebbeling et al., 2005; Foster et al., 2003; Shai et al., 2008; Strychar, 2006; Thomas, Elliott, & Baur, 2007). Such trials offer relative clarity in the absence of potential confounds, such as biased reporting of diet and physical activity, which may have clouded observational evidence in this area (see Ballard-Barbash, Graubard, KrebsSmith, Schatzkin, & Thompson, 1996; Lichtman et al., 1992; Livingstone & Black, 2003; Yoshita et al., 2010 for null, weak and inverse effects of self-reported calorie intake on obesity). Obesity, weight gain and duration of obesity are robustly associated with a number of negative health outcomes (Flegal, Graubard, Williamson, & Gail, 2005; Must, Jacques, Dallal, Bajema, & Dietz, 1992), particularly incidences of type II diabetes (Abdullah et al., 2011; E. S. Ford, Williamson, & Liu, 1997; Pontiroli & Galli, 1998; H. E. Resnick, Valsania, Halter, & Lin, 2000), numerous cancers (Calle, Rodriguez, Walker-Thurmond, & Thun, 2003; Calle, Thun, Petrelli, Rodriguez, & Heath, 1999; Must et al., 1992) and cardiovascular disease (Calle et al., 1999; Must et al., 1992; Suka, Miwa, Ono, & Yanagisawa, 2011), indicating that it is essential to moderate consumption of high calorie and glycemic-index foods.

Dietary sodium chloride intake is also associated with chronic disease prevalence. Many processed foods, consumed in abundance in economically developed nations, contain high levels of sodium (>1g/100g; Mhurchu et al., 2011; Webster, Dunford, & Neal, 2010). Regular consumption of excessive levels of sodium chloride has been associated with a number negative endpoints, particularly with gastric (Joossens et al., 1996; Kurosawa, Kikuchi, Xu, & Inaba, 2006; Tsugane,

2005) and bladder (Riboli et al., 1991; Vena et al., 1992) cancers, although though links between salt intake and cancer at other sites (breast, pharynx, lung, gastrointestinal tract) have also been reported (Strnad, 2010). Salt intake has also been associated with cerebrovascular (Ikeda, Kasahara, Koizumi, & Watanabe, 1986; Takemori, Mikami, Nihira, & Sasaki, 1993) and cardiovascular (N. R. Cook et al., 2007; Klaus et al., 2009) disorders. There is, though, extant controversy regarding the effects of sodium intake on health outcomes (Alderman, 2006, 2010), likely because reducing intake to very low levels may also precipitate negative effects (Cohen, Hailpern, & Alderman, 2008; Jessup et al., 2009).

1.4. Fruit and Vegetable Consumption and Chronic Disease

Fruit and vegetable consumption is particularly important in the prevention of chronic diseases (WHO, 2011b). Inadequate intake of this food group is held to be responsible for up to 2.6 million premature deaths per year worldwide (Lock, Pomerleau, Causer, Altmann, & McKee, 2005) and the annual loss of up to 26.7 million years of healthy life to disability (WHO, 2002). Fruit and vegetables confer active benefits to health (see review below) and, as people consume a relatively fixed weight of food per day (WHO, 2005), may also achieve positive health outcomes by precluding excessive consumption of the calorie-dense, nutritionally-poor food components outlined above (Haslam & James, 2005; WHO, 2005). As with these unhealthy food components the reported beneficial effects of fruit and vegetable consumption are subject to some controversy, however the majority of good-quality epidemiological evidence is in support of there being a broad protective effect.

Fruit and vegetable consumption has been shown in hundreds of case-control studies to be protective of cancers (see Block, Patterson, & Subar, 1992; Steinmetz &

Potter, 1996; Vainio & Weiderpass, 2006 for reviews). A number of prospective cohort studies have also revealed protective effects (see Steinmetz & Potter, 1996; Vainio & Weiderpass, 2006 for reviews). Suggesting that the link is weaker than indicated, one of the largest prospective studies of its kind (with around half a million participants recruited at baseline), the recently analysed European Prospective Investigation into Cancer and Nutrition, provides only a weak association between fruit and vegetable consumption and overall cancer risk (Boffetta et al., 2010). This study is consistent with a meta-analysis which finds discrepancy between the two main epidemiological methods in this field (Riboli & Norat, 2003). Case-control studies consistently report stronger evidence for the cancer-preventive properties of fruit and vegetables than do cohort designs. Prospective cohort studies are considered to provide the strongest epidemiologic evidence, primarily because they enable avoidance of recall bias. The results of case-control studies, conversely, are held to be more open to confounding as the dietary reports of those who contract a target disease may be subject to demand characteristics (Orne, 2009) and bias their recollection of past diet to fit beliefs about the cause of their illness. Hence prospective investigations (e.g., Kirsh et al., 2007; Sato et al., 2005; Takachi et al., 2008) are held to reflect a truly weak relationship between fruit and vegetable consumption and cancer risk.

This apparent methodological impact may, though, also reflect differential validity of exposure assessments across epidemiological techniques. Prospective designs often establish only a snapshot of diet in a cancer-free sample at baseline but diet is likely to fluctuate greatly within participants (Bohlscheid-Thomas, Hoting, Boeing, & Wahrendorf, 1997; Martinmoreno et al., 1993; Salvini et al., 1989). Case-control studies on the other hand involve long-term retrospective estimation of diet in order to establish typical pre-morbid eating habits in those diagnosed with cancer. These retrospective assessments could prove to be an accurate measure of actual long-term dietary intake, especially as a cancer diagnosis could predispose individuals to confront their past lifestyle with impartiality (Demark-Wahnefried, Aziz, Rowland, & Pinto, 2005). Cancer risk may be reduced only by a *consistently* healthy diet, and as such, case-control studies may more frequently return evidence of a protective effect than prospective studies. The inconsistency of observational designs, though, warrants yet further examination in order to elucidate the true effect of fruit and vegetables on cancer risk. Specific effort should be made to conduct prospective studies with longer-term or rolling estimations of pre-morbid dietary intake.

Even if the effect of fruit and vegetable consumption on overall cancer risk is overstated, consumption of this food group is less ambiguously associated with cancer-protective effects in at-risk individuals. For instance, high fruit and vegetable consumption is consistently protective of lung (Buchner et al., 2010; Galeone et al., 2007; C. M. Gao, Tajima, Kuroishi, Hirose, & Inoue, 1993; Jansen et al., 2001; Linseisen et al., 2007) and oropharyngeal cancer in smokers (Sanchez et al., 2003) and overall cancer risk in heavy drinkers (Boffetta et al., 2010; Sanchez et al., 2003).

Further, fruit and vegetables have also been particularly strongly associated with vascular disease prevention. Cardiovascular disease has been negatively associated with fruit and vegetable intake in a number of large case-control (La Vecchia, Chatenoud, Altieri, & Tavani, 2001; Ness & Powles, 1997; Panagiotakos, Pitsavos, & Stefanadis, 2006; Yusuf et al., 2004) and prospective cohort studies

(Bazzano et al., 2002; Hung et al., 2004; Joshipura et al., 1999; Joshipura et al., 2001; S. Liu et al., 2000; Takachi et al., 2008). Similarly, cerebrovascular disease is more frequent (Kastorini et al., 2011; Mahe et al., 2010; O'Donnell et al., 2010), and is more likely to develop (Dauchet et al., 2008; Gillman et al., 1995; He, Nowson, & MacGregor, 2006; Johnsen et al., 2003; Knekt et al., 1994; Mizrahi et al., 2009; Ness & Powles, 1997) amongst those that report low fruit and vegetable consumption. A relatively small collection of evidence suggests that there are positive or null associations between fruit and vegetable intake and vascular diseases (see Ness & Powles, 1997 for examples). However, in light of the uncertain and poor methodological quality of such studies (e.g., poor measures of diet) and the extent of evidence suggesting a negative association, it is very likely that fruit and vegetable consumption has a protective effect against vascular diseases.

Fruit and vegetable consumption is also frequently protective of obesity (Buijsse et al., 2009; Lissner, Lindroos, & Sjostrom, 1998; Maskarinec, Novotny, & Tasaki, 2000; Schroder, 2010) and its comorbidities, for instance type II diabetes (Bazzano, Li, Joshipura, & Hu, 2008; R. P. K. Ford, Cowan, Schluter, Richardson, & Wells, 2001; Harding et al., 2008) and metabolic syndrome (Baxter, Coyne, & McClintock, 2006; Esmaillzadeh, Kimiagar, Mehrabi, & Azadbakht, 2007). Null findings are also common though (see Hamer & Chida, 2007), likely because of stronger associations between excessive caloric intake (via fat and carbohydrate consumption) and these chronic diseases. There is nevertheless strong evidence that fruit and vegetable consumption protects against worsening of the comorbidities associated with obesity and diabetes (Celik, Celik, & Akpolat, 2009; Esposito et al., 2004; Giammarioli et al., 2004; Itsiopoulos et al., 2011; Nothlings et al., 2008; Takahashi et al., 2012).

Inadequate intake of fruit and vegetables is additionally linked to a host of other non-communicable chronic diseases which further contribute to DALY losses (WHO, 2003). Incidences of maculopathy (VandenLangenberg et al., 1998), cataracts (Christen, Liu, Schaumberg, & Buring, 2005; Pastor-Valero, Vioque, Navarrete-Munoz, & Monzo, 2011), inflammatory bowel disease (Hou, Abraham, & El-Serag, 2011), Parkinson's disease (X. Gao et al., 2007), gout (Schlesinger, 2005), asthma (Nja, Nystad, Carlsen, Hetlevik, & Carlsen, 2005), psoriasis (Brown et al., 2004; Naldi et al., 1996), rheumatoid arthritis (Pattison, Harrison, & Symmons, 2004) and liver cirrhosis (La Vecchia, Decarli, & Pagano, 1998) have all been independently linked to low fruit and vegetable intake.

1.5. Biological Mechanisms of Chronic Disease Prevention

The above evidence indicates that fruit and vegetable consumption is broadly protective against chronic diseases. The mechanisms responsible for these protective effects have been examined with a wide range of methodologies, from *in vitro* studies of basic biological effects to clinical trials of *in vivo* effects. Overall, this research suggests that the consumption of a wide range of phytochemicals, rather than a circumscribed subset, is necessary to convey the observed active benefits.

One of the chief mechanisms by which fruit and vegetables protect against chronic disease is likely to involve oxidative stress, a biological state in which the systemic ratio of oxidising to anti-oxidising molecules is in favour of the former (Sies, 1997). Oxidative stress precipitates deleterious oxidative modification of cellular proteins, lipid membranes and DNA (Sies, 1993), and as such is commonly implicated in a number of chronic diseases (Droge, 2002). Oxidative stress is particularly strongly implicated with atherosclerosis and vascular diseases (Cai & Harrison, 2000; Stocker & Keaney, 2004) as vascular obstructions accumulate when lipoproteins are oxidised, ultimately leading to the starvation and death of cells in critical organs such as the heart and brain (Stocker & Keaney, 2004). Oxidative stress is also held to play a major role in the development of cancers (Droge, 2002; Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006) as oxidative damage can precipitate DNA mutations. When these modifications are non-lethal to the cell, essential processes of programmed cell death (apoptosis) can be interrupted and uncontrolled, irreversible proliferation of cells may be initiated, leading to tumour growth.

Fruit and vegetables contain many antioxidant compounds which could protect against oxidative stress (Sies, 1993), chiefly by preventing the formation of reactive oxygen species and by intercepting pro-oxidants before their harmful effects manifest on cellular apparatus. Observational studies linking dietary consumption of antioxidant-rich fruit and vegetables to chronic disease protection suggest that these phytochemicals may be at least partly responsible for witnessed protective effects (e.g., Gaziano, 2004; Hertog, Feskens, Hollman, Katan, & Kromhout, 1994; Knekt et al., 1997; Kubo et al., 2008; Seddon et al., 1994), however these studies do not directly address the impact of specific antioxidants on health and may be confounded by additionally present active ingredients.

As such, clinical trials have been performed in an attempt to verify the unique impact of hypothesised compounds by orally administering specific antioxidants individually in randomised placebo-controlled trials. These though, in contradiction of observational studies of diet, have repeatedly found that dietary antioxidant supplements confer little benefit to health. Supplementation of α-tocopherol (vitamin E) has been shown in a number of large-scale clinical trials to have no protective effect against cardiovascular disease in at-risk patients (Lonn et al., 2005; Valagussa et al., 1999; Yusuf et al., 2000). A systematic review of large-scale randomised controlled trials also revealed no net benefit of β-carotene, α-tocopherol or ascorbic acid (vitamin C) antioxidant supplements on cardiovascular disease prevalence in healthy individuals (Asplund, 2002). Further, administering antioxidant supplements is unlikely to be protective of cancers. A number of clinical trials have returned null results. Supplementing healthy and diabetic participants with antioxidants (α-tocopherol, retinol, ascorbic acid, β-carotene, zinc) and antioxidant precursors (selenium) conferred no protective effect against overall, or specific cancer risk (see Myung, Kim, Ju, Choi, & Bae, 2010 for a meta-analysis incorporating over 160,000 participants; see also Collins et al., 2002; Lin et al., 2009; Papaioannou et al., 2001; Sesso et al., 2008 for examples of large-scale trials). Additionally, antioxidant supplements have been ineffective in preventing Barrett's esophagus (Kubo et al., 2008), inflammatory bowel disease (Rezaie, Parker, & Abdollahi, 2007) and arthritis (Canter, Wider, & Ernst, 2007).

A number of clinical trials have also indicated *harmful* effects of antioxidant supplementation. In a now infamous clinical trial, smokers receiving high-dose β-carotene and retinol supplements were more likely to die of lung cancer and cardiovascular disease than matched controls receiving a placebo (Omenn et al., 1996). Further, antioxidant supplements have been associated with increases in gastrointestinal (Bjelakovic, Nikolova, Simonetti, & Gluud, 2004, 2008), bladder

(Myung et al., 2010) and overall cancer mortality rates in a recent meta-analysis (Myung et al., 2010).

The contrasting impact of antioxidant supplementation and fruit and vegetable consumption may be for a number of reasons. An overarching issue is that the actions of antioxidants are better understood *in vitro* than *in vivo* (Britton & Helliwell, 2008; Burton, 1989). Trials administering antioxidant supplements did so in very high doses relative to the amounts of these substances that can feasibly be ingested via fruit and vegetable consumption (e.g., Omenn et al., 1996). The break-down products of some antioxidants are themselves toxic and pro-oxidative (Siems et al., 2005) and, as particular antioxidants may be readily expended by oxidising agents when systemic reserves of alternative antioxidants are low (Vinkler & Albrecht, 2010), administering high doses of single antioxidants could precipitate oxidative tissue damage (Siems et al., 2002). Synergistic relationships between antioxidants are likely to exist (Jeon, Kim, Lee, Shin, & Lee, 2008; C. Liu, Russell, & Wang, 2004; Sies, 1993), but these are compromised when a circumscribed subset of phytochemicals is administered. Recycling of antioxidant molecules is also likely to be critical to antioxidant defences. For instance, maintenance of the antioxidant capacity of α-tocopherol is contingent on the availability of carotenoids (Monaghan, Metcalfe, & Torres, 2009), and α-tocopherol breakdown product toxicity is mitigated by the presence of carotenoids (Monaghan et al., 2009).

Antioxidant supplementation may also fail to reap positive health outcomes due to additional health-protective aspects of fruit and vegetable consumption being absent. For instance, fruit and vegetables contain many additional non-antioxidant phytochemicals that are likely to have essential health-protective roles in combination and moderation, including a number of actively anticarcinogenic compounds (Steinmetz & Potter, 1991, 1996), such as dithiolthiones (Ansher, Dolan, & Bueding, 1983, 1986), allium compounds (Merhi, Auger, Rendu, & Bauvois, 2008), glucosinolates (I. T. Johnson, 2002) and indoles (Sarkar & Li, 2004). Fruit and vegetables also contain high amounts of fibre, which is important for gastrointestinal health (J. W. Anderson, Smith, & Gustafson, 1994; Eastwood & Kritchevsky, 2005) and glycemic control (J. W. Anderson et al., 1994). Increased intake of fibre also achieves satiety whilst precluding the consumption of calorifically high, nutritionally poor foods (J. W. Anderson et al., 1994; Gorman & Bowman, 1993).

Many fruits and vegetables are also rich in chemical elements that are essential for biological functions. For instance calcium is abundant in this food group (Health Canada, 2010) and may play an important role in blood pressure modulation (Cappuccio, Elliott, Allender, & Cutler, 1997; L. M. Resnick, 1999). Dietary copper can be acquired through fruit and vegetable consumption (Health Canada, 2010), has a key role in metabolic, antioxidant and immunological pathways (Bonham, O'Connor, Hannigan, & Strain, 2002; Uriu-Adams & Keen, 2005) and is particularly important in cardiac vasculature (Klevay, 2000). Iron deficiency is associated with anaemia, particularly in pregnant women (WHO, 2000) and iron-rich fruit and vegetable consumption increases haemoglobin production (Peneau et al., 2008).

The literature reviewed in this chapter makes it clear that increasing the percentage of calories obtained via fruit and vegetable consumption is likely to convey active benefits and simultaneously reduce the intake of calorifically-dense, nutritionally poor foods. As such, fruit and vegetables may be the most valuable food group to target in order to improve population health.

2. Current Dietary Intervention Techniques

This chapter is partially based on the following work accepted for publication in a peer reviewed journal (accepted August 4, 2011):

Whitehead, R., Ozakinci, G. Stephen, I. D. & Perrett, D. I. (2012). Appealing to vanity: Could potential appearance improvement motivate fruit and vegetable consumption? *American Journal of Public Health, 102*(2), 207-211.

2.1. Summary

The previous chapter indicated the necessity of a healthy diet, specifically identifying the importance of fruit and vegetable consumption. This chapter will begin by briefly outlining current estimates of fruit and vegetable intake per capita in developed nations, highlighting an urgent requirement to motivate improvements in consumption of this food group. The utility of current population-level and more intensive attempts to motivate dietary change will then be appraised. Significant drawbacks of each of these contemporary approaches will be identified, but key positives will also be outlined. Evidence-based models of behaviour change will be considered and it will be concluded that an intervention methodology which combines approaches across the major dietary intervention paradigms could be effective.

2.2. Estimates of Current Fruit and Vegetable Intake

The previous chapter makes it clear that fruit and vegetables have a unique beneficial impact on health, however according to self-reported and objective assessments of intake, the under-consumption of this food group (relative to the international guideline of 400g per day, WHO, 1990) is ubiquitous in economically developed nations (WHO, 2004, 2010).

Adults in the United Kingdom are commonly found to under-consume fruit and vegetables. In an intensive study of over 2,000 British adults' dietary habits in 1986-87, participants weighed and self-reported intake over a seven day period (Billson, Pryer, & Nichols, 1999). Participants consumed an average of 245g fruit and vegetables per day, with over 25 per cent consuming less than 100g. A more recent study found that over half of a sample of 16,000 English adults reported (via food frequency questionnaire) consumption of less than 400g of fruit and vegetables per day (Myint et al., 2007). The rolling Scottish Health Survey commenced in 2008 and has repeatedly found marked under-consumption of fruit and vegetables across demographic groups in Scotland. Around 80 per cent of individuals in the 2008 and 2010 Scottish samples (Scottish Government, 2008, 2010) did not consume fruit and vegetables in line with recommended levels, and the population average intake is well below 400g per day (approximately 260g per day in 2008, and 256g per day in 2010). Of particular concern, approximately 10 per cent of Scots report zero intake of fruit and vegetables per day, a figure which extends to 20 per cent of young adults between 16 and 24.

The European Prospective Investigation into Nutrition and Cancer found that a cohort of 452,187 adults across 10 European countries consumed an average of

455.6g of fruit and vegetables per day (Agudo et al., 2002), although the method of diet estimation used in this large-scale study (food frequency questionnaire) is known to overestimate intake relative to other methods (Bingham, 1997; Michels, Welch, Luben, Bingham, & Day, 2005), so it is possible that the true figure is considerably less than this. Despite this potential overestimation, approximately 50 per cent of individuals in this sample reported consumption of less than 400g per day.

The American National Health and Nutrition Examination Surveys have also consistently revealed poor fruit and vegetable consumption in the United States. In a 24-hour recall study of around 15,000 American adults between 1988 and 1994, a mere 10.9 per cent met fruit and vegetable intake guidelines, with this sample reporting the consumption of 3.06 servings per day on average (Casagrande, Wang, Anderson, & Gary, 2007). These findings were later repeated in a further sample of 8,900 reporting their dietary habits between 1999 and 2002 (Casagrande et al., 2007). A recent Canadian study also indicated that 77 per cent of a sample of over 15,000 adults reported consumption of fewer than five servings per day (Dehghan, Akhtar-Danesh, & Merchant, 2011).

In addition to the discussed overestimation issues specific to food frequency questionnaires, it is important to consider that self-reported fruit and vegetable consumption may be subject to response biases more generally. Specifically, demand characteristics may be present as fruit and vegetable consumption can be seen as socially appropriate (T. M. Miller, Abdel-Maksoud, Crane, Marcus, & Byers, 2008; Orne, 2009). Objective markers of fruit and vegetable consumption offer a means of circumventing these issues. Whilst such measures cannot fully elucidate absolute levels of fruit and vegetable intake (Jansen et al., 2004), they are potentially useful in providing guidelines to identify particularly low consumers and for validating self-report measures.

Examples of markers include blood plasma carotenoid levels (e.g., Jansen et al., 2004), adipose tissue carotenoid levels (e.g., Gomez-Aracena et al., 2003 which may be particularly useful for estimating long-term fruit and vegetable consumption), urinary flavonoid levels (e.g., Brantsaeter et al., 2007), and plasma ascorbic acid and folate concentrations (e.g., Macdonald et al., 2009). Previous studies have attempted to quantify the levels of objective markers associated with under consumption of fruit and vegetables (e.g., Bingham et al., 1995; Khaw et al., 2001). For instance levels of 50μmol of ascorbic acid per litre of blood have been taken to indicate adequate fruit and vegetable intake, where values over this threshold indicate that the individual is likely to consume over 400g of fruit and vegetables per day (Khaw et al., 2008). A 2008 study of over 20,000 British adults found that the blood of over 50 per cent of males and almost 30 per cent of females fell below this criterion, suggesting that these individuals are unlikely to consume adequate levels of fruit and vegetables. Other studies, though, have found ascorbic acid levels to be considerably greater than 50μmol/l amongst individuals that consume over five portions per day (e.g., Macdonald et al., 2009), and by considering only a single objective marker, confounding by factors other than dietary intake is likely (e.g., supplement intake Khaw et al., 2001) suggesting that in reality, a much greater proportion of this large sample may be under-consuming fruit and vegetables.

Given the benefits of fruit and vegetable consumption as detailed in Chapter 1, the above findings suggest that urgent and profound dietary change is required at a population level to prevent further escalation of chronic disease burden. The following section of this chapter will discuss contemporary population-level and more intensive strategies with this aim in mind.

2.3. Current Solutions

Current campaigns aiming to improve fruit and vegetable consumption such as the WHO's '5-a-day' scheme (WHO, 1990) and the U.S. Department of Health and Human Services' 'Fruits and Veggies – More Matters' program (Heimendinger, Van Duyn, Chapelsky, Foerster, & Stables, 1996) predominantly aim to provide individuals with information on the health benefits of fruit and vegetables alongside recommended intake levels. For instance, a National Health Service (NHS) healthy eating campaign (NHS, 2011b) details the dietary recommendations of the WHO (1990) and gives practical information about how to achieve these targets. In an attempt to drive populations towards healthier eating habits, the link between diet and wellbeing is frequently cited, with such sources commonly stating that fruit and vegetables are important for general health (e.g., NHS, 2011b). The public are also provided with more specific information about epidemiological findings relating to fruit and vegetable intake, including statistics on cancer, diabetes and cardiovascular incidence (e.g., NHS, 2011b; United States Department of Agriculture, 2005). However, as highlighted by the recent estimates of fruit and vegetable intake above, and by the Produce for Better Health Foundation's *2010 Gap Analysis* (Rosenfeld, 2010), fruit and vegetable consumption has not risen since the inception of such campaigns. This suggests that the provision of health information alone may not be sufficient to motivate adherence to recommendations at a population level.

As a result of such information provision strategies, it may be that individuals are aware of what constitutes a healthy diet, but lack motivation to act on this knowledge (Shepherd & Stockley, 1987; Shepherd & Towler, 1992). Indeed, such campaigns appear to have been relatively successful in improving nutritional knowledge. In a sample of over 3,000 British adults, 85 per cent acknowledged that they should eat more fruits and vegetables (Food Standards Agency, 2006) and 67 per cent of this sample also spontaneously reported that they should be consuming at least five portions of fruit and vegetables per day. This knowledge, though, has not translated into behavioural change: only 30 per cent of individuals in this sample reported eating five or more portions daily. Similar patterns of discrepancy between knowledge and behaviour are commonly observed worldwide (Clark, Duncan, Trevoy, Heath, & Chan, 2011; Hussein, 2011; Michaud et al., 1998; Peltzer & Promtussananon, 2004; Pollard, Miller, Woodman, Meng, & Binns, 2009; Sharma, Gernand, & Day, 2008) suggesting that the absence of motivation ubiquitously hinders fruit and vegetable consumption. Individuals cite many barriers to fruit and vegetable consumption. For instance issues of actual and perceived cost, availability, preparation time and taste preferences are given as reasons for the low intake of this food group (Glasson, Chapman, & James, 2011). The breadth of these barriers indicates that it is important to consider a wide range of incentives to overcome these obstacles and increase the value placed on a healthy diet.

Smaller-scale intervention trials that have gone beyond information provision are relatively successful in achieving improvement in fruit and vegetable consumption. A systematic review by Pomerleau, Lock, Knai and Mckee (2005) concludes that such interventions are most effective when they specifically target the individual's motivation to consume fruit and vegetables through techniques such as motivational interviewing. This approach has the drawback of being labour intensive

(W. R. Miller & Rollnick, 1991), usually requiring multiple one-on-one sessions between each participant and a trained counsellor, which is impractical for wide-scale use. Michie, Abraham, Whittington, McAteer and Gupta (2009) recently conducted a meta-regression of interventions targeting healthy eating and increased physical activity, revealing a number of further intervention techniques to be effective in achieving dietary behaviour change. Studies that encouraged participants to explicitly set specific goals (involving detailed plans of how, when and where a specific dietary behaviour will be performed, e.g., de Nooijer, de Vet, Brug, & de Vries, 2006) were found to be effective, as were those which required participants to self-monitor (precisely record personal acts of a target dietary behaviour) and review goals (in light of feedback).

Such techniques commonly form small parts of complex, multicomponent interventions, which concurrently use as many as 14 discretely identifiable techniques (Abraham & Michie, 2008; Michie et al., 2011) arguably rendering them too complex for use in a public health context and calling into question their unique contribution. For instance, a 2003 dietary intervention trial (Burke, Giangiulio, Gillam, Beilin, & Houghton, 2003) delivered information on the link between dietary behaviour and health; encouraged participants to evaluate the costs and benefits of healthy eating; prompted the identification of barriers to improved diet; provided instructions and demonstrations on how to eat healthily; encouraged participants to self-monitor behaviour; gave feedback on performance; encouraged peer comparisons; suggested social infrastructure changes that could support healthy living; offered relapse prevention advice; provided stress and time management advice and gave general encouragement to improve self-efficacy. There is an urgent call for more economically-feasible dietary interventions to be developed and tested specifically for public health applications.

2.4. Models of Behaviour Change

An effective strategy might involve augmentation of existing methods (e.g., the information provision approach of public health campaigns) with novel methods that may incentivise consumption of this food group. Evidence-based models of behaviour change are important to consider in this context as a means of identifying strategies that are likely to be effective at motivating population-level changes in dietary behaviour. It is critical to use these theories to formulate intervention strategies which capitalise on peoples' existing cognitions as these are likely to be the most effective and economical methods of behaviour change.

In line with systematic reviews and meta-analyses of effective behaviour-change techniques (Michie et al., 2009; Pomerleau et al., 2005), Carver and Scheier's (1990) *Self-Regulation Model of Health Behavior* regards behavioural change to be critically dependent on goal-directed action plans. For behaviour modification to be successful, individuals must establish a goal, which involves identification of a perceived discrepancy between the current self and a desired state. The individual must then use existing or newly acquired knowledge to determine how best to reduce this discrepancy. This suggests that fruit and vegetable consumption may improve if participants are encouraged to create strong motivational goals or reinforce extant ones. *The Health Action Process Approach* (Schwarzer, 2008) is a similar model which suggests that an initial motivational phase (in which goals are set) should be followed by phases which actively structure the initiation and maintenance of behavioural change.

The *Information-Motivation and Behavioral Skills Model* (Fisher & Fisher, 1992; Fisher, Fisher, Williams, & Malloy, 1994) was initially developed to predict sexual behaviours associated with HIV transmission, but has potential utility for a wide range of health behaviours. This model proposes that behaviour change is contingent on a participants' informedness (i.e. knowledge of the risks associated with particular behaviours) and motivation to perform that behaviour, and that these are two independent constructs; i.e. an individual can be informed, but not motivated (as appears to be the case with diet in industrialised nations) and vice-versa. The evidence behind this model further suggests that information-provision alone is an ineffective means of motivating improvements in dietary behaviour and that it is important to develop materials that are effective in encouraging fruit and vegetable consumption whilst providing information on their benefits.

The *Transtheoretical Model of Behaviour Change* (Prochaska & DiClemente, 1984) regards behavioural interventions to be most effective when materials are tailored to the individual's readiness to alter a given behaviour. According to this popular theory, individuals progress from a precontemplation stage (in which there is no desire to change behaviour) to one of contemplation (in which behaviour begins to be evaluated), followed by the preparation and completion of specific actions. Successful long-term behaviour modification is subsequently achieved via active maintenance of changes and elimination of previous habits. This theoretical framework highlights the importance of using intervention methods that are able to target behaviour change across these critical stages. Although it is important to intervene in the early, precontemplational and contemplational stages, in the interests of economy, it is also important to demonstrate that a given intervention strategy remains effective once participants advance through theoretical stages.

The concept of self-efficacy (Bandura, 1977) considers individuals' cognitions surrounding their competence in achieving behaviour-change goals. Highlighting the importance of this construct it has been incorporated into a large number of theories of behaviour change, including those outlined above, in addition to *Social Cognitive Theory* (N. E. Miller & Dollard, 1941), *Social Learning Theory* (Bandura, 1989) and *Self-Concept Theory* (Mcadam, 1986). These theories posit that individuals with low self-efficacy are likely to avoid specific tasks they perceive as difficult (even if specific plans are laid out, Richert et al., 2010), hence it is important to investigate and develop effective methods of improving confidence in achieving motivating behavioural outcomes.

The concept of social norms has also valuably contributed to understanding of human behaviour. Humans have a strong tendency to act in accordance with the perception of others' behaviour (D. Yun & Silk, 2011), hence it may be valuable to highlight to individuals that large numbers of people can and do perform target dietary behaviours, thereby making behaviour changes appear less challenging. Social norms are key to both the *Theory of Reasoned Action* (Fishbein & Ajzen, 1975) and the subsequent *Theory of Planned Behavior* (Ajzen, 1985) which posit that successful behaviour change is contingent on one's attitudes towards that behaviour and on one's beliefs about how their peers will interpret behaviour of that action. The *Theory of Planned Behaviour* (Ajzen, 1985) additionally incorporates individuals' perception of control, a concept similar to that of self-efficacy.

The above theories and theoretical constructs highlight a number of intervention components which may be useful in establishing behavioural change. Typically, these components are delivered in a particularly effortful manner (Michie et al., 2009; Pomerleau et al., 2005), but it is now important from a public health viewpoint to investigate more parsimonious and effective means of delivery. Providing information on the health benefits of certain behaviours is likely to be ineffective when in isolation, but this approach is likely to be more successful when paired with intervention techniques which encourage the development of clear and achievable goals.

3. Appearance-Based Behavioural Interventions

This chapter is partly based on the following work accepted for publication in a peer reviewed journal (accepted August 4, 2011):

Whitehead, R., Ozakinci, G. Stephen, I. D. & Perrett, D. I. (2012). Appealing to vanity: Could potential appearance improvement motivate fruit and vegetable consumption? *American Journal of Public Health, 102*(2), 207-211.

3.1. Summary

The previous chapter found scope for improvement in contemporary dietary intervention strategies and indicated that the ability to sufficiently incentivise increased fruit and vegetable consumption is absent from typical population-level campaigns. This chapter identifies physical-appearance-based behavioural interventions as a potentially powerful paradigm in this respect. Recent attempts to use physical appearance as a motivational device will be reviewed, chiefly focusing on trials which attempt to modify behaviour and cognitions relating to sun exposure. This chapter will conclude that similar strategies targeting dietary behaviour may also be effective, as may largely untested interventions which illustrate positive, rather than negative ramifications of certain behaviours for outward appearance.

3.2. Appearance-Based Behavioural Interventions

Large cross-sectional studies reveal that men and women across age and socioeconomic groups pay close attention to their outward physical appearance (Harris & Carr, 2001). As such, appearance may be a universal motivator of individuals' behaviour. Symptom-free individuals frequently value their appearance more than their health (S. J. Chung, Hoerr, Levine, & Coleman, 2006; J. L. Jones & Leary, 1994), this is particularly evident when considering sun-exposure behaviour. Many individuals actively seek ultraviolet (UV) light exposure to acquire a tan and improve personal appearance (Jorgensen, Wayman, Green, & Gelb, 2000; Robinson, Rademaker, Sylvester, & Cook, 1997), despite widespread knowledge that this behaviour is robustly associated with skin cancer risk (Armstrong & Kricker, 2001; Martin, 1995).

A novel intervention paradigm has recently attempted to motivate safer sun-exposure behaviour by highlighting that prolonged sun-exposure in fact has *negative* consequences for long-term physical appearance (J. L. Jones & Leary, 1994; Mahler, Fitzpatrick, Parker, & Lapin, 1997). The *Self-Regulation Model of Health Behavior* (Carver & Scheier, 1990), and similar evidence-based frameworks suggest that such an approach could be effective. The high value placed on own appearance may facilitate the development of a goal state, which people may strive towards using knowledge of the link between behaviour and appearance. Indeed, this approach has experienced success in motivating cognitive and behavioural changes relating to sun-exposure.

Jones and Leary (1994) pioneered this strategy by explaining to individuals in writing how outward appearance is harmed by excessive sun exposure. They found that these messages about appearance motivated sun-protection intentions significantly more than did health-based messages (which emphasised the negative consequences of sunbathing for health). More recently, participants have been shown graphical illustrations of the negative consequences of UV light on facial appearance (Mahler, Kulik, Gibbons, Gerrard, & Harrell, 2003). This technique involves showing participants UV-filtered photographs which strikingly depict the epidermal hyperpigmentation which arises through excessive sun exposure. This approach has been successful in encouraging beneficial changes to sun-exposure intentions (Mahler, Kulik, Gerrard, & Gibbons, 2006) and is also sufficient to motivate lasting behavioural changes. Longitudinal studies indicate that safer sun-exposure behaviour is sustained for at least four months in a young adult sample (Mahler, Kulik, Gerrard, & Gibbons, 2007) and up to one year in a study of male highway workers (Stock et al., 2009). Critically, these appearance-based sun-exposure interventions illustrated the deleterious consequences of sunbathing on images of the participants' *own face*. This may further strengthen goal formation, and additionally increase the salience and perceived relevance of the intended health message.

A similar approach has also recently been used to target tobacco use. A number of studies have employed graphical simulations of the negative consequences of smoking on facial skin wrinkling and oral disfigurement, according to empirical measurement of these parameters (Hysert, Mirand, Giovino, Cummings, & Kuo, 2003). These illustrations have been successful in motivating adolescents to attend smoking cessation programs (Semer et al., 2005) and create lasting anti-smoking intentions (Grogan, Flett, Clark-Carter, Conner, et al., 2011; Grogan, Flett, Clark-Carter, Gough, et al., 2011), though no investigations have verified non-smoking status using objective detection methods (such as salivary cotinine assays).

Interventions highlighting the negative impact of sun exposure and cigarette use on outward appearance may be particularly effective in reducing the prevalence of these behaviours as there is a common conception that these behaviours improve physical appearance and social image, respectively (Grogan, Fry, Gough, & Conner, 2009; Jorgensen et al., 2000; Robinson et al., 1997). These beliefs are likely to potentiate incidences of these actions, but by highlighting the *deleterious* consequences of these behaviours for appearance, participants may be led to re-evaluate their performance of these behaviours. In this context, an alternate strategy involving illustration of links between healthy lifestyles and appearance *benefits* could be effective in motivating the adoption of desirable behaviours. Dietary behaviour is strongly motivated by appearance (S. J. Chung et al., 2006; Hayes & Ross, 1987), hence an appearance-based intervention strategy could also be beneficial for motivating individuals to consume fruit and vegetables in line with recommendations, provided that there are demonstrable associations between healthy diet and desirable aspects of appearance.

An appearance-based intervention strategy is likely to be particularly effective in motivating behaviour change in adolescents, as a group particularly concerned about their appearance (S. J. Chung et al., 2006). Further, typical incentives to lead a healthy lifestyle focus on the consequences of behaviour for long-term health and overlook short-term incentives. This may motivate some, but behavioural economics and psychosocial research reveals that humans are particularly present-focused. Empirical evidence indicates that people prefer rewards that are temporally proximal compared to those that are more distal, largely irrespective of relative value (Frederick, Loewenstein, & O'Donoghue, 2002). This suggests that existing population-level campaigns have not achieved necessary lifestyle improvements amongst young adults because health-based incentives are particularly distant rewards for these individuals. An appearance-based strategy is potentially capable of offering more immediate incentives, which may lead to the development of healthy lifestyle habits and a healthy lifestyle trajectory from an early age.

In addition to illustrations of outward appearance, the use of visual stimuli has recently been employed as an effective behaviour change tool more broadly. A Cochrane review found that illustrating the results of medical imaging (via techniques such as arterial scanning and computed tomography) to participants was an efficient way of motivating the adoption of relevant healthier behaviours in some situations (Hollands, Hankins, & Marteau, 2010). Further, the use of graphic warning labels on cigarette packets has been shown to increase the salience of dangers associated with tobacco use (McCool, Webb, Cameron, & Hoek, 2012; Ng, Roxburgh, Sanjay, & Eong, 2010).

As indicated by the recent success of this paradigm, the general use of images is a potentially powerful adjunct to behavioural intervention strategies. Image-based intervention messages are straight-forward to implement, may be particularly effective in communicating complex messages rapidly and as such, are likely to represent a valuable strategy for economically establishing healthy lifestyle habits.

4. An Appearance-Based Dietary Intervention

This chapter is partly based on the following works accepted for publication in peer reviewed journals (accepted August 4, 2011):

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(accepted July 13, 2012):

Whitehead, R. Ozakinci, G. & Perrett, D. I. (In press). Attractive skin colouration: Harnessing sexual selection to improve diet and health. *Evolutionary Psychology.*

4.1. Summary

The previous chapters highlighted the requirements for, and merits of, appearance-based behavioural interventions. This chapter demonstrates that an appearance-based dietary intervention strategy could be based on the impact that dietary carotenoid pigments have on human skin. Potential mechanisms responsible for the dermal deposition of these pigments will be considered and a body of research will be reviewed which suggests that carotenoid ornamentation is an ‘honest’ indicator of health in the animal kingdom. It will be concluded that carotenoid-based skin pigmentation is also a reliable cue of human condition which could be valuable in shaping an appearance-based dietary strategy. Individuals reliably perceive carotenoid-based skin colouration as healthy and attractive in others’ skin. This is likely to generalise to self-perception and act as an incentivising goal. Potential means of implementing the discussed strategy will be considered.

4.2. An Appearance-Based Dietary Intervention

An appearance-based dietary intervention approach may be practicable due to the impact that dietary carotenoid pigments have on skin colour, and the general influence of skin colour on human appearance and attractiveness (Fink, Grammer, & Thornhill, 2001; B. C. Jones, Little, Burt, & Perrett, 2004; Scott, Pound, Stephen, Clark, & Penton-Voak, 2010; Stephen, Coetzee, & Perrett, 2011; Stephen, Law-Smith, Stirrat, & Perrett, 2009; Stephen et al., 2012). Carotenoids are yellow-red organic pigments, which cannot be synthesized *in vivo*, but are abundant in, and impart colour to a wide range of fruit and vegetables (Health Canada, 2010). These phytochemicals are found in all layers of human skin (Lademann, Meinke, Sterry, & Darvin, 2011; R. Lee, Mathewsroth, Pathak, & Parrish, 1975) and contribute to normal skin colour (Alaluf, Heinrich, Stahl, Tronnier, & Wiseman, 2002).

4.3. Carotenoids and Human Skin Colour

The mechanism by which carotenoid pigments impart colouration to human skin is largely unexamined. There are, however, two viable pathways which are not necessarily mutually exclusive. Carotenoids have been detected in abundance in sweat (Darvin et al., 2009) and sebum (Prince & Frisoli, 1993), therefore one possibility involves the excretion of carotenoids onto the skin's surface via eccrine and sebaceous activity. These pigments would then reabsorbed by the outermost skin layer, the *stratum corneum*, imparting colouration. Carotenoids are fat-soluble and hydrophobic, thus to exist and be transported *in vivo*, they must interact with lipids or lipoproteins (Britton & Helliwell, 2008). The *stratum corneum* is lipophilic, facilitating absorption of these lipids, along with associated carotenoid pigments. Supporting this view, carotenoid colouration is particularly prevalent in body areas with a thicker *stratum corneum*, for example at volar (*palmar* and *plantar*) surfaces (Edwards & Duntley, 1939). This may also be due to increased eccrine activity at these surfaces; carotenodermia, a harmless yellow colouration of the skin caused by excessive carotenoid consumption, is commonly comorbid with hyperhidrosis (Jeghers, 1943). Individuals with this condition exhibit abnormally high levels of perspiration, providing evidence for the hypothesis that carotenoids are deposited via sweat.

Carotenoids are also detected in deeper skin layers (e.g., Alaluf, Heinrich, et al., 2002), suggesting that this mechanism is not the only means by which skin yellowness is affected. Lipid-dissolved carotenoids may also be deposited via a rich capillary network into dermal and subcutaneous skin, staining viable tissues in these layers. This mechanism may be facilitated by lipid transporters, which are likely to be responsible for the accumulation of specific carotenoids in particular body tissues which are particularly at risk of oxidative stress. For instance lutein and zeaxanthin accumulate preferentially in the retina (Bone et al., 1997; Snodderly, 1995) which is subject to oxidative stress via UV exposure (Young, 1988). β-carotene also builds up in the prostate gland (Clinton et al., 1996) which may be subject to increased oxidative stress due to high metabolic rates (Costello & Franklin, 2000; Franklin & Costello, 2008). Carotenoid transporters are found at the gut-blood barrier, and are held to be responsible for the transportation of lipid-dissolved carotenoids from the small intestine to the blood stream (Richelle, Sabatier, Steiling, & Williamson, 2006). These transporters remain to be discovered in the skin, however passive transportation via a concentration gradient is a further plausible mechanism by which carotenoids may leave the blood stream and pigment dermal and subcutaneous skin.

Individual differences in fruit and vegetable consumption are associated with variation in skin carotenoid concentrations (Mayne et al., 2010) and skin yellowness (Stephen et al., 2011). Carotenoid supplementation also has been shown to impact skin yellowness (Stephen et al., 2011). Further, within-person dietary changes have been qualitatively linked with changes in skin carotenoid levels within a matter of days (Darvin et al., 2008), although whether skin *colour* changes are this rapid or perceptible remains to be determined (see Chapter 7).

Skin yellowness and carotenoid pigmentation contribute beneficially to the apparent health and attractiveness of human faces. When asked to manipulate facial skin colour and optimise the appearance of healthiness and attractiveness in Caucasian faces, observers increase skin yellowness, mimicking the appearance of increased carotenoid pigmentation (Stephen et al., 2011; Stephen, Law-Smith, et al., 2009). The degree of natural facial skin yellowness is also strongly associated with rated attractiveness (Scott et al., 2010). Whilst these perceptual effects have been shown to hold when images of Caucasian and African (Stephen et al., 2011) individuals are assessed, comprehensive cross-cultural investigations remain to be performed and colour changes associated with diet have yet to be assessed cross-culturally. It may be the case that skin colour related appearance-gains are less visible or motivational in individuals with heavily pigmented skin. It is also possible that own ethnicity affects the perception of carotenoid-based pigmentation on faces of other ethnicities, because of familiarity effects.

Whilst factors besides skin colour contribute to the appearance of health and attractiveness in humans, for instance skin texture (Fink et al., 2001) and face shape/structure (Coetzee, Chen, Perrett, & Stephen, 2010; Perrett et al., 1998), recent studies suggest that skin colour has greater influence on facial attractiveness than some morphological cues of quality (e.g., masculinity, Scott et al., 2010 for Caucasian male faces; Coetzee in prep for African female faces). It is widely held to be the case that carotenoid-based colouration is a sexually-selected cue of condition in the animal kingdom amongst species with appropriate colour vision (e.g., Lozano, 1994). It is possible that the mechanisms propagating carotenoid pigmentation as a cue of health in animals will also apply to humans. Review of this animal literature will identify the pathways which are likely to render carotenoid pigmentation of human skin as healthy and attractive.

4.4. Carotenoid Ornaments as Sexually Selected Cues to Condition

Many vertebrate species exhibit carotenoid-based yellow-red colouration of their skin, beaks, feathers, scales or ornaments (Fox, 1976; Goodwin, 1984). A wealth of observational and experimental data indicates that the extent and intensity of carotenoid pigmentation reliably reflects the bearer's condition, particularly in bird and fish species. For instance, the carotenoid-based yellow breast plumage of great tits (*Parus major*) is duller in parasited birds and brighter in those free of infection (Horak, Ots, Vellau, Spottiswoode, & Moller, 2001). Experimentally, inducing a parasite load in blackbirds (*Turdus merula*) leads to decreases in carotenoid-based bill colouration (Baeta, Faivre, Motreuil, Gaillard, & Moreau, 2008) and similar manipulations reduce the intensity of orange carotenoid spots in male guppies (*Poecilia reticulata*) (Houde & Torio, 1992) and red colouration in male sticklebacks (*Gasterosteus aculeatus*) (Milinski & Bakker, 1990). Removal of parasites via antihelmintic treatment increases the redness and size of carotenoid-based combs in red grouse (*Lagopus lagopus*) (Mougeot et al., 2010). Further, male greenfinches (*Carduelis chloris*) with larger carotenoid-based plumage patches are less susceptible to, and exhibit faster clearance of, viral infection (Lindstrom & Lundstrom, 2000).

It follows that preferences for this overt indicator of health may have evolved via sexual selection, as preferentially mating with an extravagantly coloured partner is likely to confer direct and indirect fitness benefits to the observer (Hamilton & Zuk, 1982; G. E. Hill, 1991). A further body of evidence indeed suggests that perceived mate value is contingent on natural and experimentally induced variation in carotenoid colouration. For example, female guppies preferentially mate with males exhibiting brighter carotenoid-based colouration (Kodric-Brown, 1985). Male house finches (*Carpodacus mexicanas*) with naturally brighter carotenoid plumage were more frequently selected as sexual partners (G. E. Hill, 1990) and when the carotenoid colouration of plumage in this species was artificially brightened, males were more likely to find a mate than controls (G. E. Hill, 1991).

Carotenoids are expended in their role as antioxidants (Sies, 1993), and cannot be re-synthesized *in vivo* by animals (McGraw, 2006). Consequently, the systemic level and hence the availability of carotenoids for deposition is widely held to be contingent on a trade-off between their expenditure as antioxidants and display (Lozano, 1994). Carotenoid colouration can thereby provide an ‘honest’ cue to the bearer’s condition. Supporting this hypothesis, the experimental administration of a redox-active herbicide (which raises oxidative stress) is associated with reduced intensity of carotenoid-based plumage in partridges (*Alectoris rufa*) (Alonso-Alvarez & Galvan, 2011). Also, male sticklebacks with a greater buffer of non-carotenoid antioxidants exhibit more intense carotenoid-based redness (Pike, Blount, Lindstrom, & Metcalfe, 2007).

In line with this hypothesis, carotenoid-based colouration may be a strong indicator of prevailing infection levels due to the nature of primary phagocytic mechanisms. “Respiratory burst” is a process in which pathogens are neutralized by high levels of reactive oxygen species (Babior, Kipnes, & Curnutte, 1973). The non-targeted nature of this defence mechanism necessitates increased expenditure of carotenoids and other antioxidants during periods of infection to mitigate oxidative damage to the host’s tissues. Individuals that regularly experience infections and hence oxidative stress will consequently exhibit reduced levels of circulating carotenoids, which is likely to detract from carotenoid ornamentation (Lozano, 1994).

A further hypothesis proposes that carotenoid colouration may reflect actual condition because of the toxicity of carotenoid breakdown products (Vinkler & Albrecht, 2010). The carotenoid maintenance handicap hypothesis postulates that the toxic by-products of carotenoid oxidation are more likely to be formed when systemic antioxidant reserves are low (Vinkler & Albrecht, 2010). This theory also proposes that the relationship between oxidative stress and carotenoid pigmentation is linked to testosterone, which increases carotenoid bioavailability (Blas, Perez-Rodriguez, Bortolotti, Vinuela, & Marchant, 2006), but simultaneously increases oxidative stress (Wikelski, Lynn, Breuner, Wingfield, & Kenagy, 1999). The honesty of carotenoid ornamentation is preserved as only the individuals with competent antioxidant systems may exhibit intense carotenoid colouration; the oxidation challenge posed by testosterone cannot be endured by individuals with inadequate antioxidant resources (Vinkler & Albrecht, 2010).

A number of endogenous and exogenous factors could, in principle, affect the quantity and quality of antioxidant reserves which, through either of the mechanisms proposed above, may contribute to carotenoid colouration being a reliable cue of condition (e.g., heritable enzymic antioxidant capacity, Cheng, Aggrey, Nichols, Garnett, & Godin, 1997). Dietary quality (as a function of foraging competence or an ability to maintain a food-bearing territory) is likely to be a key determinant of apparent condition in this respect, as carotenoids and a number of additional, colourless, antioxidant phytochemicals are largely or exclusively obtained through consumption of food items containing these important molecules (Rietjens et al., 2002; Smith, Ungnade, & Prichard, 1938). This suggestion is supported by observations that carotenoid colouration is contingent on the abundance of carotenoid-rich food items in the individual's habitat (Horak et al., 2001; Slagsvold & Lifjeld, 1985) and studies which link experimentally-manipulated consumption of carotenoids and non-pigmented antioxidants with plumage, integument and beak colouration (e.g., Bertrand, Faivre, & Sorci, 2006; G. E. Hill, 1992, 1993; Jouventin, McGraw, Morel, & Celerier, 2007; Kodric-Brown, 1989; Pike et al., 2007).

4.5. Implementation of an Appearance-Based Dietary Intervention

The literature reviewed above suggests that fruit and vegetable consumption may be perceived as healthy in human skin due to sexual selection of this cue as an 'honest' indicator of condition. Humans may have evolved to be sensitive to this cue and/or learned by association that bearers of skin rich in carotenoid pigments are likely to be healthy, hence increasing their value as a mate (Thornhill & Gangestad, 1999).

It is likely that the positive perception of carotenoid pigmentation in others' skin generalises across cultures (explored in Chapter 5) and to individuals' perception of their own appearance, though this presupposition requires empirical investigation (Chapters 6 and 9). In contrast to the majority of existing appearance-based interventions (see Chapter 3), which focus on the *damage* associated with non-compliance, an approach targeting fruit and vegetable intake could capitalise on the appearance *gains* associated with increased consumption of this food group (Stephen et al., 2011). Individuals can be shown how their appearance may benefit from dietary change, providing a salient goal that they can strive towards. This represents a more palpable, gratifying, and potentially quicker-to-realize benefit than current attempts to improve diet.

Illustrating the benefits of healthy eating for appearance may be a particularly valuable approach as it may facilitate the simultaneous delivery of a number of proven behaviour change techniques (Abraham & Michie, 2008; Michie et al., 2011). The strategy proposed here is capable of delivering many of the effective intervention techniques identified in a recently published taxonomy of behaviour change techniques (Michie et al., 2011). Namely, a motivational incentive will be provided for participants to set appearance-related *goals*; participants will receive *behaviour-contingent rewards*, as appearance gains are dependent on the level of dietary improvement. Further, lapses in fruit and vegetable consumption are likely to lead to appearance deterioration, thus witnessing the negative impact of worsened diet may motivate maintenance of behavioural modification. Information will be given about others' ubiquitous *approval of behavioural outcomes*, as it can be highlighted that skin pigmentation conferred by fruit and vegetable consumption is reliably perceived as healthy and attractive. *Self-monitoring* of behavioural outcomes can be encouraged as participants may constantly 'evaluate' their skin tone, though whether participants will themselves be able to perceive appearance improvements over the timescale involved requires investigation. Given an appropriate intervention design, this approach could also provide *feedback on performance*, based on objective measurements of skin colour changes.

In practice, implementation of such a strategy requires empirical investigation of diet-linked appearance changes. Published cross-sectional studies that investigate the impact of diet on appearance (Stephen et al., 2011) can be used to quantify the typical skin colour change associated with a portion difference in fruit and vegetable consumption. Though it will be valuable to utilise data from studies examining the effect of diet change on skin colour change *within-subject*, such investigations do not yet exist (explored in Chapter 7).

Given empirically-derived colour-change values, facial images can be manipulated with appropriate computer software (Burt & Perrett, 1995) in order to illustrate the impact of an improved or worsened diet in a portion-wise fashion. Such illustrations could involve comparison between an individual's current appearance and that which is potentially achievable through a specified increase in fruit and vegetable consumption. For instance, those individuals that consume fewer than five portions a day could be shown images which demonstrate the appearance-gains associated with consuming fruit and vegetables in line with this WHO recommendation (WHO, 1990). Alternatively, using an interactive computer program, individuals could manipulate their own facial image along a fruit and vegetable consumption colour axis. Such a set-up could, at a range of colour-change intervals, indicate the change in diet necessary to achieve the illustrated skin colouration. This would allow participants to explore their facial appearance in various guises and help shape the diet change required to achieve the facial appearance that they themselves consider to be the healthiest or most attractive in this range.

Diet is recognized as a key factor in non-communicable disease prevalence and has proved challenging to ameliorate with current methods. Visualisation of potential appearance benefits associated with healthy diet could offer a valuable and novel incentive, which added to other methods may ultimately serve to reduce the prevalence of disease associated with inadequate fruit and vegetable consumption. Randomised controlled trials are required to investigate whether the strategy outlined here is sufficient to motivate increased fruit and vegetable consumption (see Chapters 6 and 9). It will also be important to investigate the efficacy of such a technique relative to existing dietary intervention approaches.

5. Cross-Cultural Perception of Carotenoid Based Skin Colouration

5.1. Summary

Increased skin yellowness, as a proxy of carotenoid deposition has previously been shown to contribute beneficially to the appearance of health in Caucasian faces, when evaluated by Caucasian individuals (Stephen, Law-Smith, et al., 2009). This chapter investigates preferences for skin yellowness cross-culturally in order to replicate and extend previous findings. The research reported here finds that, in addition to Caucasian faces, African and Asian faces also benefit from increased skin yellowness. This was the case across Caucasian and Asian observer ethnicities, suggesting that there is a universal preference for carotenoid colouration that humans may have learned or evolved to be sensitive to, in order to selectively mate with healthy individuals.

5.2. Introduction

When Caucasian observers are given the opportunity to manipulate the colour of Caucasian facial skin, yellowness (a proxy of carotenoid pigmentation) is reliably increased to optimise the appearance of health (Stephen, Law-Smith, et al., 2009). Chapter 4 indicates that the degree of carotenoid ornamentation reliably reflects the condition of animal species, it is also likely that this trait is utilised by humans. To support this hypothesis, the preference for carotenoid colouration is expected to be apparent across cultures.

Potentially precluding such generalisability is the increased levels of melanisation in non-Caucasian skin. Melanin darkens and yellows skin (Stamatas, Zmudzka, Kollias, & Beer, 2004), potentially masking the appearance of carotenoid colouration in non-Caucasian skin. There are substantial cross-cultural differences in the degree of constitutive melanisation (Alaluf, Atkins, et al., 2002), due to differences in melanocyte activity (Iozumi, Hoganson, Pennella, Everett, & Fuller, 1993) rather than variation in the density of melanin-producing melanocytes (Szabo, 1954). Africans typically exhibit substantially darker skin colouration than Caucasians, due to greater production of the dark melanin pigment; 5, 6-dihydroxyindole (DHI) enriched eumelanin (Alaluf, Atkins, et al., 2002). Asian skin is lighter than African, but darker and yellower than Caucasian skin, due to an intermediate amount of this type of melanin. It is necessary to investigate preferences for yellow colouration within faces across these skin types, in order to determine the generalisability of preferences initially reported in Caucasian skin (Stephen, Law-Smith, et al., 2009). It is also critical to investigate cross-cultural preferences for carotenoid pigmentation to determine the extent to which an appearance-based dietary intervention could be used to effectively motivate dietary improvement in non-Caucasian individuals.

Here perceptual experiments are conducted in which Asian and Caucasian observers' preferences for skin yellowness are assessed with African, Asian and Caucasian facial stimuli. It is expected, given likely universal mechanisms preserving carotenoid pigmentation as a reliable cue of health (Chapter 4), that there will be cross-cultural preferences for increased skin yellowness when observers across ethnicities are tasked with optimising apparent health.

5.3. Methods

Ethical approval for the reported procedures was sought from the University of St Andrews Teaching and Research Ethics Committee, and prior informed written consent was obtained from all participants.

Photographs were taken of 51 Caucasian (21 male, 30 female), 48 African (23 male, 25 female), 7 West-Asian (2 male, 5 female) and 39 East-Asian (10 male, 29 female) participants, all aged 18-35.

Participants were asked to remove spectacles and visible jewellery, and maintained a neutral expression. Photographs were taken using a Fujifilm FinePix S5Pro digital SLR camera with a fixed-length 60mm lens in a booth painted on all surfaces with Munsell N5 grey. Illumination was exclusively provided by three Verivide 6504K daylight simulation bulbs. Participants held a Munsell N5 painted board over their shoulders to obscure reflections from clothing. A GretagMacbeth Mini ColorChecker chart was included in each image in order to colour-calibrate images. Images were colour-corrected by transforming observed RGB values of each of the 24 colour-checker patches towards manufacturer specified CIE L*a*b*

(Commission Internationale de l'Eclairage, 1976) values of these same patches using a least-squares transform of an 11-expression polynomial expansion (Hong, Luo, & Rhodes, 2001). This resulted in a mean colour error (ΔE) of 2.4, where ΔE is a standard means of presenting colour-error in the CIE L*a*b* colour space and here represents the Euclidean distance between calibrated image and reference colour patches. The CIE L*a*b* tristimulus colour space approximates human trichromatic vision. Colour is determined by values on three axes: L* represents lightness (0-100); a* represents position on a green-red axis (-60 to +60) where positive values are red; and b* represents position on a blue-yellow axis (-60 to +60) where positive values are yellow (see also Figure 8.3). Skin b* values have been positively associated with carotenoid concentrations (Alaluf, Heinrich, et al., 2002), therefore here we examine the effect of b* colouration alone on the perception of healthiness cross culturally.

Two face-shaped endpoint colour masks were created in Matlab (Figure 5.1), one of which represents a decrease in yellowness (average face colour -8 b* units) and another representing an increase in yellowness (average face colour +8 b* units). Masks were Gaussian blurred at the edges of the face, in order to prevent final images having an obvious colour border. The skin portions (including lips and ears, excluding eyes, hair and background) of all collected images were manipulated by the colour difference between these two endpoints (Burt & Perrett, 1995) in order to obtain a set of 13 images per individual, ranging from -16 to +16 units of b* relative to the individual's initial face colour (Figure 5.2), this colour range was chosen to exceed the normal range. The initial colour of each face was calculated by obtaining CIE L*a*b* values for all facial skin pixels (again including lips and ears, excluding eyes, hair and background) in calibrated digital photographs.

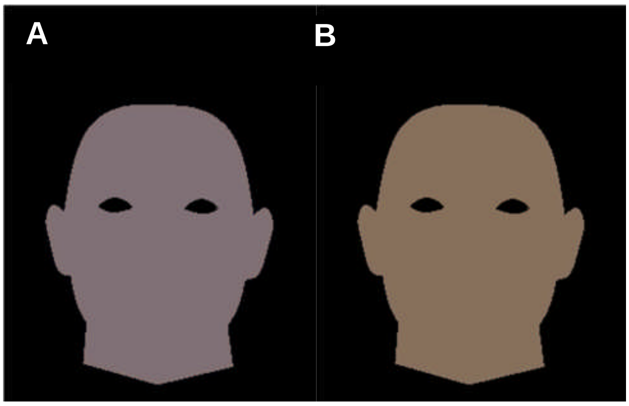

Figure 5.1. Endpoint masks representing (A) average face colour -8 b* units and (B) average face colour +8 b* units.

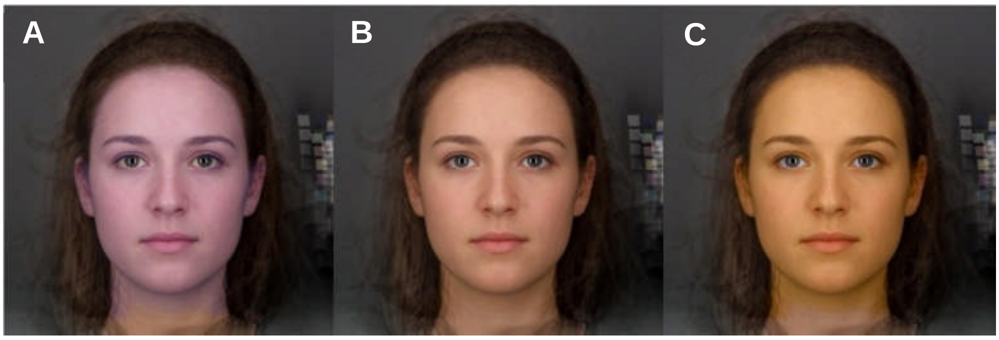

Figure 5.2. Skin yellowness transforms, (B) Original image (A) -16 b* units and (C) +16 b* units. A set of 13 images was produced per face identity within this colour range of 32 b* units, resulting in a change between adjacent images of 2.46 b* units.

Observers used a computer program to manipulate the colour of face images, one at a time, along this colour range and were instructed to optimise the appearance of health for each image. A randomly selected image within the colour range was displayed and horizontal mouse cursor movements made by observers allowed them to manipulate the displayed image sequentially along the colour range. Each of the 13 images within the colour range was pre-loaded in order to have no delay between the presentation of successive images. The direction of colour change was randomised across trials, in order to eliminate any effect of systematic left or rightward movement through the image sequence. The cursor position required to display the midpoint image was offset randomly across trials, thus the original face colour was not consistently displayed when the cursor was in the centre of the screen. The colour scale was also wrapped, such that when mouse movements took the cursor to the edge of the scale, it reappeared at the opposite edge of the scale, this was configured in order to prevent observers easily finding either maxima and together these measures meant that participants had to actively seek out their perceived optimum skin colour on each trial. Observers were instructed to click the mouse button when they were satisfied with face colour, the computer program would then record the selected image and advance to the next face, chosen randomly.

Stimuli were presented on a Sony GDM-F500R cathode-ray tube monitor colour-calibrated to a Δu'v' of less than 0.5 using Spyder S2 Pro software and hardware (Δu'v' represents the Euclidean difference in the 1976 CIE u'v' colour space; Wyszecki & Stiles, 1982). Trials were conducted within a darkened booth, in order to prevent light falling on the monitor.

Using this paradigm, 89 Caucasian, and 52 Asian (10 West-Asian, 42 East-Asian) observers with normal trichromatic vision chose optimum yellowness for 51 Caucasian and 48 African faces. Additionally 18 Caucasian and 9 Asian (2 West-Asian, 7 East-Asian) participants chose optimum yellowness for a second set of faces (7 West-Asian, 39 East-Asian).

5.4. Results

Mean yellowness change per face was calculated, in addition to mean yellowness change per observer. One-sample *t*-tests were conducted to investigate whether faces, across all observers had yellowness added relative to the starting colour of the face. One-way ANOVAs were carried out to investigate main effects of face and observer ethnicity.

Repeated measures ANOVAs (within-subjects factor = face ethnicity; between-subjects factor = observer ethnicity) were used to investigate main effects of face ethnicity and examine interactions between face and observer ethnicity. One way ANOVAs were carried-out to investigate differences in mean yellowness added within observer ethnicity across face ethnicity, and separately within face ethnicity, across participant ethnicities. Pearson correlations were used to investigate the relationship between starting face yellowness and mean yellowness added across all participants. Mean starting colour was calculated per ethnicity for each of the CIE L*a*b* axes, and independent-samples *t*-tests were conducted to investigate differences in these dimensions between ethnicities.

Mean yellowness change for all face and observer ethnicities is summarised in Tables 5.1 and 5.2. Across all observer ethnicities, faces of all ethnicities were significantly increased in yellowness to optimise healthiness (all $p < .001$).

A one-way ANOVA revealed a significant main effect of face ethnicity ($F_{3,334} = 47.221$, $p < .001$), and this difference remained after removing the small group of West-Asian Faces ($F_{2,304} = 65.562$, $p < .001$). The greatest yellowness change was applied to West-Asian, East-Asian and Caucasian faces, between which post-hoc *t*-tests were non-significant (all $p > .2$). African faces received the smallest yellowness change, and post-hoc *t*-tests reveal significant differences between these and all other face ethnicities (all $p < .001$). The smaller yellowness change in African faces therefore appears to be driving this main effect. A one-way ANOVA shows no significant main effect of participant ethnicity ($F_{2,164} = 1.244$, $p = .291$), and removing the small group of West-Asian participants, an independent samples *t*-test revealed no significant difference between the remaining Caucasian and East-Asian participants ($t_{303} = 1.012$, $p = .312$).

A repeated-measures ANOVA revealed a significant interaction between face and observer ethnicity for the first set of faces rated ($F_{2,134} = 5.027$, $p = .008$), and this interaction remained when West-Asian observers were removed ($F_{1,125} = 10.043$, $p = .002$), thus this effect is driven by differences between Caucasian and East-Asian observers across face ethnicities. For Caucasian faces, Caucasian observers add significantly more yellowness than East-Asian observers ($t_{68.4} = 2.795$, $p = .011$), but this difference does not exist when African faces are rated ($t_{61} = -0.0428$, $p = .670$).

No interaction was observed between face and observer ethnicity for the second set of faces rated ($F_{2,24} = 0.069$, $p = .933$) and this remains the case when West-Asian observers are removed from the analysis ($F_{1,23} = 0.144$, $p = .708$). West-Asian faces are hereafter omitted from analysis due to small sample size.

Table 5.3 summarises initial starting colour of faces of in CIE L*a*b* space. Independent samples *t*-tests reveal significant differences in the starting lightness (all $p < .01$) and yellowness (all $p < .01$) between all face ethnicities, such that Africans exhibit significantly darker and yellower facial skin than all other ethnicities, and Caucasians are lighter and less yellow than all other ethnicities.

Figure 5.3 shows that the yellowness added to a face across all observers positively correlates with the face's starting yellowness, within Caucasian, African and East-Asian faces. Table 5.3 also summarises the Pearson's correlation coefficients between yellowness change and initial face yellowness.

Table 5.1. Colour change applied to Caucasian and African faces, by observers of Caucasian, West-Asian and East-Asian ethnicities.

	Face ethnicity	**Mean yellowness change (Δb*± *SE*)**	**One-sample *t*-test**	**Caucasian observers (*n* = 89)**	**West-Asian observers (*n* = 10)**	**East-Asian observers (*n* = 42)**
Set 1	Caucasian (*n* = 51)	6.94 ± 0.28	t_{139} = 24.45, *p* < .001	7.31 ± 0.31	8.63 ± 1.42	5.66 ± 0.55
	African (*n* = 48)	2.39 ± 0.33	t_{138} = 7.20, *p* < .001	2.07 ± 0.36	4.23 ± 1.70	2.41 ± 0.71

Table 5.2. Colour change applied to West and East-Asian faces, by a second set of observers of Caucasian, West-Asian and East-Asian ethnicities.

	Face ethnicity	**Mean yellowness change (Δb*± *SE*)**	**One-sample *t*-test**	**Caucasian observers (*n* = 18)**	**West-Asian observers (*n* = 2)**	**East-Asian observers (*n* = 7)**
Set 2	West-Asian (*n* = 7)	7.76 ± 0.54	t_{27} = 14.37, *p* < .001	6.96 ± 0.94	5.14 ± 1.33	7.84 ± 0.71
	East-Asian (*n* = 39)	7.12 ± 0.64	t_{27} = 11.10, *p* < .001	7.58 ± 0.70	5.61 ± 3.42	8.11 ± 0.54

Table 5.3. Initial starting colour of facial skin in CIE L*a*b* space, split by face ethnicity.

Face ethnicity	Initial starting colour ± *SE*			Pearson's correlation between starting b* and Δb*
	L*	**a***	**b***	
Caucasian (n = 51)	66.9 ± 0.42	14.4 ± 0.23	16.2 ± 0.23	r = -0.83, p <.001
African (n = 48)	41.8 ± 0.66	15.9 ± 0.36	22.3 ± 0.69	r = -0.96, p < .001
West-Asian (n = 7)	46.6 ± 1.45	12.9 ± 0.41	18.9 ± 0.59	r = -0.05, p = .909
East-Asian (n = 39)	56.9 ± 0.52	13.9 ± 0.18	20.4 ± 0.22	r = -0.69, p < .001

Note. L* represents lightness (0-100), a* and b* represent degrees of redness and yellowness, respectively (0-60).

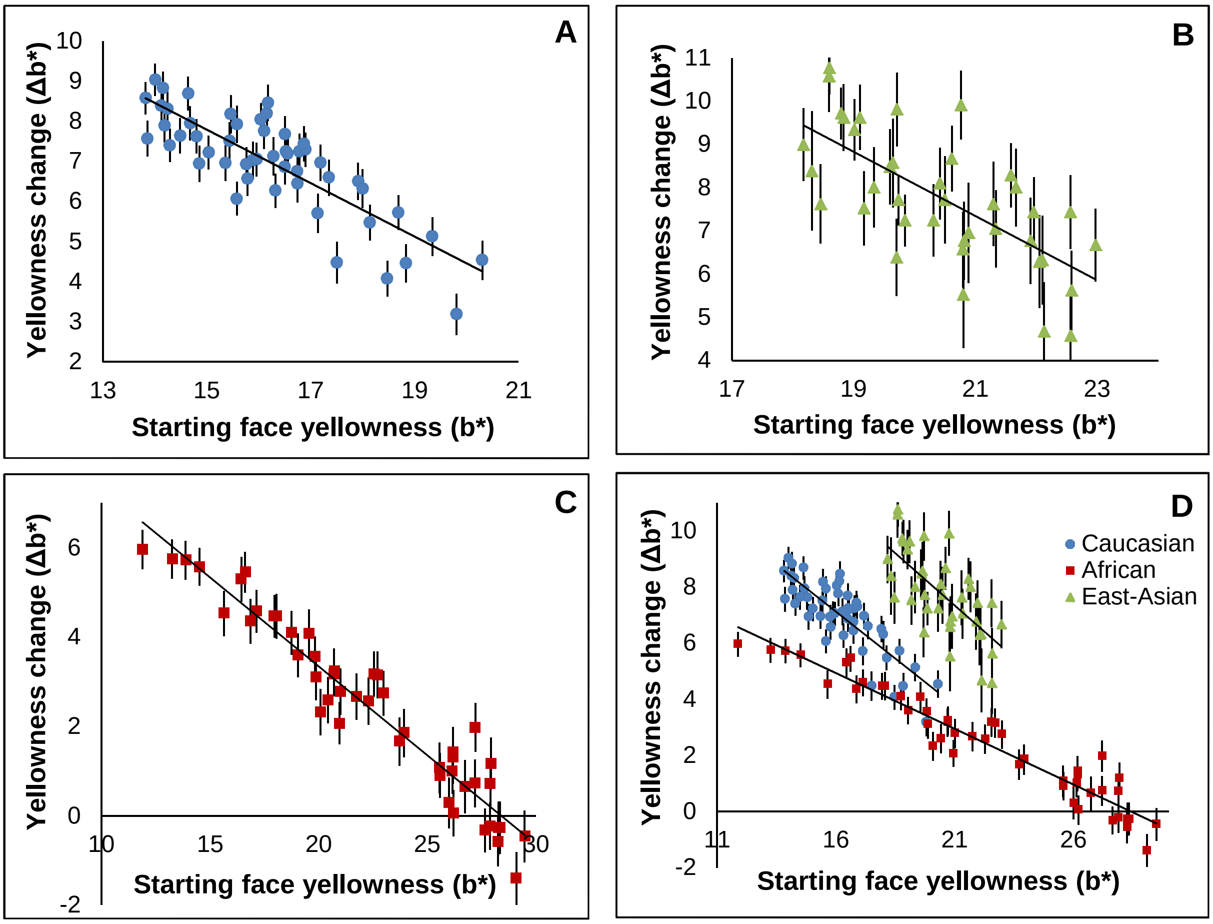

Figure 5.3. Effect of initial face yellowness on mean yellowness change (± *SE)* across all participants. Initial yellowness negatively correlates with yellowness added in (A) Caucasian (R^2 = 0.69); (B) East-Asian (R^2 = 0.47); and (C) African faces (R^2 = 0.93). (D) The relationship between faces of different ethnicities.

5.5. Discussion

This study replicates the previous finding that Caucasian observers increase the yellowness of Caucasian faces when optimising the appearance of health (Stephen, Law-Smith, et al., 2009). African and Asian faces were also found to benefit from increased yellow colouration, and Asian observers were found to perceive yellow colouration as healthy in both Caucasian and Asian faces. The finding of yellowness preference across face ethnicities is interesting, due to the varying degrees of melanin present in non-Caucasian skin. East-Asian skin is significantly darker and yellower than Caucasian skin, due to a slightly higher amount of DHI-enriched eumelanin (Alaluf, Atkins, et al., 2002). Despite this, East-Asian faces are not found here to receive significantly less yellowness than Caucasian Skin. This suggests that in East-Asian skin, the increased level of melanin is not sufficient to mask the beneficial appearance of carotenoid-based colouration.

African faces, however, received significantly less yellowness change than Caucasian and Asian faces across all observers. This is perhaps due to the extreme abundance of DHI-enriched eumelanin (Alaluf, Atkins, et al., 2002) in these faces. As shown above, faces heavily pigmented with this type of melanin begin significantly yellower, therefore it may be the case that less yellowness is required in these faces in order to reach an optimum level of yellowness. Yellowness above a hypothetical optimum may present a cue of illness rather than health, as bilirubin, an intensely yellow product of red blood cell breakdown fails to be excreted during various pathophysiological conditions (Pashankar & Schreiber, 2001), and is consequently visibly deposited in the skin. Excessive skin yellowness therefore may be a sign of conditions such as hepatitis, sepsis, alcohol-induced liver cirrhosis and acute liver failure (M. W. Whitehead, Hainsworth, & Kingham, 2001). It is assumed that observers in the current study manipulated face yellowness in order to sufficiently convey the positive appearance benefits of carotenoid colouration, whilst remaining below a level associated with jaundice. Supporting this, observers were given the opportunity to increase the yellowness of faces by up to 16 b* units, however across all races, substantially less than this was chosen as the healthiest colour. Furthermore, within each face ethnicity the yellowness added is strongly contingent upon the face's starting yellowness. Faces that are originally paler benefit from more yellowness being added, than do initially yellower faces. Indeed the yellowest original faces featured in this experiment, a group of seven African faces all originally above 26 units of b*, had yellowness decreased across all observers.

The preference for increased skin yellowness exists across Caucasian and Asian observer ethnicities, and this is consistent with the hypothesis that carotenoid colouration is a universal cue of health that humans have evolved to be sensitive to. Fitness-linked theories of attractiveness (e.g., Gangestad & Buss, 1993) are rooted in evolutionary theory, and posit that the construct of attractiveness is inextricably linked to health. According to these theories, a trait is attractive to the opposite sex if it is able to convey the healthiness and thus reproductive value of its bearer. Such theories are fuelled by findings of cross-cultural similarities in judgements of attractiveness (e.g., Langlois et al., 2000; Thakerar & Iwawaki, 1979). Mate-healthiness is beneficial directly and indirectly, regardless of culture, thus a trait is likely to be universally regarded as attractive if it honestly signals the health of the bearer. Selective pressures also critically operate regardless of culture, thus propagating the genes of individuals who preferentially select healthier mates on the basis of such traits. One of the key results of the current experiment agrees with such theories. Skin yellowness, a proxy for carotenoid colouration as a trait which potentially indicates healthiness, is found here to be cross-culturally regarded as healthy. It is true that Caucasian participants add more yellow colouration than Asian participants do to Caucasian faces, in order to optimise health. This may be explained by Caucasian individuals having an accentuated preference for melanisation (A. G. Miller, Ashton, Mchoskey, & Gimbel, 1990), due to the social association between sun-tanning and wealth (see Chapter 8 for an investigation into the relative impacts of carotenoid and melanin pigmentation on perceived health).

These results are encouraging for the possibility of an appearance-based dietary intervention, as they suggest that individuals, regardless of their ethnicity, will desire the appearance changes conferred by carotenoids, potentially motivating increased consumption of fruit and vegetables. This study, however, makes it clear that a blanket yellowness transformation should not be applied to faces of all ethnicities. The levels of yellowness increase preferred in some faces may lead to *excessive* yellowness in more melanised faces, hence decreasing, rather than increasing apparent health. An intervention attempting to motivate fruit and vegetable intake should bear this in mind and moderate the colour-changes used to illustrate the impact of a healthy diet accordingly.

Despite these promising findings, this study has a number of limitations that need to be addressed before the conclusions drawn can be concretely accepted. First, it is important to replicate these findings across further face and observer ethnicities. This study does not present the preferences of African individuals. It is potentially the case that this group will exhibit stronger preferences for yellowness than seen for participants of other ethnicities, due to a familiarity with faces that are initially substantially yellower. It is also important to establish preferences for yellowness in own-race faces, in order to better determine whether African individuals may desire this trait in their own face. This result is critical for the efficacy of a carotenoid-centred appearance intervention amongst these individuals. Furthermore, carotenoid colouration preferences may be exaggerated in cultures where it is more important to secure a healthy mate. Yellowness preferences may, therefore, vary according to the level of infection and parasitism present in a participant's environment (DeBruine, Little, & Jones, 2012). Also in order to corroborate the hypothesis that this cue is involved in mate-choice, skin yellowness preferences should be investigated when individuals are asked to optimise the attractiveness, rather than the healthiness of facial stimuli.

This study allowed participants to manipulate facial skin colour along the CIE b* axis alone, this is an adequate substitute for carotenoid colouration as positive b* values have been previously shown to be strongly correlated with skin carotenoid concentration (Alaluf, Heinrich, et al., 2002). It is, however, important to establish whether the precise colour changes associated with both carotenoid, and fruit and vegetable consumption are also preferred cross-culturally. Although this field would strongly predict that this is the case, there may be additional ramifications of diet for skin colour. Carotenoids are not exclusively yellow pigments (e.g., lycopene and torulene are red). Fruit and vegetable consumption may also be associated with health benefits such as increased cardiovascular fitness (Cesarone et al., 2008; Ghosh & Scheepens, 2009). Thus, in addition to the yellowness that consumption of carotenoid-rich fruit and vegetables confers, skin redness may also be altered.

Increased redness is known to improve the apparent health of Caucasian facial skin (Stephen, Law-Smith, et al., 2009), but cross-cultural preferences for such colouration should be examined with further perceptual studies.

The results of this study suggest that the preference for increased yellowness exists cross-culturally, lending credence to the hypothesis that carotenoid skin colouration is a universal cue of healthiness used in human mate choice. Such a universal preference for carotenoid colouration may be used advantageously in an attempt to motivate diet change worldwide, however a further experiments are required to fill pertinent knowledge gaps.

6. Does Seeing Potential Appearance-Gains Motivate Fruit and Vegetable Consumption

This chapter is partially based on the following paper accepted for publication in the conference proceedings of the 32nd Annual meeting of the Society for Behavioral Medicine (accepted December 1, 2010):

Whitehead, R., Perrett, D. I. & Ozakinci, G. (2012). Appealing to vanity: does seeing the potential appearance-benefits of fruit and vegetable consumption motivate dietary change? *Annals of Behavioral Medicine*, *41*(S1), S214.

6.1. Summary

The previous chapters suggest that an appearance-based dietary intervention approach could motivate improvements in fruit and vegetable consumption, and that such an intervention could be based on the impact that this food group has on skin colour. Here a quasi-randomised controlled trial is conducted. This trial examines the motivational value of witnessing the benefits of fruit and vegetable consumption on images of one’s own face. Dietary change was examined relative to a group of individuals receiving either no intervention or health information in line with existing public health campaigns. The results of this initial pilot trial were encouraging; controlling for baseline fruit and vegetable consumption, a main effect of intervention group was found in favour of the appearance-based intervention group, whilst receiving a health-information intervention conveyed no measurable benefit to fruit and vegetable intake.

6.2. Introduction

The previous chapters indicate that illustrating the beneficial impact of fruit and vegetable consumption on skin pigmentation may be an effective dietary intervention strategy. Here we present a suite of experiments designed to investigate this hypothesis. In Experiment i, we quantified the impact that fruit and vegetable consumption has on skin colour. We then investigated in Experiment ii, using a quasi-randomised controlled trial, the effectiveness of an appearance-based dietary intervention.

Participants were allocated to three groups receiving; a NHS information-only intervention; this information in addition to an appearance-based intervention; or a no-intervention control condition. We hypothesise that fruit and vegetable consumption will improve in the group receiving the appearance-based intervention, relative to the other groups. We explore baseline diet quality and financial status as variables that may limit responsiveness to intervention.

6.3. Experiment i: Quantifying the Impact of Fruit and Vegetable Consumption on Skin Colour

6.3.1. Methods

All procedures obtained ethical approval from the University of St Andrews Teaching and Research Ethics Committee, and prior informed written consent was obtained from all participants. All individuals were reimbursed financially for their participation at the rate of £5 per hour.

6.3.2. Participants

Sixty Caucasian undergraduate students (25 females, 35 males, mean age = 20.61, age range: 18-26), recruited via advertisement, completed food frequency questionnaires (Margetts, Cade, & Osmond, 1989) to determine typical daily fruit and vegetable consumption. Participants were photographed and completed brief questionnaires, providing information on demography, hours of strenuous exercise and self-tanning product and solarium use.

6.3.3. Skin colour measurement

Skin colour was recorded using a Konica Minolta CM-2600d spectrophotometer. An 8mm diameter aperture was used for all measurements. CIE L*a*b* tristimulus values were recorded (excluding specular reflection) for each participant at seven body locations (left cheek, right cheek, forehead, volar forearm, outer bicep, shoulder and palmar thenar eminence). Care was taken to ensure that the aperture was lightly held against skin, in order to minimise blanching. White-point calibration was conducted before each recording session according to a white reference tile.

Participants reporting the use of self-tanning products (n = 3) or solariums (n = 3) were excluded from analysis. Remaining participants were split into two groups according to self-reported fruit and vegetable consumption. High and low fruit and vegetable consumption groups were constructed using participants in the upper and lower quartiles, respectively. The resultant groups were equivalent in terms of gender (5 males, 10 females in each group), age [low = 20.75 ± 1.67 (mean ± *SD*), high = 20.99 ± 2.29], hours of vigorous exercise per week (high = 0.87 ± 0.52, low = 0.80 ±

0.94) and body mass index (high = 22.0 ± 2.65, low = 22.55 ± 3.43, Mann-Whitney *U* test, all $p \geq .475$).

6.3.4. Results

The high consumption group reported consuming an average of 8.63 portions per day (*SD* ± 2.18), whereas the low consumption group consumed 3.08 portions per day (*SD* ± 0.75). With all seven measured body regions combined, participants in the high consumption group exhibited significantly redder (higher a*; $t_{18.83} = -2.635$, $p = .016$) and yellower skin (higher b*; $t_{28} = -2.873$, $p = .008$) than the low fruit and vegetable consumption group. Those in the high consumption group were also marginally darker (lower L*; $t_{23.32} = 1.849$, $p = .077$) than the low consumption group.

6.3.5. Conclusion

The results of Experiment i reveal, in line with Stephen et al. (2011), that increased fruit and vegetable consumption is linked with increased skin yellowness. The empirically-derived colour values acquired here make it possible to accurately illustrate, in a portion-wise manner, the likely impact of increasing fruit and vegetable consumption on human skin. In Experiment ii we investigate whether these skin colour changes can motivate increased fruit and vegetable consumption.

6.4. Experiment ii: Appearance-Based Dietary Intervention

6.4.1. Participants

Seventy-three undergraduate students (55 females, 18 males, mean age = 21.06, age range: 18-43, 83.6% Caucasian) at the University of St Andrews participated between March and June 2010. Twenty-two of these individuals were photographed as part of Experiment i and formed the majority of the appearance-based intervention group (see Figure 6.1 for participant flow diagram). The additional 51 participants were recruited via advertisement, which described the study as an investigation of diet and health, and were quasi-randomly allocated (via alternation at sign-up) to one of three conditions individually [information-only n = 20; information plus appearance-based intervention n = 28 (henceforth “appearance-based intervention”); or control n = 25]. Allocation was initially closed to the appearance-based intervention condition, but was opened after a sufficient number of participants had signed up for the other conditions. Neither the experimenter, nor participants could be blinded to study condition assignment. At baseline, these three groups were equivalent in terms of body mass index, household income during childhood, self-rated health, hours of strenuous exercise and fruit and vegetable intake (Kruskal-Wallis H test, all $p \geq .312$). Participants reported consuming an average of 4.14 (*SEM* ± 0.30) fruit and vegetable portions per day.

6.4.2. Fruit and vegetable information

Selected pages from the NHS information booklets [“5 A Day, Just Eat More (fruit & veg)”; pages i, ii, 12-15, 20 & 21] and [“5 A Day, Just Eat More (fruit & veg): What’s it all about?”; pages i-ii)] were provided to participants (except controls) on completion of the initial measurement session. The pages (NHS, 2004a, 2004b) provided information on recommended portion sizes, meal planning and health benefits and answered frequently asked diet-related questions.

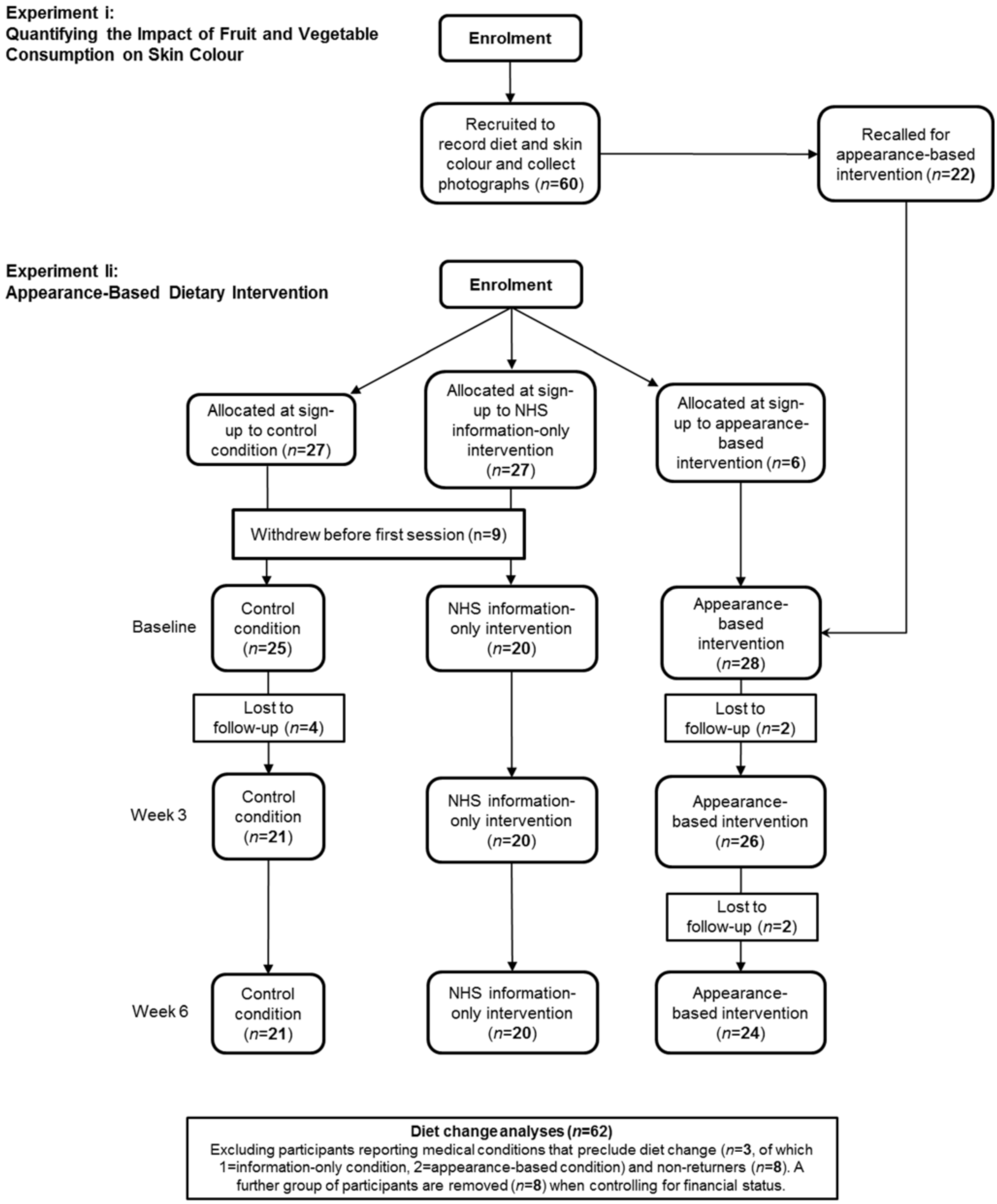

Figure 6.1. Flow diagram of participant involvement in Experiments i and ii.

6.4.3. Appearance-based intervention

Photographs of participants in the appearance-based intervention condition were collected, calibrated and manipulated (see Chapter 5 for methods), to illustrate the impact of changes in fruit and vegetable consumption on skin colour, based on the results from Experiment i. The average volar forearm skin colour values in CIE L*a*b* space (Table 6.1) within each group were used to create endpoint masks as this area is relatively free of habitual sun-exposure and make-up. A continuum of 13 images was created per participant, representing a total range of ± 2.00 L* units, ± 2.28 a* units and ± 4.25 b* units, and was equivalent to a change of ± 11.11 fruit and vegetable portions per day. Participants in this condition used a computer program (as in Chapter 5) to manipulate the colour of their own face image along the fruit and vegetable colour axis, and were instructed to select what they perceived as the healthiest face colour using horizontal movements of a computer mouse. The participant's selected face colour was recorded. The experimenter (R.W.) subsequently explained how fruit and vegetable consumption changes skin colour in the illustrated fashion and that in independent experiments, participants indicate that faces manipulated in a similar manner appear healthier than their un-manipulated versions (Stephen et al., 2011). Participants were also given an approximate quantification of how many fruit and vegetable portions are required to achieve their desired skin colour.

Table 6.1. Mean volar forearm colour in CIE L*a*b* space ± SD.

	L*	**a***	**b***
Low fruit and vegetable consumption group (*n* = 15, 3.08 ± 0.75 portions/day ± *SD*)	69.56 ± 2.41	5.28 ± 1.51	13.18 ± 2.18
High fruit and vegetable consumption group (*n* = 15, 8.63 ± 2.18 portions/day ± *SD*)	68.56 ± 1.85	6.42 ± 0.74	15.30 ± 1.44
***Δ* (High – low)**	-1.00	1.14*	2.12**

Note. L* represents skin lightness (0 -100), a* represents position on a green-red axis (-60 to +60) where positive values are red and b* represents position on a blue-yellow axis (-60 to +60) where positive values are yellow. *p < .05, **p < 0.005.

Participants in the appearance-based intervention group also received a take-home photo-quality leaflet containing images of their manipulated facial images, to further illustrate the effect of fruit and vegetable consumption on skin colour (Appendix A). The leaflet presented the participant's current facial appearance, alongside manipulations illustrating the effect of eating approximately five more, or five fewer fruit and vegetable portions per day (Figure 6.2). Printed images were colour calibrated using a 140 patch GretagMacbeth chart to an average a *ΔE* of 3.56 for 14 skin colour patches.

6.4.4. Procedure

Participants completed all parts of the experiment individually in a quiet office. In the initial session, all participants completed a questionnaire to establish recent diet (Margetts et al., 1989) and health; household income during childhood (quartiles), recent sun exposure and use of make-up, self-tanning products and solariums. At the end of this session, depending on allocated condition, participants received either no additional information (control group), the fruit and vegetable information pack only (information-only group), or this pack in addition to the computer-based skin colour transformation session and personalised leaflet (appearance-based intervention group).

Participants then returned for two follow-up sessions after approximately 3 and 6 weeks (1st interval mean = 21.07 days, *SEM* ± 0.42; 2nd interval = 20.29 days, *SEM* ± 0.36), in order to complete further questionnaires collecting information on their diet, use of make-up, and use of self-tanning products or solariums in the last weeks. At the end of their final session, participants were asked "Please give brief details if you believe your diet changed for any other reasons (e.g., medical advice, personal/religious reasons, media influence)".

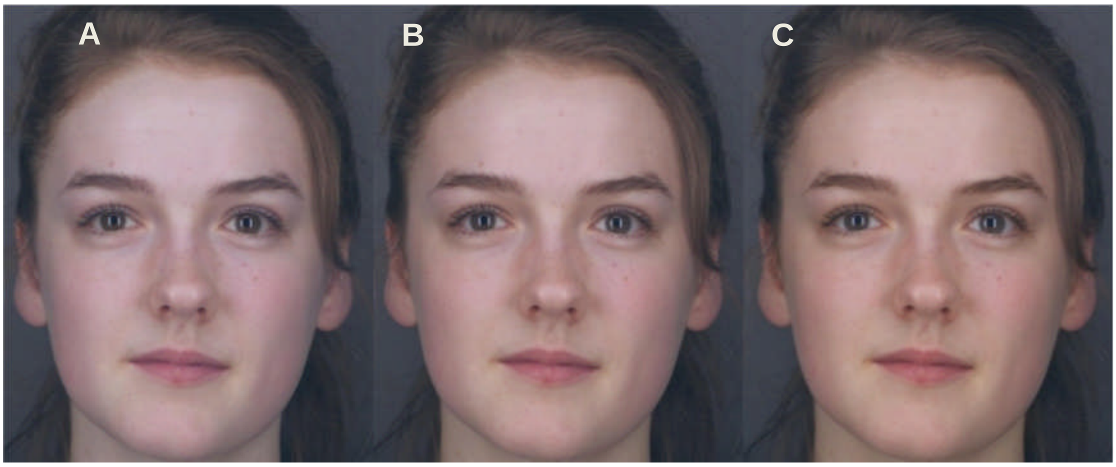

Figure 6.2. Example face stimuli provided to participants in the appearance-based intervention condition. Images reflect (B) original, calibrated face image and face colour manipulated according to a 5.5 fruit and vegetable portion per day (A) decrease or (C) increase.

6.4.5. Results

6.4.5.1. Statistical methods.

SPSS v.17 was used for main analyses. G*Power v.3.1.2 (Faul, Erdfelder, Buchner, & Lang, 2009) was used to calculate effect sizes where appropriate. Where necessary, a log transform was used to successfully normalise data, failing this, non-parametric statistical tests were used. Three participants reporting medical conditions (e.g., fructose intolerance) that preclude diet change were eliminated from appropriate analyses. Two participants (of which 1 = control, 1 = information-only group) completed the week 6 session via the internet.

6.4.5.2. Computer-based colour manipulation.

When asked to optimise the appearance of health in their own face, participants in the appearance-based intervention group ($n = 28$) chose to significantly add colouration associated with increased fruit and vegetable consumption (Wilcoxon signed-rank test, $Z = -4.080$, $p < .001$). Participants manipulated their face colour by an average ΔE of 2.55 (*SEM* ± 0.37), equivalent to an increase in fruit and vegetable consumption of 5.41 (*SEM* ± 0.78) portions per day.

6.4.5.3. Fruit and vegetable consumption.

We analysed the self-reported diet of only those participants that completed both the first and final sessions. No significant difference was seen between returners ($n = 65$) and non-returners ($n = 8$, of which 4 = control, 4 = appearance-based intervention) in terms of intervention group, gender or age and the baseline measures of body mass index, ethnicity, hours of exercise per week and fruit and vegetable intake per day (Mann-Whitney *U* test, all $p > .18$), suggesting no systematic effects of these variables on repeated participation. Childhood household income was marginally higher in returning participants compared to non-returners ($Z = -1.781$, $p = .075$).

To investigate effects of the interventions on fruit and vegetable consumption after 3 and 6 weeks (Figure 6.3A), a repeated measures analysis of covariance (ANCOVA) was performed, with intake at 3 and 6 weeks as the repeating factor and intervention group as a between-subjects factor. Controlling for a significant relationship with baseline fruit and vegetable intake ($F_{1,58}$ = 63.315, p < .001, η_p^2 = .52) as a covariate, a significant main effect of intervention group was seen on fruit and vegetable intake ($F_{2,58}$ = 4.177, p = .020, η_p^2 = .13). Independent samples *t*-tests revealed no significant difference in fruit and vegetable consumption between the control and information-only groups at 3 ($t_{37.05}$ = -0.298, p = .768, d = *0.09*) or 6 weeks (t_{38} = -0.239, p = .813, d = *0.07*). Consumption was marginally higher in the appearance-based intervention group than in the control and information-only groups combined at 3 weeks (t_{60} = -1.972, p = .053, d = *0.52*) and significantly higher at 6 weeks (t_{60} = -2.224, p = .030, d = *0.59*). The lack of interaction between group and session ($F_{2,58}$ = 0.309, p = .735, η_p^2 = .01), suggests the dietary changes within groups were stable between weeks 3 and 6.

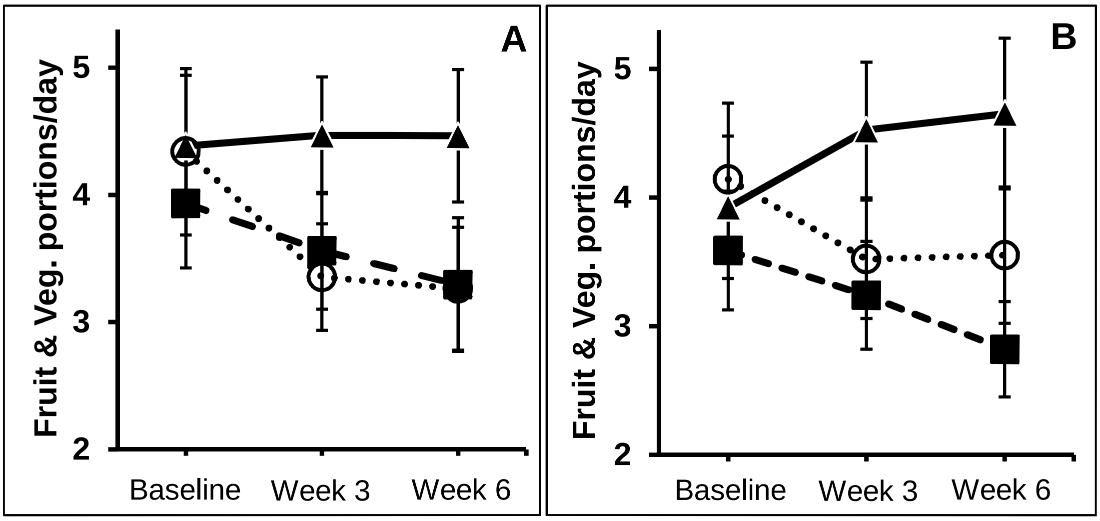

Figure 6.3. Mean (± *SEM*) fruit and vegetable consumption per day, per group across intervention sessions. Filled triangles – appearance-based intervention condition; filled squares – information-only condition; hollow circles – control condition. (A) Appearance-based intervention condition (n = 22); information-only condition (n = 19); control condition (n = 21). (B) Including only participants reporting childhood household income in upper three quartiles, Appearance-based intervention condition (n = 19); information-only condition (n = 17); control condition (n = 18).

Paired samples *t*-tests were used to investigate fruit and vegetable intake changes between sessions, within each of the three groups. The control group reported a significant decline in fruit and vegetable consumption between baseline and week 6 (t_{20} = 2.639, p = .016, dz = 0.58) and baseline and week 3 (t_{20} = 2.518, p = .020, dz = 0.55), but not weeks 3 and 6 (t_{20} = 1.199, p = .245 dz = 0.26). The information-only group reported no significant changes in diet between baseline and week 6 (all p > .152). The appearance-based intervention group reported no significant changes in diet between baseline and week 6 (all $p \geq 0.479$).

Diet change could be limited by finance. We therefore reanalysed fruit and vegetable intake, excluding students reporting household income during childhood in the lowest quartile (n = 4) and those not reporting household income (n = 4). We assume that the remaining sample retains the participants most likely able to afford diet change (Figure 6.3B). Further repeated measures ANCOVA replicated the significant effects of baseline consumption ($F_{1,50}$ = 70.201, p < .001, η_p^2 = .58) and intervention group ($F_{2,50}$ = 5.618, p = .006, η_p^2 =.18). The stability of group effect across weeks 3 to 6 was also replicated ($F_{2,50}$ = 0.616, p = .544, η_p^2 = .02).

Further paired-samples t-tests revealed that with this exclusion criterion, the control group exhibited a marginally significant decline in fruit and vegetable consumption between baseline and week 6 (t_{17} = 1.773, p = .094, dz = 0.42) and baseline and week 3 (t_{17} = 1.934, p = .070, dz = 0.46), but not weeks 3 and 6 (t_{17} = 0.531, p = .602, dz = 0.13).

The information-only group reported no significant change in consumption between baseline and week 6 (all $p \geq .146$). The appearance-based intervention group reported no significant change in consumption between baseline and week 6 (ps ≥

0.127), but reported a marginal increase between baseline and week 3 (t_{18} = -1.732, p = .100, dz = 0.40).

6.5. General Discussion

This suite of experiments developed and evaluated an appearance-based intervention, which aimed to promote fruit and vegetable consumption in a student population. We first empirically quantified the impact of fruit and vegetable consumption on skin colour. In line with Stephen et al. (2011), high fruit and vegetable consumers exhibited significantly yellower skin than individuals who consumed 5.5 fewer portions per day on average. Furthermore, when given the opportunity to manipulate own face colour to optimise the appearance of health, individuals in the appearance-based intervention condition chose to significantly increase this colouration. Such a result is critical to the efficacy of an appearance-based intervention based on this effect as, for effective goal formation, the participant themselves should desire the illustrated skin colour changes.

Accordingly, a significant effect of intervention group, favouring the appearance-based condition, was seen on fruit and vegetable consumption over the course of the study. Participants receiving no intervention reduced their fruit and vegetable consumption over the six-week period. This may be due to the study being carried out towards the end of the academic year, which imposes financial constraints, due to the exhaustion of support funds. This period also is associated with increased anxiety due to examinations. Both of these issues may cause participants to sacrifice a healthy diet for one that is cheap, quick to prepare and high in energy (Epel, Lapidus, McEwen, & Brownell, 2001; Nelson, Lust, Story, & Ehlinger, 2008; Oliver & Wardle, 1999). Indeed at completion, a number of participants commented that the exam period had negatively affected their diet, e.g., “Towards the end of the study I entered the exam period at school and attended less to maintaining a healthy diet”; “Increased workload. So less time/inclination to prepare healthy food.”; “Exams - fast food”.

Participants receiving only NHS dietary advice tended to report a similar decline in fruit and vegetable intake; consumption in this group was not significantly greater than that of the control group at any session. This supports the conclusions of Chapter 2 that the information-provision approach of current healthy-eating campaigns is insufficient to effectively motivate increased fruit and vegetable consumption.

The addition of the appearance-based intervention protected against the seasonal decline observed in control and information-only participants. This group may also be subject to financial limitations, indeed one participant in this group wrote at debrief “[*my*] diet did not change much mostly due to money restrictions, but the appearance benefits have made me want to eat more fruit and veg once I can afford them”. Further, restricting analysis to participants reporting childhood household income in the upper three quartiles tentatively suggests that the appearance-based intervention may be capable of motivating *increased* fruit and vegetable consumption in these individuals. These results warrant further investigation, but indicate that any attempt at persuading individuals to improve their diet will critically depend on general and seasonal factors affecting financial status and stresses to lifestyle.

The declining fruit and vegetable consumption in the control and information-only groups may have an additional or alternative explanation. Participants may have overestimated fruit and vegetable intake at first report, and subsequently self-adjusted their report of diet in further sessions, potentially serving to be more accurate as attention is drawn to their diet (Golembiewski, Billingsley, & Yeager, 1976). If so, this would indicate that the control and information groups had more consistent fruit and vegetable consumption than their self-report conveys, and that the appearance-based intervention produced a larger increase in fruit and vegetable consumption than immediately obvious. To circumvent this potential confound in future study, all interventions could be administered following a period in which participants are given diet questionnaires, thereby providing the opportunity to self-adjust reported dietary intake.

In terms of the *Self-Regulation Model of Health Behavior* (Carver & Scheier, 1990), these results suggest that the appearance-based intervention was sufficient to establish a motivational goal, and that participants strived to reduce the discrepancy between their current state and this goal. To increase the efficacy of this intervention technique, participants may be instructed to form plans of specifically when, where and how they aim to consume fruit and vegetables. Such implementation intentions are regarded as being important in promoting health behaviour due to their ability to act as a mnemonic device (Sheeran & Orbell, 1999), which reminds the individual of their goals or intentions in situations where the opportunity arises to achieve them. This suggests that implementation intentions are most effective when the individual is sufficiently motivated to change the behaviour of interest. The current study presents a novel means of motivating individuals to consume more fruit and vegetables, and thus may be a valuable addition to interventions which utilise implementation intentions.

Whilst these results are encouraging, it is necessary to address a number of limitations before such an intervention can be utilised at a population level. As fruit and vegetable consumption was recorded via self-report, and participants were not blinded to intervention condition, we cannot be confident that this measure was immune to demand characteristics or overestimation. An objective estimate of dietary intake is required to eliminate this potential source of biased or selective reporting, for example, measurement of blood plasma carotenoids may be used as a biomarker of fruit and vegetable consumption (Dauchet et al., 2008).

It is chiefly important to determine whether appearance-based intervention can motivate increases in fruit and vegetable consumption amongst individuals with a more stable diet, to examine this, future studies should investigate dietary intake of a non-student population. It is also important to determine whether the technique can motivate long-term dietary changes, as sustained change is required for lasting good health. In the current study, our method of stimulus collection and calibration limited sample size and recruitment. Our encouraging results may now justify investment in automating the stimulus creation and colour calibration required for accurate appearance-based demonstration, permitting investigation in a larger, stringently randomised sample. We envisage that application of the knowledge acquired in this proof-of-concept study will serve to bolster the efficacy of this technique, potentially indicating suitability for wider-scale dietary intervention.

7. You Are What You Eat: Within-Subject Increases in Fruit and Vegetable Consumption Confer Beneficial Skin Colour Changes

This chapter is largely based on the following work accepted for publication in a peer reviewed journal (accepted February 8, 2012):

Whitehead, R. D., Re, D. R., Xiao, D., Ozakinci, G. & Perrett, D. I. (2012). You Are What You Eat: Within-Subject Increases in Fruit and Vegetable Consumption Confer Beneficial Skin colour Changes. *PLoS ONE*, *7*(3), e32988.

7.1. Summary

Fruit and vegetable consumption and ingestion of carotenoids have been found to be associated with human skin colour (yellowness) in recent cross-sectional studies (including Chapter 6). Here we investigate the effects of fruit and vegetable consumption on skin colour longitudinally to determine the magnitude and duration of diet change required to change skin colour. We use psychophysical methods to investigate the minimum colour change required to confer perceptibly healthier and more attractive skin colouration. Together, the results of these experiments suggest that modest dietary changes are required perceptibly to enhance apparent health (2.91 portions per day) and attractiveness (3.30 portions) within six weeks. These results may be used as further incentive in an appearance-based dietary intervention strategy.

7.2. Introduction

Carotenoids are yellow-red organic pigments which are abundant in and impart colour to fruit and vegetables. These phytochemicals are efficient singlet oxygen quenchers (Sies, 1993) which enables them to protect tissue against oxidative stress, arising when the balance of oxidants to antioxidants *in vivo* is in favour of the former. Such conditions can precipitate damage to cellular proteins, lipids and DNA (Sies, 1993) and consequently may contribute to a variety of age-related degenerative processes (Frisard & Ravussin, 2006), cardiovascular disease (Sies, Stahl, & Sevanian, 2005), diabetes and related complications (Ceriello, 2005; Dierckx et al., 2003) and possibly some cancers (Martinez-Outschoorn et al., 2010).

In addition to the endogenous oxidants produced as part of normal metabolic (Valko et al., 2007) and immunological processes (Dahlgren & Karlsson, 1999), skin is directly exposed to a number of environmental oxidants, such as UV radiation, nitrogen oxides, cigarette smoke and ozone (Cross, van der Vliet, Louie, Thiele, & Halliwell, 1998). As antioxidants, carotenoids are important for skin health, serving a protective role by virtue of their relatively high concentration in all layers of this organ (Alaluf, Heinrich, et al., 2002; R. Lee, Mathews-Roth, Pathak, & Parrish, 1975; Richelle et al., 2006). Illustrating their importance in this capacity, carotenoids reduce UV light sensitivity, increasing the minimum level of UV exposure required to induce erythema (Alaluf, Heinrich, et al., 2002; Bouilly-Gauthier et al., 2010; Rizwan et al., 2011). Carotenoids are expended in their antioxidative role (Krinsky, 1998), potentially leading to organism-wide deficits which, if not restored via dietary intake, could precipitate conditions associated with elevated oxidative stress (Ceriello, 2005; Dierckx et al., 2003; Frisard & Ravussin, 2006; Martinez-

Outschoorn et al., 2010; Sies et al., 2005). Further, carotenoids are implicated in immune-cell activity (Bendich, 1991; Fuller, Faulkner, Bendich, Parker, & Roe, 1992), increasing the cell-surface expression of MHC class II signaling molecules (Alexander, Newmark, & Miller, 1985; Hughes et al., 1997). Consequently, carotenoid deficits may also lead to immune suppression.

Individual differences in dietary intake of carotenoids, which occur chiefly via fruit and vegetable consumption, have been linked to between-subject variation in skin yellowness (Stephen et al., 2011; Chapter 5), in particular the b* axis of the CIE 1976 L*a*b* colour-opponent space (Commission Internationale de l'Eclairage, 1976). Studies using Raman spectroscopy (Darvin et al., 2008; Meinke, Darvin, Vollert, & Lademann, 2010) indicate that skin carotenoid concentrations are also subject to short-term fluctuation within individuals. This variation has been linked to diet change, carotenoid supplementation and other important lifestyle factors such as tobacco use, alcohol consumption and infectious illness which are known to affect oxidant and antioxidant levels (Dahlgren & Karlsson, 1999; Dragsted et al., 2006; McDonough, 2003; Polidori, Mecocci, Stahl, & Sies, 2003; Vassalle, Maffei, Ndreu, & Mercuri, 2009). Despite qualitative evidence that skin carotenoid levels can be affected by diet (e.g., Darvin et al., 2008), it is not known whether dietary variation is sufficient to confer visible skin colour changes within-subject. The quantitative level of diet change and timescale required to achieve skin colour change also remain obscure.

Here we address these issues using reflectance spectrophotometry to measure skin colour (CIE L*a*b*) and spectral reflectance at three time points over a six-week period. Diet was self-reported at each session via food frequency questionnaire to estimate daily fruit and vegetable consumption. We hypothesise that changes in fruit and vegetable consumption will correlate positively with changes in skin yellowness over this six-week study, and that diet-linked skin reflectance changes over this period will parallel the absorption spectra of common carotenoids.

We also present a psychophysical study which aims to estimate the level of diet change associated with perceptibly healthier or more attractive skin colouration. We used a two-alternative forced-choice staircase design to determine colour thresholds. Participants were asked to select the yellower, healthier or more attractive of two sequentially presented face stimuli, which differed in colour according to an empirically derived fruit and vegetable colour axis. In accordance with previous studies (Stephen et al., 2011; Stephen, Law-Smith, et al., 2009) and the results of Chapter 5 and 6, we predict that participants will choose to increase colouration associated with carotenoid pigmentation.

7.3. Experiment i: The Effect of Changes in Fruit and Vegetable Consumption on Skin Colour

7.3.1. Methods

All procedures obtained ethical approval from the University of St Andrews Teaching and Research Ethics Committee, and prior informed written consent was obtained from all participants. All individuals were reimbursed financially for their participation at the rate of £5 per hour.

7.3.1.1. Participants.

Sixty-three undergraduate students at the University of St Andrews each participated in three sessions between March and June 2010. Twenty-five participants reporting recent sunbathing and/or use of self-tanning products, solariums and/or facial make-up (e.g., blusher/foundation) at any session were eliminated from data analyses. Three additional participants with initial overall skin lightness (see procedure) outside of two standard deviations from the mean were also excluded, leaving 35 participants in the final analyses (21 females, 14 males, mean age = 20.74, age range: 18-25, 34 (97.1%) Caucasian, 1 (2.9%) East Asian). These participants reported consuming an average of 3.41 (*SEM* ± 0.32, min = 0.94, max = 7.80) fruit and vegetable portions per day over three sessions. Overall skin lightness, redness and yellowness were normally distributed in this sample (Kolmogorov-Smirnov tests all $p \geq .134$).

7.3.1.2. Procedure.

Participants attended an initial measurement session and returned for two follow-up sessions after approximately three and six weeks (1st interval mean days (± *SEM*) = 20.8 ± 0.81; 2nd interval = 20.01 ± 0.63), in which all measurements and questions were repeated. Participants completed a food frequency questionnaire (Margetts et al., 1989) to establish daily fruit and vegetable intake. The questionnaire contained nine items pertaining to common fruits and vegetables, not including potatoes. At each session participants were asked "How often do you currently eat each of the following food and drink items?" (2 or more times a day, once a day, 3-5 times a week, 1-2 times a week, 1-3 times a month, rarely/never). Daily consumption of these items was estimated and summed to achieve an estimate of daily fruit and vegetable intake. Participants were able to supplement the questionnaire with up to four additionally consumed items. Fruits or vegetables among these added items were included in the daily total.

Skin colour was recorded via spectrophotometry (as in Chapter 6). Spectral reflectance between 400 and 540nm at 10nm intervals were also recorded (excluding specular reflection) for each participant across the seven measured body locations (left cheek, right cheek, forehead, volar forearm, outer bicep, shoulder and palmar thenar eminence).

7.3.2. Results

Mean baseline skin colour values and six-week changes are presented in Table 7.1. To investigate the effect of diet on skin colour, we examined the impact of changes in fruit and vegetable consumption within-subjects using linear regression (skin colour change over each three-week interval or the entire six-week study the dependent variable, and change in fruit and vegetable consumption over these periods as the independent variable). Spearman correlation was used to investigate the link between diet-change and skin reflectance change at wavelengths between 400nm and 540nm. We then investigated, using further Spearman correlations, whether the strength of this relationship followed the absorption spectra of common carotenoids (E. S. Miller, 1937) or melanin (Sarna & Swartz, 1988), which also affects skin yellowness (Stamatas et al., 2004).

Over the six-week study period skin lightness decrease averaged across all seven measured regions was significantly associated with increase in fruit and vegetable consumption (b = -0.333, SEM_b = 0.163, p = .049, r^2 = .11; Figure 7.1A). Skin redness (b = 0.224, SEM_b = 0.108, p = .045, r^2 = .12; Figure 7.1B) and yellowness (b = 0.251, SEM_b = 0.116, p = .038, r^2 = .12; Figure 7.1C) changes were significantly associated with increase in fruit and vegetable intake. Averaged across the three facial measurements, neither skin lightness (b = -0.299, SEM_b = 0.212, p =

.169, r^2 = .06) nor redness (b = 0.151, SEM_b = 0.200, p = .456, r^2 = .02) changes were significantly associated with change in fruit and vegetable consumption.

Table 7.1. Mean initial CIE L*a*b* values. n = 35.

	Mean initial value (± *SEM*)	**Minimum**	**Maximum**	**Mean *Δ* (± *SEM*)**	**Minimum *Δ***	**Maximum *Δ***
Overall L*	66.95 ± 0.31	62.83	69.64	-0.07 ± 0.18	-3.08	1.92
Overall a*	9.24 ± 0.24	5.97	12.51	+0.20 ± 0.12	-1.29	2.04
Overall b*	14.50 ± 0.25	10.68	17.52	+0.51 ± 0.13	-1.16	2.65
Face L*	65.94 ± 0.39	59.41	69.53	-0.40 ± 0.23	-3.45	1.93
Face a*	11.48 ± 0.33	7.42	16.98	+0.30 ± 0.21	-2.09	2.39
Face b*	14.66 ± 0.28	11.09	18.02	+0.74 ± 0.17	-1.43	2.54

Note. L* represents skin lightness (0 -100), a* represents position on a green-red axis (-60 to +60) where positive values are red and b* represents position on a blue-yellow axis (-60 to +60) where positive values are yellow. Overall values represent average skin colour across seven body regions (left cheek, right cheek, forehead, volar forearm, outer bicep, shoulder and palmar thenar eminence). Face values represent the average of the three facial measurements. Delta (*Δ*) values represent six-week changes in skin colour values.

Increase in fruit and vegetable intake over the six-week period was marginally associated with increases in facial skin yellowness (b = 0.312, SEM_b = 0.154, p = .051, r^2 = .11; Figure 7.1D). Examination of changes over the three-week periods between participants' first two, or last two sessions revealed no significant relationships between fruit and vegetable intake change and skin colour change (all $p \geq .229$).

To investigate whether the observed relationships between diet change and skin colour change were contingent on baseline diet a series of ANCOVA models were constructed, each with skin colour change over the six-week period as the dependent variable and change in fruit and vegetable consumption over this period and initial fruit and vegetable consumption as covariates. These models revealed no impact of initial fruit and vegetable consumption on overall or facial skin lightness, redness or yellowness change (all $F \leq 2.321$, $p \geq .137$, $\eta_p^2 \leq 0.07$). The effect of six-week diet change on change in skin colour remained as above i.e., diet change was significantly associated with change in overall lightness, redness and yellowness (all $F \geq 4.181$, $p \leq 0.049$, $\eta_p^2 \geq 0.116$) and marginally significantly associated with change in facial yellowness ($F = 3.974$, $p = .055$, $\eta_p^2 = 0.11$). Over the six-week period, the skin colour change (*ΔE)* associated with an increase of one portion of fruit and vegetables per day was 0.47 over all seven body measurements and 0.46 for facial skin (here *ΔE* represents the Euclidean distance between zero and the unstandardised beta values for L*, a* and b* change derived in the linear regression analyses).

To investigate further the basis of the observed skin colour changes over this six-week period we examined changes in skin reflectance at 400-540nm, the wavelengths associated with peak light absorption by carotenoids (E. S. Miller, 1937). To control for the impact of between-subject differences in overall skin reflectance, recorded values were normalised by dividing each raw reflectance value by a participants' average reflectance across all measured wavelengths (Stephen et al., 2011).

Following a modified version of the methods used in Stephen et al (2011), at 10nm intervals, we obtained Spearman's rank correlation coefficients for the relationships between change in skin reflectance and change in fruit and vegetable consumption over the six-week period of the study ($\rho_{\Delta\ reflectance\ vs.\ \Delta\ diet}$). Negative correlations are expected because, if an increase in the consumption of carotenoid-rich fruit and vegetables leads to the deposition of these pigments in the skin, then the skin's absorption will increase at these wavelengths, producing a concurrent decrease in reflectance.

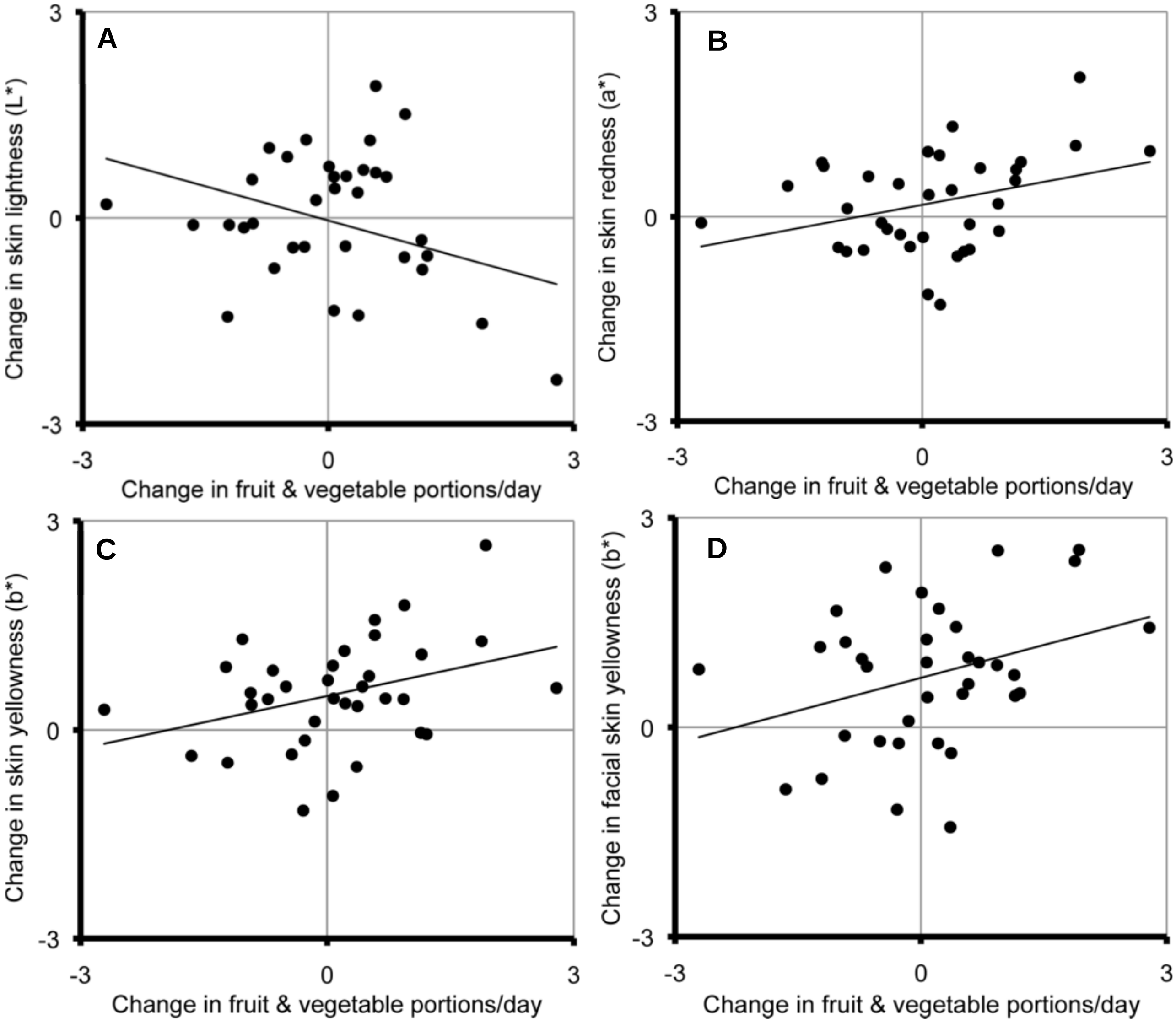

Figure 7.1. The relationship between six-week changes in fruit and vegetable intake and average skin (A) lightness (L*), (B) redness (a*), (C) yellowness (b*) and (D) facial skin yellowness (b*).

Spearman correlation was then used to examine whether the strength of this relationship ($\rho_{\Delta\ reflectance\ vs.\ \Delta\ diet}$) is associated with carotenoid absorption across the 400-540nm wavelength range (E. S. Miller, 1937). We expect the relationship to be strongest (more negative) at wavelengths associated with the greatest light absorption by carotenoids, and weaker at wavelengths associated with lower absorption. Across wavelengths 400-540nm, the relationship between overall skin reflectance change (average of all seven measured skin regions) and fruit and vegetable consumption change from week zero to six was not significantly correlated with the absorption spectra of α-carotene (ρ = -0.232, p = .404) but was marginally significantly correlated with the absorption spectra of β-carotene (ρ = -0.454, p = .089) and significantly correlated with the absorption spectra of lycopene (ρ = -0.846, p < .001) and the mean absorption across these three common carotenoids (ρ = -0.539, p = .038; Figure 7.2A).

Over the six-week duration, the strength of relationship between *facial* (average of three face regions) skin reflectance change and fruit and vegetable consumption change was not significantly correlated with the absorption spectra of α-carotene (ρ = -0.386, p = .156), but was significantly correlated with the absorption spectra of β-carotene, lycopene and the mean absorption of these three carotenoids (all ρ < -0.588, $p \leq$ .021; Figure 7.2B). The relationship between average skin, or facial skin reflectance change and fruit and vegetable consumption change was not significantly correlated with the absorption spectrum of melanin (E. S. Miller, 1937) over these wavelengths (both $\rho \leq$ 0.399, $p \geq$ .140).

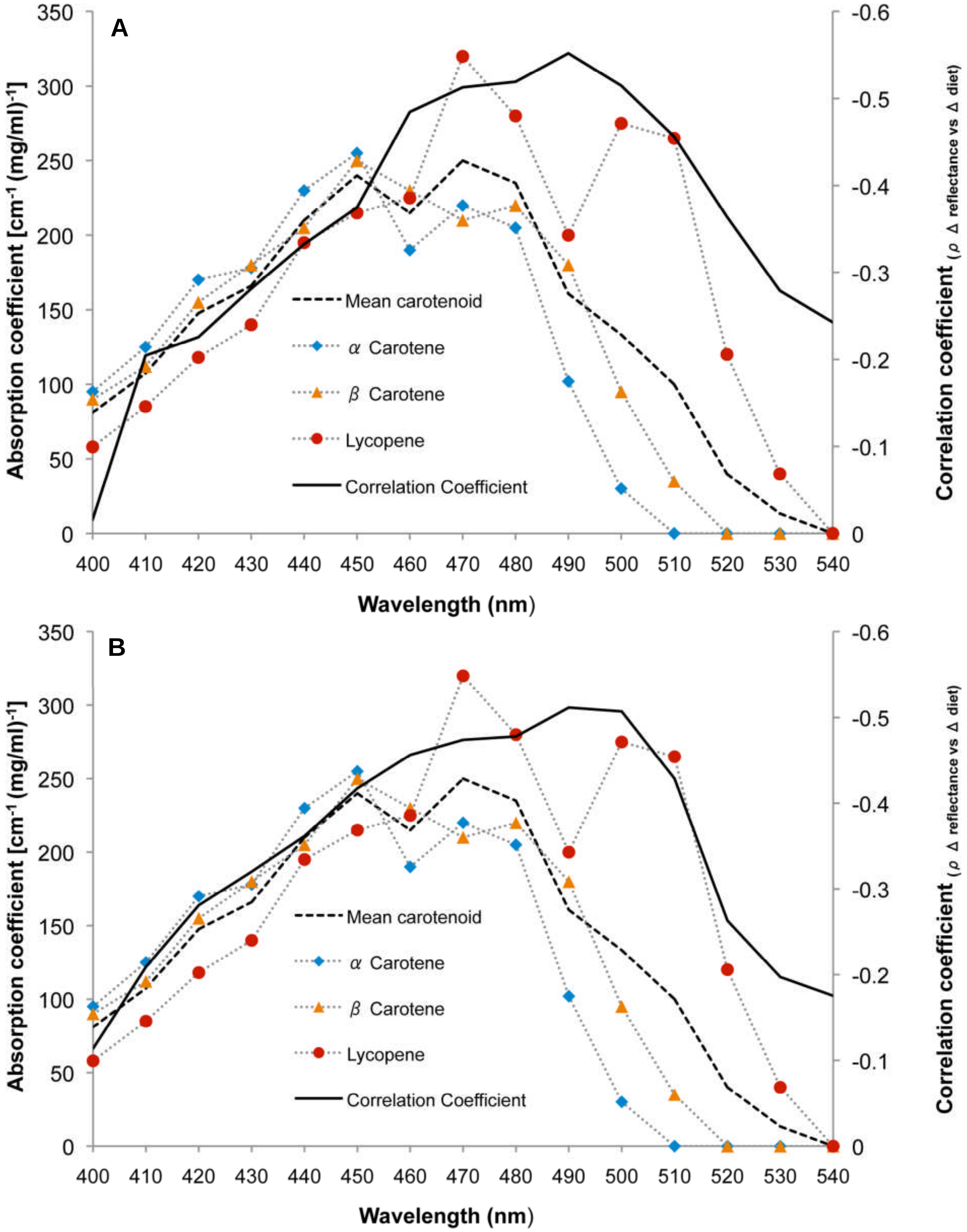

Figure 7.2. Solid black line - Spearman correlation coefficients ($\rho_{\Delta \text{ reflectance vs. } \Delta \text{ diet}}$) of the relationships between fruit and vegetable consumption change (from baseline to week 6) and (A) average skin reflectance change and (B) facial skin reflectance change. Dashed bold line shows the mean absorption spectra [cm^{-1} $(mg/ml)^{-1}$] for three common carotenoids, which are individually plotted (blue rhombi - α-carotene, orange triangles - β-carotene and red circles - lycopene).

The results remain similar when we repeated the above analyses controlling for the potential effect of initial fruit and vegetable consumption. To achieve this we conducted Spearman correlations between skin reflectance change and standardised residuals derived from a non-significant linear regression with six-week diet change as the dependent variable and initial diet as the independent variable (b = -0.004, SEM_b = 0.103, p = .970, r^2 = .00). This partials out any effect of starting diet on diet change. Over all measured body regions, the strength of these correlations was not significantly associated with the absorption spectra of α-carotene (ρ = -0.205, p =.463) or β-carotene (ρ = -0.427, p =.113), but was significantly associated with the absorption spectra of lycopene (ρ = -0.829, p < .001) and marginally correlated with the mean absorption spectra of these three carotenoid pigments (ρ = -0.511, p = .052). For average facial skin reflectance, no association was seen with the absorption spectra of α-carotene (ρ = -0.386, p =.156) but the correlation strength was associated with the absorption spectra of β-carotene (ρ = -0.588, p =.021), lycopene (ρ = -0.893, p < .001) and the mean absorption spectra of these three carotenoids (ρ = -0.668, p = .007). No association with the absorption spectra of melanin was seen for overall (ρ = 0.435, p =.105) or facial skin (ρ = 0.138, p = .623).

7.3.3 Conclusion

The results of Experiment i reveal that fruit and vegetable consumption changes over a six-week period are sufficient to confer measurable skin colour changes over this interval. No effect of initial diet was seen on observed skin colour changes. Diet-linked skin reflectance changes were associated with the absorption spectra of common carotenoids, but not that of melanin and this pattern of results was similar once controlling for initial diet. Experiment ii investigates the level of skin colour change required to perceptibly improve apparent healthiness and attractiveness.

7.4. Experiment ii: Determining Perceptual Thresholds

7.4.1. Methods

7.4.1.1. Participants.

Twenty-four undergraduate students (19 females, 5 males, mean age = 18.88, age range: 18-22) at the University of St Andrews participated in an investigation of the effect of skin colour changes on the perception of health and attractiveness.

7.4.1.2. Procedure.

Psychophysical methods were used to determine the minimum colour change associated with fruit and vegetable consumption necessary to change viewers' perception of facial skin colour, health and attractiveness.

Images of four Caucasian individuals (2 females, 2 males, age range: 19-21) were captured and calibrated as in Chapter 5. Two face-shaped uniform colour masks were created in Matlab (Chapter 5; Stephen, Law-Smith, et al., 2009) to represent the average skin colour of 15 high and 15 low Caucasian fruit and vegetable consumers, according to the empirically derived values obtained in Chapter 6. The two groups were equivalent in terms of gender (5 males, 10 females in each group), age [low = 20.75 ± 1.67 (mean ± *SD*), high = 20.99 ± 2.29], hours of vigorous exercise per week (high = 0.87 ± 0.52, low = 0.80 ± 0.94) and body mass index (high = 22.0 ± 2.65, low = 22.55 ± 3.43, Mann-Whitney *U* test, all $p \geq .475$). Individuals were without self-tanning agents, make-up and recent intensive sun exposure. In terms of per-portion difference, the colour-values between the two groups are very similar to those obtained for diet change in Experiment i of this chapter.

The skin colour (including lips and ears, excluding eyes, hair and background) of calibrated facial photographs were then manipulated according to the colour difference between the two endpoint masks (Chapter 5; Burt & Perrett, 1995) in order to obtain a set of 22 images for each face, the middle of which was the original un-manipulated face. The masks applied were Gaussian blurred at the edges of the face (*SD* ± 3 pixels), to prevent final images having an obvious colour border. This continuum represented a total range of +/- 1.00 L* units, +/- 1.14 a* units and +/-2.12 b* units, equivalent to a change of +/- 5.55 fruit and vegetable portions per day. Each of these continua reflected the colour changes between high and low fruit and vegetable consumers, and within these, skin lightness, redness and yellowness changed simultaneously.

These continua were then used in a two-alternative forced-choice psychophysics computer program (Re, Whitehead, Xiao, & Perrett, 2011). Face images were presented on a Sony GDM-F500R cathode ray tube monitor, colour-calibrated to a Δu'v' less than 0.5 using Spyder S2 Pro software and hardware (Pantone, Inc.). Sessions were conducted within a darkened booth, to minimise the effects of light falling on the monitor. The program sequentially showed two versions of the same face, each displayed for 750ms, with a random dot mask presented for 100ms in-between. Participants were asked in three separate tasks to choose the face that appeared more yellow, healthier or more attractive. Each of these tasks was tested in a separate block in random order. A staircase design (Re et al., 2011) was used. Participants were initially shown two versions of the same face at the extreme ends of the fruit and vegetable colour spectrum. The colour difference between sequentially displayed faces was halved if the participant chose the face associated with greater fruit and vegetable consumption. A ‘reversal’ occurred when a participant chose a face colour associated with reduced fruit and vegetable consumption, after which the program doubled the colour difference of the previous trial. A further ‘reversal’ occurred once participants chose the face associated with greater fruit and vegetable consumption, again halving the colour difference of the previous trial. Discrimination thresholds were defined as the average colour difference after three reversals.

7.4.2. Results

Discrimination thresholds for each of the four faces were examined across all three blocks (skin yellowness, health, and attractiveness). Repeated-measures ANOVAs found no differences in thresholds across the four faces in the tasks for skin yellowness (F_3 = 0.34, p = .80), health (F_3 = 1.43, p = .24), or attractiveness judgements (F_3 = 2.10, p = .11). We therefore collapsed thresholds across the four face stimuli when analysing thresholds across tasks. When asked to determine the yellower face, the average discrimination threshold was ΔE 0.89 ± 0.08 (± *SEM*), equivalent to a between-subjects change of 1.89 ± 0.17 fruit and vegetable portions. For healthiness and attractiveness, the average thresholds were ΔE 1.37 ± 0.15 and ΔE 1.55± 0.15, equivalent to changes of 2.91 ± 0.31 and 3.30 ± 0.31 portions per day, respectively.

The yellowness discrimination threshold was significantly lower than the health (t_{23} = -3.44, p = .002) and attractiveness discrimination thresholds (t_{23} = -3.67, p = .001) which were not different (t_{23} = -0.76, p = .45).

7.4.3. Conclusion

The results of Experiment ii showed that the skin colour change associated with fruit and vegetable consumption is seen as healthy and attractive, and is detectable at a relatively modest level of dietary change.

7.5. General Discussion

We find here that self-reported changes in diet significantly correlate with objectively measured changes in skin colour. In addition to a positive correlation between fruit and vegetable intake changes and skin yellowness changes, we find that when all measured skin areas are combined, an increase in fruit and vegetable consumption correlates with an increase in skin redness. Such colouration is held to contribute beneficially to the appearance of health in human faces (Stephen, Coetzee, Law-Smith, & Perrett, 2009; Stephen, Law-Smith, et al., 2009) as is the case with skin yellowness (Stephen et al., 2011; Stephen, Law-Smith, et al., 2009).

The spectral reflectance analysis conducted here indicates that the observed diet-linked changes in skin colour are attributable to the impact of carotenoids, as the relationship between diet change and skin reflectance change is strongest at wavelengths associated with light absorption by these pigments. The observed relationship between diet change and skin yellowness change is not attributable to sun exposure as we found that the diet-linked changes in skin reflectance were not associated with the absorption spectra of melanin (Sarna & Swartz, 1988).

We were unable to investigate the potential influence of melanin-based constitutive pigmentation on the relationship between diet change and skin colour change as our sample was almost exclusively Caucasian and thus was limited in initial skin lightness variation. We expect our reported perceptual effects will hold in a more heterogeneous sample across the spectrum of human skin pigmentation because perceptual studies indicate that yellow (carotenoid) skin colouration is perceived as healthy cross-culturally (Chapter 5; Stephen et al., 2011). The physiological effect is also expected to exist across ethnicities, but it may be the case that the magnitude of diet change required to achieve perceptible skin colour change is contingent upon initial skin lightness (i.e. the change may be less evident in dark skin). Therefore it is important to establish with further research the nature of dietary effects in non-Caucasians.

The observed relationship between overall skin redness and diet changes may reflect several processes that are not mutually exclusive. The skin's redness may be affected by fruit and vegetable consumption due to the influence of pigments such as lycopene, a red carotenoid that imparts colouration to fruits and vegetables, for example tomatoes and red peppers. This carotenoid is common in human diet (Johnson-Down, Saudny-Unterberger, & Gray-Donald, 2002) and skin (Hata et al., 2000) and is likely to contribute to skin redness via the same mechanism by which other carotenoids such as β-carotene impact skin yellowness. In support of this view, we find that the relationship between diet changes and skin reflectance changes shows the strongest association with the absorption spectra of lycopene (Figure 7.2).

The relationship between diet and skin redness changes also potentially reflects the influence of fruit and vegetable consumption on the skin's blood perfusion. Polyphenols, contained in abundance in fruit and vegetables may contribute beneficially to artery elasticity and endothelium health (Ghosh & Scheepens, 2009), properties that may positively affect blood perfusion in the skin (Cesarone et al., 2008).

In the present study we found no effect of initial diet on the observed relationship between diet change and skin colour change. It is important for further work to establish the generality of these results. We might expect that individuals starting with an extremely high fruit and vegetable consumption will exhibit weaker skin colour change than those starting with a lower fruit and vegetable consumption as there may be a point at which the skin becomes saturated with dietary carotenoids. We could not find any evidence for this hypothesis within our sample but further work with a more heterogeneous sample is necessary. As population estimates of fruit and vegetable consumption (Guenther, Dodd, Reedy, & Krebs-Smith, 2006; NHS, 2007) are comparable to the range of our sample's initial self-reported intake, we hold that our results are applicable to the majority of individuals.

Studies utilizing Raman spectroscopy reveal that skin carotenoid concentrations can vary over a relatively short timescale. For example, Meinke et al (2010) revealed that a carotenoid-rich dietary supplement significantly increased palm and forehead skin carotenoid concentrations within 14 days, with further accumulation when supplementation was continued for a total of 28 days. Darvin et al (2008) showed carotenoid concentrations to fluctuate in palmar skin over a one to three-day period. We have found that diet and skin colour changes were only significantly correlated over a six-week interval, and not over a three week period. It may be the case that carotenoid-based changes in outwardly visible skin colour occur only via the longer-term accumulation of carotenoid pigments in the most superficial skin layers. Diet-linked decreases in skin colour may also be relatively slow, and linked to the loss of superficial skin layers via abrasion, a process that may take up to three weeks (Cowen, Imhof, & Xiao, 2001). Further, subcutaneous adipose tissue accumulates lipophilic carotenoids (Parker, 1988) and may act as a buffer, releasing these pigments to blood and subsequently the dermis when skin concentrations reduce. Hence skin carotenoid levels may diminish only once adipose tissue is depleted of carotenoids. The mechanism responsible for more rapid fluctuation in skin carotenoid levels (diffusion of carotenoids from blood to the dermis and epidermis, Darvin et al., 2008) may contribute only weakly to skin colour changes visible to an observer.

The psychophysical results in this study suggest that diet-linked skin colouration is perceptibly healthier and more attractive at ΔE of 1.37 and 1.55, respectively. This is in line with the results of Re et al.'s study (2011) which recently investigated the level of oxygenated blood-colour change associated with perceptible benefits in skin colour, finding that average colour differences of ΔE 1.44 and 1.38 are required between faces for one to be reliably deemed more healthy and attractive, respectively. The regression analyses in the current study reveal that an additional fruit or vegetable portion per day results in a colour change of ΔE 0.47 over all skin and 0.46 for facial skin over a six-week period. Taken together, these results suggest that perceptibly healthier and more attractive skin colouration is achievable through relatively modest increases in fruit and vegetable consumption (of fewer than four portions per day). Lower thresholds may be found using alternate methodology. For example, reducing the interstimulus interval (100ms) and removing the visual mask may decrease any attenuating effect caused by short-term memory. Thus, the thresholds reported here are conservative, and it is possible that even smaller dietary changes are able to produce perceptible benefits to skin colouration. It must be noted, however, that our psychophysical study utilised a sample with a mean age of 18.9. It is possible that older participants will be less sensitive to skin colour changes as colour acuity is reported to decline with age (Kinnear & Sahraie, 2002).

The current study provides further evidence that fruit and vegetable consumption affects skin colour. Diet-linked skin colour changes occurred over a relatively short time period and were attainable through relatively modest dietary changes. These conditions suggest potential utility as a dietary intervention tool. In order to verify whether wide-scale public health benefits could be reaped, it is important for further research to demonstrate that the effects extend to non-Caucasian individuals and to populations with a greater range in initial diet.

8. Perceptual Effects of Melanin versus Carotenoid Pigmentation on Caucasian Faces

This chapter is largely based on the following work accepted for publication in a peer reviewed journal (accepted July 13, 2012):

Whitehead, R., Ozakinci, G. & Perrett, D. I. (In press). Attractive Skin Colouration: Harnessing Sexual Selection to Improve Diet and Health, *Evolutionary Psychology*.

8.1. Summary

Most of the perceptual work in this growing field has investigated the perceptual impact of skin yellowness (CIE b*) and pigmentation associated with β-carotene supplementation. Fruit and vegetable consumption, though, confers wider health benefits and is likely to confer qualitatively different colouration. It is therefore important to evaluate the perceptual impact of fruit and vegetable consumption *relative* to single carotenoid colour transforms. Further it is important to establish whether this dietary effect is perceived more favourably than melanin pigmentation, as β-carotene pigmentation is known to. We find here that skin pigmentation associated with dietary intake of fruit and vegetables (and the range of phytochemicals therein) confers added benefits to those associated with a single carotenoid (β-carotene), and that fruit and vegetable consumption benefits apparent health to a greater extent than melanin pigmentation upon Caucasian faces.

8.2. Introduction

There are significant gaps in the evidence that would accompany a public health intervention such as that outlined in Chapter 4. It is important that participants are motivated to increase their fruit and vegetable consumption, rather than opt for a course of β-carotene supplementation alone, as fruit and vegetable consumption confers health benefits beyond those associated with dietary supplementation. This is briefly discussed here and more fully in Chapter 1. There exist important synergistic relationships between antioxidants (Shixian et al., 2005), therefore high-dose supplementation of a circumscribed subset of phytochemicals is unlikely to be beneficial. Fruit and vegetables are rich in other beneficial non-antioxidant phytochemicals (de la Rosa, Alvarez-Parrilla, & González-Aguilar, 2010) and supplements ingested in tablet or capsule form are unable to provide sufficient levels of dietary fibre. People also tend to eat a relatively consistent weight of food each day (WHO, 2005), thus a diet that includes a greater proportion of fruit and vegetables will reduce the consumption of foods high in energy and saturated fats which are associated with disorders such as cardiovascular diseases and hypertension (Chapter 1; R. K. Johnson et al., 2009).

Carotenoids have diverse absorption spectra and will therefore affect skin colour in subtly different ways. The research reviewed in Chapter 4 suggests that a range of the 600-plus carotenoid pigments could confer benefits to the observer via sexual selection. As such, we hypothesise that skin colouration associated with fruit and vegetable consumption will confer added benefits to apparent health over and above those from β-carotene pigmentation alone. We investigate this hypothesis in the study below using a perceptual paradigm in which participants are able to simultaneously manipulate skin colour along separate β-carotene and fruit and vegetable consumption skin colour axes.

The perception of colouration induced by fruit and vegetable consumption has yet to be compared with melanin pigmentation. Many Caucasian individuals actively seek melanisation via UV light exposure with the aim of improving appearance (J. L. Jones & Leary, 1994). An appearance-based dietary intervention may be additionally useful in reducing the prevalence of this dominant cause of skin cancer (Armstrong & Kricker, 2001). Individuals may be motivated to seek alternate, dietary means of improving their skin appearance if dietary impacts on skin colour are perceived as healthier than the impact of melanisation. We also investigate this possibility in the perceptual study below by enabling participants to simultaneously manipulate skin colour along separate melanin and fruit and vegetable consumption skin colour axes in order to optimise the appearance of healthiness.

8.3. Methods

All procedures were subject to ethical approval from the University of St Andrews Teaching and Research Ethics Committee.

We used existing spectrophotometer measurements to define the impact of β-carotene, melanin and fruit and vegetable consumption on skin colour (in CIE L*a*b* colour space, where L* reflects degrees of lightness and positive values of a* and b* reflect degrees of redness and yellowness, respectively). The impact of β-carotene on skin colouration was determined by amplifying the within-subject colour change arising after a course of β-carotene supplementation (Table 8.1; Stephen et al., 2011). Melanin pigmentation was determined by measuring the difference between a sun-exposed outer arm skin region and an occluded shoulder region (Table

8.1; Stephen et al., 2011). The impact of fruit and vegetable consumption on skin colour was determined by measuring mean change in facial skin colour per portion change in fruit and vegetable consumption (Table 8.1).

Table 8.1. Maximum and minimum spectrophotometer-derived transformation values along each empirically-derived axis in CIE L*a*b* colour space.

		Δ*L	***Δ*a***	***Δ*b***	***ΔE***
β-carotene	+	1.3	4.3	10.5	11.42
	-	-1.3	-4.3	-10.5	
Melanin	+	-5.2	-1.21	7.1	8.9
	-	5.2	1.21	-7.1	
Fruit and Vegetable consumption	+	-2.06	2.58	7.98	8.64
	-	2.06	-2.58	-7.98	

Note. L* reflects degrees of lightness (0-100) and positive values of a* and b* reflect degrees of redness and yellowness, respectively (0-60). Each pigment continuum comprised 13 images, representing equal steps between these L*a*b* maxima and minima. The centre image of each continuum represented no change from the original photograph. *ΔE* is a standard way of representing colour differences in CIE L*a*b* space and here represents the maximum and minimum colour change applied to original photographs. β-carotene transform (extrapolated from Stephen et al., 2011) represents ±150mg β-carotene supplementation per day. Fruit and vegetable consumption colour-value ranges represent increased and decreased consumption of +20 and -20 portions per day over a six week period, respectively.

Colour-calibrated facial photographs were taken of 16 Caucasian participants (9 female, 7 male, mean age = 20.31, see Chapter 5 for methods). The skin portions of these images were transformed according to the empirically-derived pigment colour ranges in Table 8.1 (see Chapter 5 for methods). Initially, faces were transformed according to the impact that fruit and vegetable consumption has on skin colour. A continuum of 13 images was created for each of the 16 identities. The skin colour of face images were transformed in 13 equal steps across the continuum and lightness, redness and yellowness were manipulated simultaneously. Each continuum was organised such that the endpoints represented the maximal colour changes and the centre image of each set represented no colour change from the original photograph.

Per identity, each of the resulting 13 images in the fruit and vegetable skin colour continuum were then similarly transformed along the β-carotene and separately the melanin colour axes. This resulted in two 13x13 matrices of 169 images for each of the 16 identities. One matrix contained independent skin colour manipulations associated with fruit and vegetable consumption and β-carotene pigmentation (Figure 8.1A). A second matrix contained independent skin colour manipulations associated with fruit and vegetable consumption and melanin pigmentation (Figure 8.1B).

A computer program controlled matrix display. At any one time, only one face image was visible. Observers selected, via horizontal movements of a mouse cursor, the position along one pigment axis. Simultaneously, vertical movements of the cursor allowed independent selection along a second pigment axis. Direction of movement, axis centre location and axis arrangement were randomised to prevent learning effects. All 32 matrices were sequentially presented to a separate group of 62 Caucasian observers (46 female, 16 male, mean age = 20.11) asked to "Make the face look as healthy as possible". Observers viewed stimuli on a colour-calibrated monitor in a darkened booth. The computer program stored the chosen position on each of the pigment axes and advanced to the next matrix, selected at random.

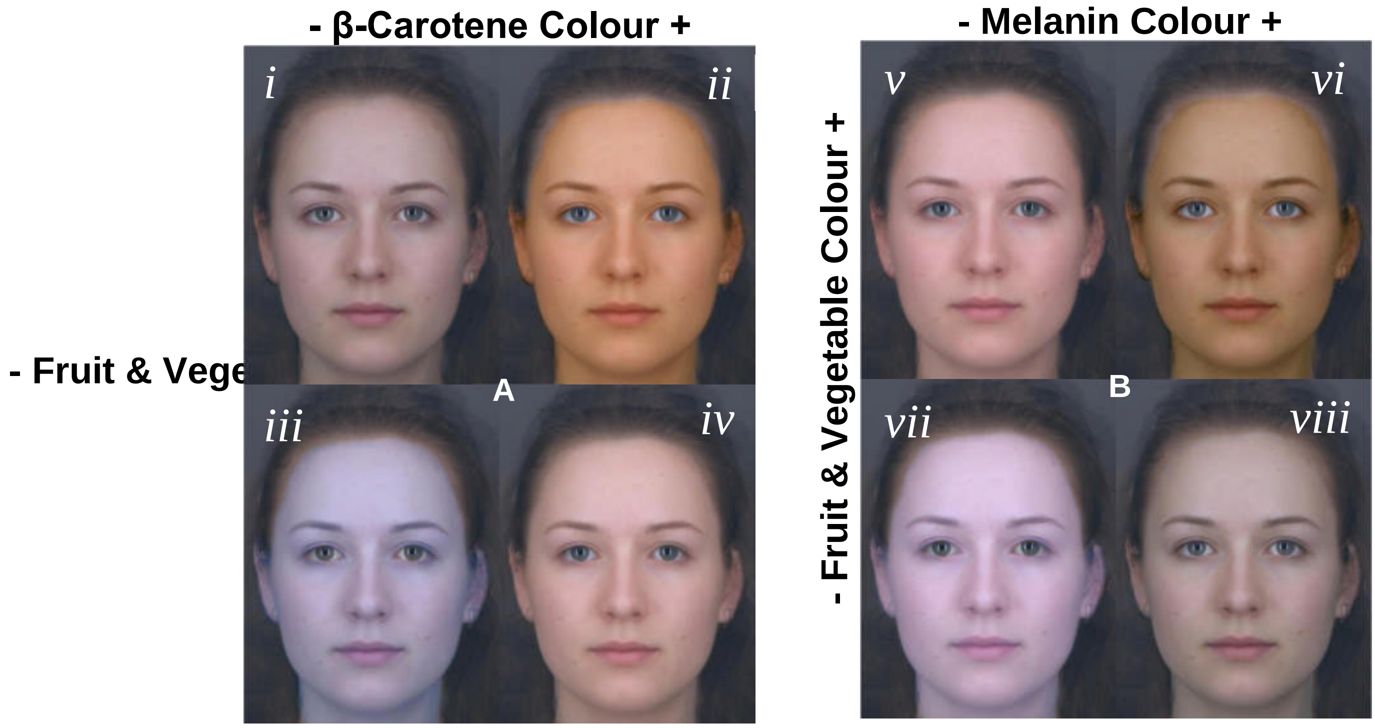

Figure 8.1. (A) Maximum and minimum colouration applied to faces along a β-carotene skin colour axis and fruit and vegetable skin colour axis. Images *i* and *iii* reflect low β-carotene colouration, images *ii* and *iv* reflect high β-carotene colouration. Images *i* and *ii* additively reflect high fruit and vegetable colouration, images *iii* and *iv* reflect low fruit and vegetable colouration. (B) Maximum and minimum colouration applied to faces along a melanin skin colour axis and fruit and vegetable skin colour axis. Images *v* and *vii* reflect low melanin colouration, images *vi* and *viii* reflect high melanin colouration. Images *v* and *vi* additively reflect high fruit and vegetable colouration, images *vii* and *viii* reflect low fruit and vegetable colouration. See Table 8.1 for pigment colour values in CIE L*a*b* colour space.

8.4. Results

8.4.1. Fruit and vegetable induced skin colouration and β-carotene pigmentation

When manipulating facial skin colouration associated with fruit and vegetable consumption alongside a β-carotene colour axis, Caucasian observers significantly increased levels of pigmentation along both continua (fruit and vegetable colouration: t_{15} = 11.45, p < .001, approximately associated with an increased consumption of 4.6 portions per day, β-carotene: t_{15}= 13.17, p < .001) to optimise apparent health across the 16 Caucasian faces (Figure 8.2A). All faces on average benefited from increased colouration along both colour axes. Comparing the two continua, observers added significantly greater colour change with the β-carotene colour axis (Figure 8.2A; $t_{15} = 4.36$, $p = .001$). Manipulation of faces along both of these pigment axes conferred an average additive skin colour change of 4.6 ΔE (-0.2 L*, +1.6 a* and +4.3 b* units change applied to the original images).

8.4.2. Fruit and vegetable induced skin colouration and melanin pigmentation

When observers were able to manipulate the level of melanin skin colouration alongside the fruit and vegetable skin consumption colour axis, we again saw significant increases in pigmentation along both continua (melanin: $t_{15} = 6.31$, $p < .001$, fruit and vegetable colouration: $t_{15} = 17.44$, $p < .001$, approximately associated with an increased consumption of 7.2 portions per day, Figure 8.2B). All faces benefited from increases in skin colouration associated with fruit and vegetable consumption, and 15 of 16 benefited from increased melanin colouration. In order to optimise apparent health, observers added significantly less melanin colouration than fruit and vegetable skin colouration (Figure 8.2B; $t_{15} = 8.23$, $p < .001$). Manipulation of images along both continua conferred an average additive skin colour change of 4.2 ΔE (-1.5 L*, +0.8 a* and +3.9 b* units change applied to the original images).

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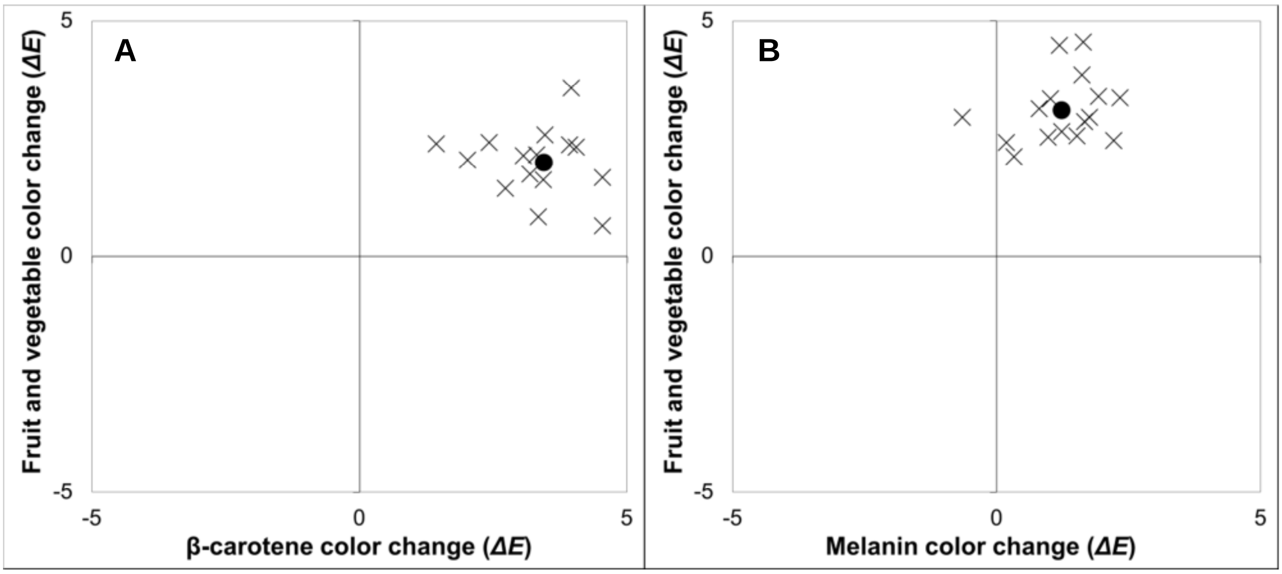

Figure 8.2. (A) Colour change applied (*ΔE*, a standard way of representing differences in CIE L*a*b* colour space) along a β-carotene skin colour axis and a fruit and vegetable skin colour axis to original face images to optimise apparent health. (B) Colour change applied along melanin skin colour axis and a fruit and vegetable skin colour axis. Crosses represent individual identities and filled circles represent mean chosen position across identities on both axes.

8.5. Discussion

In line with previous research (Stephen et al., 2011; Stephen, Law-Smith, et al., 2009; R. D. Whitehead, Re, Xiao, Ozakinci, & Perrett, 2012), this study demonstrates that the skin colouration associated with β-carotene supplementation and increased dietary consumption of fruit and vegetables is perceived as healthy in Caucasian faces. β-carotene colouration was shifted significantly more than diet-linked pigmentation to enhance the appearance of health but despite participants being able to choose higher levels of β-carotene pigmentation, they chose instead to simultaneously increase colouration associated with increased fruit and vegetable consumption. These results suggest that observers target an optimum ratio of lightness, redness and yellowness which is not satisfied by increasing β-carotene colouration alone. As β-carotene is one of the most abundant dietary carotenoids (Ben-Amotz & Fishler, 1998), we propose that recommending a rich fruit and vegetable intake will convey the skin colour benefits associated with β-carotene and a host of additional carotenoids, as well as the health benefits linked with dietary improvement. Further, the level of β-carotene supplementation required to achieve the skin colour changes applied here (Table 8.1) are likely to be harmful (Tanvetyanon & Bepler, 2008), whereas the dietary change necessary to induce optimum skin colouration is in line with guidelines for recommended intake (WHO, 1990).

Previous research finds that β-carotene pigmentation is perceived as healthier than melanisation of skin (Stephen et al., 2011). Our research suggests that this generalises to dietary consumption of carotenoids, as we find that fruit and vegetable consumption influences apparent health to a greater extent than melanin pigmentation.

The study conducted here further suggests that human perception of diet-linked changes in skin colour could be used to shape a dietary intervention strategy. As elsewhere in the animal kingdom, human skin colouration reflects dietary consumption of carotenoids in a manner that improves apparent condition. Future appearance-based interventions can advise increased consumption of a wide variety of fruit and vegetables, both because this strategy ensures the maximum health benefit and because such dietary improvement has additional benefits to skin appearance. Diet-linked skin colouration also has a larger impact on apparent health than melanisation. We therefore also conclude that advertising the results of the present study could be persuasive in motivating Caucasian individuals to eschew the dangers of excess UV exposure in favour of improving diet, which we show to be a more effective way of improving appearance amongst these individuals.

Further work is required to investigate the relative perceptual influence of skin pigmentation induced by dietary and melanin pigments upon skin that is initially more heavily melanised than Caucasian skin. Stephen et al (2011) find that African and Caucasian observers increase the lightness and yellowness of black South African faces. This suggests that carotenoid pigmentation is preferred to melanisation, which yellows but also darkens skin (Stamatas, 2004). These findings may also be partly due to the nature of the (approximately) spherical colour space in which faces are manipulated and perceived (CIE L*a*b*; Commission Internationale de l'Eclairage, 1976). When skin lightness (L*) is manipulated towards either pole of this sphere, the possible saturation on the green-red and blue-yellow axes (a* and b* respectively) is reduced (Figure 8.3). Maximal colour saturation is thus only possible with an intermediate degree of lightness. Hence when allowed to do so, observers may manipulate skin melanisation only in order to optimise lightness, allowing maximum visibility of redness and yellowness as cues of health (Stephen et al., 2011; Stephen, Law-Smith, et al., 2009). This suggests that melanin pigmentation *per-se* plays a less important active role health perception and may explain preferences for tanned skin amongst Caucasians (Jorgensen et al., 2000; Robinson et al., 1997) and skin-lightening agents amongst darker-skinned individuals (Olumide et al., 2008). Such preferences though, are also confounded by cultural influences. For instance tanned Caucasian skin may be a cue to one’s ability to afford a vacation and outdoor leisure time. Further work is required to determine the role of skin colour in relation to skin lightness across different cultures. Such work could confirm the interplay between low-level visual perception of colour saturation and preferences for skin redness and yellowness.

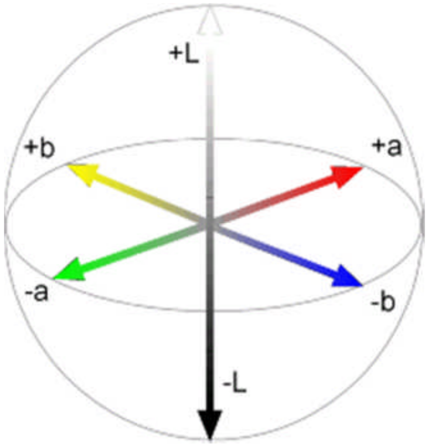

Figure 8.3. CIE L*a*b* colour space schematic. Lightness (L*) is represented on the vertical axis (0-100). Green-red and blue-yellow axes (a* and b* respectively) run horizontally (-60 to +10). Maximal saturation on the a* and b* axes is only possible around the equator of this colour sphere (where L* = 50).

9. A Randomised Controlled Trial of an Appearance-Based Dietary Intervention

This chapter is partially based on the following work invited for resubmission in a peer reviewed journal (submitted September 4, 2012):

Whitehead, R., Ozakinci, G. & Perrett, D. I. (Under review). Brief report: A randomised controlled trial of an appearance-based dietary intervention, *Health Psychology*.

Trial Registration: www.clinicaltrials.gov, trial ID: NCT01511484

9.1. Summary

Although encouraging, the previous trial of an appearance-based dietary intervention (Chapter 6) requires further investigation. Because of the way in which participants were recruited to the study, it was not possible to achieve strict randomisation to intervention conditions, potentially clouding the findings from this study. The trial reported here eliminates this potential source of bias by exclusively recruiting naïve participants and randomly assigning participants to intervention conditions. The initial trial reported in Chapter 6 also indicated that demonstration of the consequences of behaviour for participants' *own* appearance was an effective intervention strategy, it remains to be seen whether non-personalised materials are similarly effective. The previous chapters also have important physiological (Chapter 7) and perceptual (Chapters 7 & 8) findings which, when explicitly advertised to participants, may further improve intervention efficacy. Here we present a randomised controlled trial in which participants received either an information-only intervention, or a 'generic' or 'personalised' appearance-based intervention. This trial again yielded promising results; controlling for baseline diet, a significant effect of intervention group was found. The 'personalised' appearance-based intervention group exhibited significant, sustained improvement in fruit and vegetable consumption.

9.2. Introduction

Current appearance-based interventions typically use manipulated images of the participant’s own face to demonstrate the consequences of behaviour for appearance (Chapter 3). Personalising intervention materials in this manner may serve to enhance the salience, novelty and perceived relevance of the intervention administered, increasing the likelihood of active contemplation of the intended health message (Petty & Cacioppo, 1981). Personalisation is also likely to confer lasting behaviour change as increased contemplation can facilitate retention in long-term memory (T. D. Cook & Flay, 1978; Petty, 1977). Chapter 6 indeed found a significant effect of intervention group, such that a personalised appearance-based intervention conferred the most beneficial dietary changes. Whilst these are desirable qualities from a public health perspective, it is also important to develop a strategy that is as cost-effective and parsimonious as possible. The infrastructure and effort required to realise a ‘personalised’ appearance intervention strategy may be redundant if similar demonstrations in generic (i.e., non-personalised) facial stimuli are also effective in advertising important health messages. The use of non-personalised imagery can potentially be distributed via the visual media at a very low cost per participant.

Recent empirical evidence finds that small improvements in fruit and vegetable consumption are sufficient to confer perceptible benefits to skin colour over a six-week period (Chapter 7). By making it clear that relatively modest dietary changes are sufficient to benefit appearance, participants’ self-efficacy is likely to be increased as the required change in lifestyle is made less daunting. Belief in one’s competence to perform a particular behaviour is a critical determinant of whether behaviour change is achieved (Bandura, 1977), hence we expect that advertising this result to participants as part of an appearance-based intervention may further improve intervention effectiveness.

Further, the dietary problems associated with an innate focus on the present (Frederick et al., 2002) are likely to be circumvented by advertising benefits which are known to be achieved more rapidly than distal health benefits (Chapter 4). We imagine that an appearance-based dietary intervention will be optimally effective if participants are explicitly informed of the rapidity of the potential benefits for appearance.

Chapter 8 also finds that diet-linked changes in skin colour are perceived as healthier and more attractive than melanisation, which itself offers strong motivation to participate in hazardous sun-exposure behaviours (Jorgensen et al., 2000; Robinson et al., 1997). Highlighting that fruit and vegetable consumption represents a less harmful opportunity to improve appearance may be a valuable strategy for improving diet.

In the present study, we evaluated the impact of two appearance-based dietary interventions, one of which demonstrates the impact of increased and decreased fruit and vegetable consumption upon images of the participant’s own face, the other demonstrating the same skin colour effects upon generic young adult faces. These interventions were both evaluated over a ten-week period relative to an information-provision-only intervention, which is in line with current NHS advice (NHS, 2011b).

We encouraged all participants to monitor their fruit and vegetable consumption by completing a weekly diet questionnaire for the duration of the experiment. Based on literature which highlights the benefits of self-monitoring (Michie et al., 2009), we expect to observe increases in fruit and vegetable consumption regardless of intervention group. We expect that self-reported fruit and vegetable consumption will further increase when individuals receive an appearance-based intervention and that personalisation of this intervention will improve outcome yet further. We hypothesise that individuals receiving an information-only intervention will exhibit comparatively little dietary improvement.

9.3. Methods

All procedures obtained ethical approval from the University of St Andrews Teaching and Research Ethics Committee, and prior informed written consent was obtained from all participants. All individuals were reimbursed financially for their participation at the rate of £5 per hour.

9.3.1. Participants

Seventy-three students or employees at the University of St Andrews (49 females, 24 males, mean age = 23.01, age range: 18-61, 1.4% African, 72.6% Caucasian, 17.8% East Asian, 1.4% West Asian, 6.8% mixed ethnicity) participated between February and June 2011. Sample size was determined by sign-ups to one-hour time-slots made available over this period during University term-time. Participants were recruited via advertisement, which described the study as an investigation of diet and health, and were randomly allocated (via sequential look up of a computer-generated random number table) to one of three conditions individually (information-only n = 25; information plus generic appearance-based intervention n = 23 (henceforth “generic appearance intervention”); or information plus personalised appearance-based intervention $n = 25$ (henceforth "own-face appearance intervention"). Neither the experimenter nor participants could be blinded to study condition assignment, although participants ostensibly remained unaware of the two intervention groups that they were not assigned to until debriefing. One experimenter (RW) generated the random number table and enrolled and assigned participants.

At baseline, these three groups were equivalent in terms of fruit and vegetable consumption, age, gender and body mass index (Kruskal-Wallis H test, all $p \geq .356$). Participants reported consuming an average of 4.97 (*SEM* ± 0.38) fruit and vegetable portions per day at baseline.

9.3.2. Fruit and vegetable information

Selected pages from NHS information booklets "*5 A Day, Just Eat More (fruit & veg)*" (pages i, ii, 12-15, 20 & 21) and "*5 A Day, Just Eat More (fruit & veg): What's it all about?*" (pages i-ii) were provided to all participants on completion of baseline questionnaires. The pages (NHS, 2004a, 2004b) provided information on recommended portion sizes, meal planning, health benefits and answered frequently asked diet-related questions.

9.3.3. Generic appearance intervention

In addition to NHS information, participants in the generic appearance intervention group also received images to illustrate the impact of fruit and vegetable consumption on skin appearance. Participants in this group were presented with gender-congruent stimuli, which were constructed by averaging the facial shape and colour of four male or female identities (Figure 9.1). Both resulting base faces were manipulated according to an empirically-derived fruit and vegetable skin colour axis

(Table 9.1) to simulate changes in skin colour (in CIE L*a*b* space) that had been measured within-subject as a result of changes in fruit and vegetable consumption over a six-week period (Chapter 7). Two face-shaped uniform colour masks were created in Matlab (Stephen, Law-Smith, et al., 2009) according to these derived colour values. Masks applied were Gaussian blurred at the edges of the face to prevent final images having an apparent colour border. The skin colour (including lips and ears; excluding eyes, hair and background) of each base face was then individually manipulated according to the colour difference between these two endpoint masks (Chapter 5; Burt & Perrett, 1995; Stephen et al., 2011; Stephen, Law-Smith, et al., 2009) in order to obtain a continuum of 13 images for each face. The resulting image sets represented a total range between the first and thirteenth images of +/- 1.03 L* units, +/- 1.29 a* units and +/- 3.99 b* units, equivalent to a change of +/- 10 fruit and vegetable portions per day (Table 9.1). The center image of this continuum represented the colour of the original base face.

Participants viewed only the gender-congruent set of the resulting stimuli in two forms. First, after completion of baseline questionnaires, these images were displayed on a Sony GDM-F500R cathode ray tube monitor, colour calibrated to a Δu’v’ of less than 0.5 using Spyder S2 Pro software and hardware (Pantone, Inc.). Viewing sessions were conducted within a darkened booth to minimise the effects of light falling on the monitor. As in Chapter 5, a computer program was used to sequentially display the set of 13 images, enabling participants to advance through the empirically-derived fruit and vegetable colour continuum with horizontal movements of a mouse cursor. The middle position of the sequence was randomly horizontally offset in order to prevent participants easily finding the central or extreme stimuli. Participants were instructed to select what they perceived as the healthiest face colour, which was recorded by the computer program over two trials. The experimenter (RW) then explained that the illustrated changes in skin colour are linked to changes in fruit and vegetable consumption and perceived health (Chapters 5 & 7; Stephen et al., 2011). Participants were given an approximate quantification (see Table 9.1) of the difference in fruit and vegetable consumption between the central baseline and the skin colour they found healthiest.

Table 9.1. Empirically-derived fruit and vegetable skin colour axis.

Step number	**ΔL***	**Δa***	**Δb***	**Δ Fruit and vegetable portions/day**
1	1.03	-1.29	-3.99	-10.00
2	0.86	-1.08	-3.33	-8.33
3	0.69	-0.86	-2.66	-6.67
4	0.52	-0.65	-2.00	-5.00
5	0.34	-0.43	-1.33	-3.33
6	0.17	-0.22	-0.67	-1.67
7	0.00	0.00	0.00	0.00
8	-0.17	0.22	0.67	1.67
9	-0.34	0.43	1.33	3.33
10	-0.52	0.65	2.00	5.00
11	-0.69	0.86	2.66	6.67
12	-0.86	1.08	3.33	8.33
13	-1.03	1.29	3.99	10.00

Note. Across the range of 13 images created, L* (lightness), a* (redness) and b* (yellowness) vary simultaneously. These values were used to manipulate facial stimuli to reflect diet change as specified in the rightmost column.

Participants in this group also received a take-home photo quality leaflet (Appendix B) to further illustrate the effect of fruit and vegetable consumption on skin colour (Figure 9.1). The leaflet presented the skin colour changes associated with eating approximately 6.7 more, or 6.7 fewer fruit and vegetable portions per day, relative to the central starting image (Figure 9.1). Printed images were colour-calibrated using a 24-patch GretagMacbeth chart to an average *ΔE* of 3.55. *ΔE* here represents the Euclidean distance between the calibrated image and known ColourChecker patch values. This leaflet also informed participants that the illustrated skin colour changes occur within six weeks following modest dietary changes. Participants were also informed that the illustrated skin colouration improves appearance to a greater extent than melanisation.

9.3.4. Own-face appearance intervention

Participants in the personalised appearance intervention group received similar materials to the generic appearance intervention group but, for these individuals, illustrations were performed upon images of their own face. Images of individuals were taken using a Fujifilm FinePix S5Pro digital SLR camera (60mm fixed length lens) in a booth painted with achromatic matt white paint. Illumination was exclusively provided by nine diffused d65 bulbs (VeriVide, Ltd). The camera was white-balanced according to a GregtagMacbeth white balance card in these lighting conditions. Participants held a white painted board over their shoulders to occlude reflections from clothing. A GregtagMacbeth Mini ColourChecker was included in each image to colour-calibrate images. Colour-correction was performed as in Chapter 5, resulting in a final mean colour error (*ΔE*) of 2.4 (*SD* ± 0.06). Individuals in this group viewed an on-screen demonstration (as described above and in Chapter 5) in which they were able to manipulate the colour of their own skin according to the empirically-derived fruit and vegetable skin colour axis. These participants were initially asked to select what they perceived to be closest to their current skin colour on two trials, followed by two trials in which they were instructed to select what they perceived to be the healthiest skin colour in the available range.

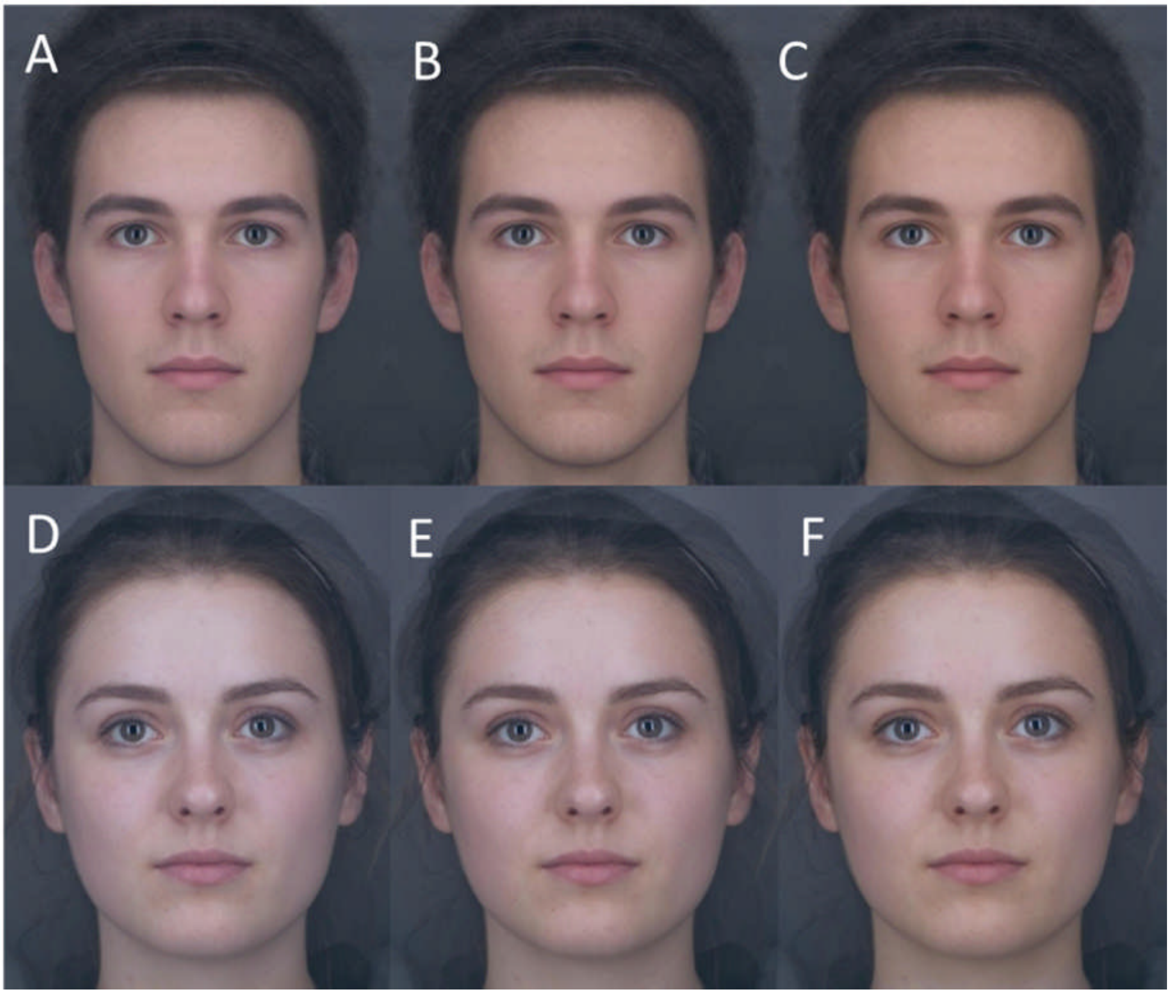

Figure 9.1. Example face stimuli provided to participants receiving the generic appearance intervention. Images reflect the average facial shape and color of four male (A-C) or female (D-F) identities, manipulated to illustrate a 6.7 portion increase (C,F) or decrease (A,D) in fruit and vegetable consumption per day relative to a hypothetical starting appearance (B,E). In the personalized appearance intervention, participants received similarly manipulated images of their own face.

Own-face intervention group participants also received a photo quality leaflet (Appendix B), which was identical to that received by the generic appearance intervention group, except the images were replaced with similarly manipulated versions of the participant's own face.

9.3.5. Procedure

Participants completed a baseline measurement session in a quiet office. Before the receipt of any intervention materials, all participants completed a computerised food frequency questionnaire (adapted from Block, Gillespie, Rosenbaum, & Jenson, 2000) to establish baseline fruit and vegetable consumption. Participants were asked to retrospectively report consumption frequency of fruit juice, fruit, vegetable juice, salad, vegetable soup and vegetable items over the past seven days. Participants reported consumption of standard portion sizes and were provided with NHS illustrations of portion size guidelines (NHS, 2003) to assist estimations. The following response categories were available for each item: “None in week”, “once in week” (coded as 0.14 portions per day), “2-3 times in week” (coded as 0.36 portions per day), “4-6 times in week” (coded as 0.71 portions per day), “1 per day”, “2 per day”, “3 per day”, “4 per day”, “5 per day” and “more than 5 per day” (coded as 6 portions per day). Participants’ responses were summed across items and used as an estimate of total fruit and vegetable consumption per day over the previous seven-day period, thus a range of 0 – 36 portions per day was able to be specified.

Body height (± 1 cm) and weight (± 0.1 kg) measurements were recorded using a wall-mounted stadiometer and Tanita SC-330 body composition analyser. To enable elimination of individuals unable to make dietary changes, participants were asked “Is there any reason, medical or otherwise, that prevents you from making changes to your diet?”

Although not used for participants in either the information-only or generic appearance intervention groups, photographs were taken of individuals in all intervention groups (as outlined above) to maintain participants' experience constant between groups. All participants were asked to cleanse their face with an alcohol-free hypoallergenic face wipe; this was done at the beginning of the participant's session, approximately 15 minutes before photographs were taken to allow any erythema caused by this process to subside.

Participants received intervention materials and demonstrations as appropriate at the end of an initial session that lasted approximately 30 minutes in total. Participants then completed up to ten-weekly online follow-up questionnaires in which they retrospectively self-reported seven-day fruit and vegetable consumption using the measure detailed above. Participants received weekly emails for the study duration to remind them to complete questionnaires. Email content was identical for all participants and contained only information pertaining to the completion of weekly questionnaires.

9.4. Results

PASW v.18 was used for main analyses. G*Power v.3.1 (Faul et al., 2009) was used for effect size calculations. Where necessary, a square-root transform was applied to successfully normalise data. Failing this, non-parametric statistical tests were used.

9.4.1. Computer-based colour manipulation

Participants in the generic appearance intervention group manipulated a gender-congruent base face stimulus along an empirically-derived fruit and vegetable skin colour axis (Table 9.1). The data reported here reflect the average of two trials per participant, in each of which the instruction was to select the healthiest skin colour. Participants in the generic appearance intervention group chose to decrease skin lightness (L*) relative to the starting base faces by 0.64 ± 0.39 units (mean ± SD), whilst increasing skin redness and yellowness by 0.80 ± 0.49 and 2.49 ± 1.51 units respectively. This colour change was significantly different from no change (Wilcoxon $Z = -3.931$, $p < .001$) and was equivalent to an increased consumption of 6.23 ± 3.79 portions of fruit and vegetables. No significant difference was seen in the degree of colour change applied by male and female participants (Wilcoxon $W = 186.5$, $p = .711$).

Participants in the own-face appearance intervention group manipulated an image of their own face along the empirically-derived fruit and vegetable skin colour axis. These participants were first asked to select what they perceived as closest to their current skin colour on two trials. Participants selected images that were on average 0.40 ± 0.44 L* units lighter, 0.50 ± 0.55 a* units less red (greener) and 1.56 ± 1.67 b* units less yellow (bluer) than their actual starting appearance. These difference were significant (Wilcoxon $Z = -3.476$, $p = .001$) and were associated with a decreased fruit and vegetable consumption of 3.9 ± 4.23 portions per day.

Participants in this group also selected what they perceived as the healthiest skin colour along the empirically-derived fruit and vegetable skin colour axis. Relative to each individual's original photograph, participants selected images that were on average 0.33 ± 0.59 L* units darker, 0.41 ± 0.74 a* units redder and 1.26 ± 2.28 b* units yellower as optimally healthy-looking. The colour change from actual starting appearance was significant ($p = .011$) and was associated with an increased fruit and vegetable consumption of 3.17 ± 5.72 portions per day.

Relative to each individual's perceived current appearance, participants selected images that were on average 0.73 ± 0.68 L* units darker, 0.91 ± 0.85 a* units redder and 2.82 ± 2.64 b* units yellower. The colour difference between perceived current appearance and perceived healthiest was significant (Wilcoxon Z = -3.661, $p < .001$) and was associated with an increased fruit and vegetable consumption of 7.07 ± 6.62 portions per day.

9.4.2. Fruit and vegetable consumption

For the purposes of evaluating intervention efficacy, we eliminated from analyses nine participants who indicated before baseline dietary measurements that they could not make changes to their diet (information-only n = 2, generic appearance intervention n = 5, own-face appearance intervention = 2; Figure 9.2). As we are concerned with investigating the long-term efficacy of any intervention effects, we excluded a further 18 participants whom did not complete the study (information-only n = 6, generic appearance intervention n = 7, own-face appearance intervention = 5, Figure 9.2). Completers and non-completers were equivalent in terms of baseline fruit and vegetable consumption, gender and BMI (all Mann-Whitney $U > 335.5$, $p > 0.23$). A marginal difference in age was seen, such that completers were marginally older than non-completers (Mann-Whitney $U = 315$, p = .069).

The remaining 46 participants (information-only n = 17, generic appearance intervention n = 11, own-face appearance intervention = 18) completed an average (± *SD*) of 10.6 (± 0.68) weekly questionnaires. The number of sessions completed by participants was marginally inconsistent across intervention group ($\chi^2 = 5.3$, $p = .071$; Table 9.2). Baseline fruit and vegetable consumption, gender, age and BMI were consistent across intervention group for these remaining participants (all $\chi^2 < 2.4$, $p > .306$; Table 9.2). This sample was 69.6% Caucasian, 19.6% East Asian and 10.9% mixed ethnicity (Table 9.2).

Table 9.2. Baseline demographic characteristics.

	Information only (*n*=17)	**Generic appearance intervention (*n*=11)**	**Own-face appearance intervention (*n*=18)**	**Overall (*n*=46)**
Fruit and vegetable consumption (Portions/day ± *SEM*)	5.07 ± 0.80	4.71 ± 0.73	3.90 ± 0.53	4.53 ± 0.40
Age (years ± *SEM*)	21.88 ± 0.74	22.64 ± 2.75	27.83 ± 3.38	24.39 ± 1.53
Gender (*n* females, *n* males)	11, 6	10, 1	13, 5	34,12
Caucasian (*n*, %)	10, 58.8	10, 90.9	12, 66.7	32, 69.6
East Asian (*n*, %)	5, 29.4	0, 0	4, 22.2	9, 19.6
Mixed ethnicity (*n*, %)	2, 11.8	1, 9.1	2, 11.1	5, 10.9
Body mass index (Kg/M^2 ± *SEM*)	22.68 ± 0.91	21.00 ± 0.71	24.17 ± 1.84	22.86 ± 0.82
Questionnaires completed overall (*n* ± *SEM*)	10.35 ± 0.21	10.91 ± 0.09	10.72 ± 0.14	10.63 ± 0.10
Questionnaires completed weeks 1-5 (*n* ± *SEM*)	4.89 ± 0.08	5.00 ± 0.00	4.83 ± 0.12	4.89 ± 0.06
Questionnaires completed weeks 6-10 (*n* ± *SEM*)	4.47 ± 0.15	4.91 ± 0.09	4.89 ± 0.08	4.74 ± 0.07

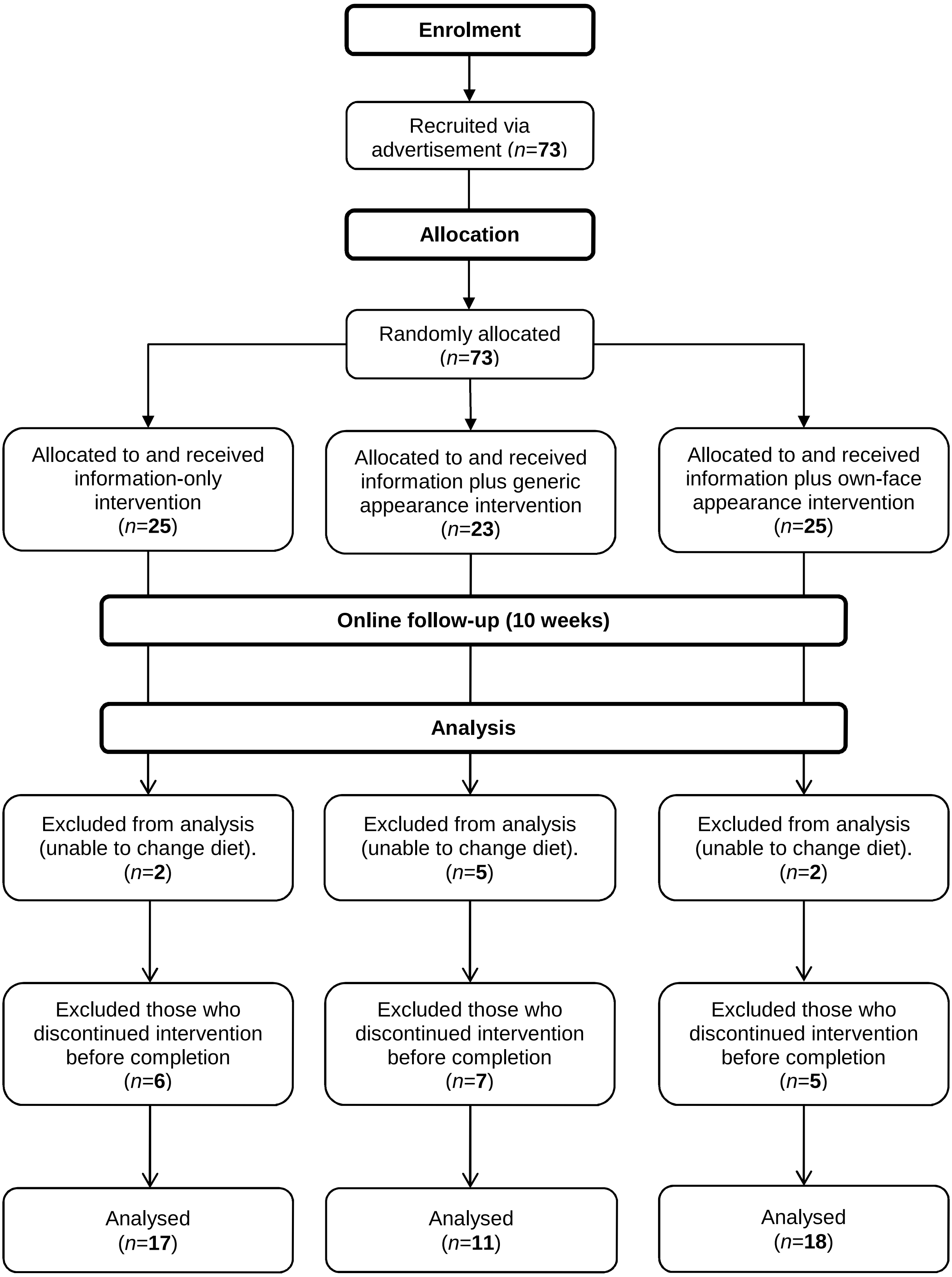

Figure 9.2. Flow diagram of participant involvement, attrition and analysis for fruit and vegetable consumption.

An intent to treat (ITT) analysis was performed on weekly fruit and vegetable consumption data. Missing data were imputed by carrying forward the last observed report, (including the participant's baseline report where necessary) until a response was received. The resulting dietary consumption data were square-root transformed to achieve a normal distribution. A repeated measures analysis of covariance (ANCOVA) was conducted on this data with weekly reports of fruit and vegetable intake as the repeating factor (ten weeks), baseline intake as a covariate and intervention group as a between-subjects factor.

A significant effect of baseline intake was found ($F_{1,42} = 126.189$, $p < .001$, $\eta_p^2 = .75$), such that baseline intake was ~~significantly~~ positively correlated with mean imputed post-baseline intake ($r = 0.84$, $p < .001$). Controlling for this relationship, a main effect of intervention group was seen on fruit and vegetable consumption over the ten-week period ($F_{2,42} = 3.774$, $p = .031$, $\eta_p^2 = .15$). No main effect of time was found ($F_{4.73,198.51} = 1.757$, $p = .127$, $\eta_p^2 = .04$) and no interaction between time and intervention group was found ($F_{9.45,198.51} = 0.538$, $p = .854$, $\eta_p^2 = .03$).

Bonferroni-corrected post-hoc pairwise comparisons revealed that across the ten-week period and controlling for baseline consumption, there was no significant difference in average fruit and vegetable intake between individuals in the generic and own-face appearance intervention groups ($p = 1.0$). No significant difference was seen between individuals in the information-only group and the generic appearance intervention group ($p = .147$), but the own-face appearance intervention group exhibited significantly greater fruit and vegetable consumption than the information-only group ($p = .042$).

Since imputation could have influenced results, we also analysed average *non-imputed* fruit and vegetable consumption across two five-week blocks. All 46 participants completed a minimum of three weekly questionnaires within each block (Table 9.2). An ANCOVA was conducted with both five-week consumption averages as the repeating factor, baseline intake as a covariate and intervention group as a between-subjects factor. Controlling for a significant relationship with baseline intake ($F_{1,42}$ = 116.364, p < .001, η_p^2 = .74), a significant main effect of intervention group was seen on overall fruit and vegetable consumption ($F_{2,42}$ = 3.458, p =.041, η_p^2 = .14; Figure 9.3). No main effect of time was seen ($F_{1,42}$ = 1.526, p =.224, η_p^2 = .04) and no interaction between time and intervention group was seen ($F_{2,42}$ = 0.088, p = .916, η_p^2 = .004).

Bonferroni-corrected post-hoc pairwise comparisons revealed that, across the ten-week period, there was no significant difference in average fruit and vegetable intake between individuals in the generic and own-face appearance intervention groups (p = 1.0). No significant difference was seen between individuals in the information-only group and the generic appearance intervention group (p = .174), but the own-face appearance intervention group exhibited marginally significantly greater fruit and vegetable consumption than the information-only group (p = .055).
Bonferroni-corrected paired-samples *t*-tests were conducted to investigate whether significant dietary changes were observed from baseline within each intervention group.

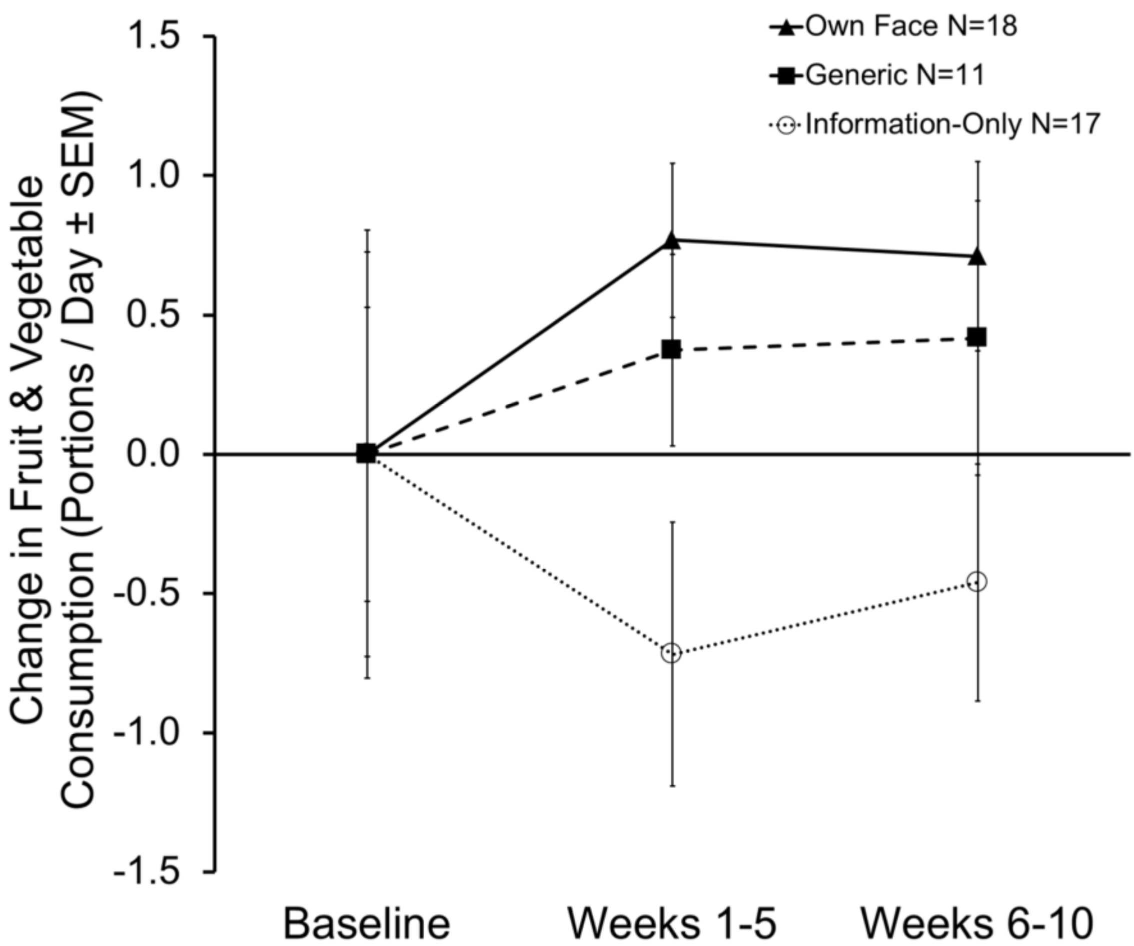

Figure 9.3. Change in fruit and vegetable consumption (non-imputed) from baseline per intervention group (± *SEM*). Error bars for baseline measurement reflect baseline consumption *SEM* per intervention group. Participants each completed at least three weekly follow up questionnaires per week-block.

The information-only group (t_{16} = 1.404, *p* = .537, *dz* = 0.34) and generic appearance intervention group (t_{10} = -0.976, *p* = 1.0, *dz* = 0.29) exhibited no significant dietary change between baseline and ten-week average non-imputed post baseline reports. The own-face appearance intervention group significantly increased their fruit and vegetable consumption (t_{17} = -2.726, *p* = .042, *dz* = 0.65).

We examined whether dietary change in the two appearance intervention groups was related to individual differences in participants' perception of the illustrated skin colour effects. Spearman correlations between diet change (between baseline and either five-week non-imputed average) and optimum colour chosen in the computer-based manipulation task were non-significant (all $\rho < 0.216$, $p > .270$). Further, in the own-face appearance intervention group we found no relation between diet change and the difference in skin colour between participants' perceived current and optimally healthy appearance (both $r < 0.393$, $p > .107$).

9.5. Discussion

The present study was the first randomised controlled trial to investigate the impact of an appearance-based dietary intervention. Our novel methodology involved brief visualisation of the impact that fruit and vegetable consumption has on skin appearance, according to an empirically-derived colour transform. We compared the efficacy of personalised versus generic stimuli. One group of individuals received illustrations upon same-sex generic faces, another witnessed identical illustrations performed upon images of their own face. Our results further suggest that an appearance-based dietary intervention may be a valuable motivational tool. Participants perceived the illustrated skin colour transform as healthy-looking (in line with previous perceptual studies; Chapters 5 & 8; Stephen et al., 2011; Stephen, Law-Smith, et al., 2009), and after viewing this transform initiated positive dietary changes, particularly when illustrations were performed upon images of the individual's own face. Those who witnessed the potential effects of improved diet to their own skin colour showed a significant elevation in their consumption of fruit and vegetables. Changes in diet produced by the intervention appeared sustained over the ten-week study period. Further, it is encouraging that positive dietary changes were seen despite this population beginning at a level close to the WHO recommendation (WHO, 1990) of five portions of fruit and vegetables per day. The results of Chapters 5 and 7 suggest that individuals consuming fewer fruit and vegetables will benefit more, in terms of appearance improvement, from increases in fruit and vegetable consumption. This intervention may, therefore, be particularly effective amongst low consumers, though further empirical work is required to confirm this hypothesis.

Although we found no difference in fruit and vegetable consumption between the generic and own-face appearance intervention groups, only those receiving personalised images exhibited significant dietary improvement within our study period and the own-face group was the only group that exhibited greater post-baseline consumption compared to the information-only group. These results concur with previous research which indicates that tailored interventions can effectively motivate changes in diet, physical activity, alcohol consumption and tobacco use (A. S. Anderson, Caswell, Wells, Steele, & MacAskill, 2010; Armitage & Conner, 2001; Bewick, Trusler, Mulhern, Barkham, & Hill, 2008; Craigie et al., 2011; E. S. Ford & Mokdad, 2001; Hollands et al., 2010). Personalisation may be particularly beneficial in the context of an appearance-based intervention as appearance is a potent motivator of our behaviour (H. Y. Chung et al., 2009; Clarke, 2002; Markland & Hardy, 1993; Mcauley, Wraith, & Duncan, 1991) and illustrating the consequences of diet on an individual's own appearance may serve to enhance the salience and perceived relevance of the intended message. Further investigations are necessary to investigate the cognitive basis of this effect. For instance it would be useful to determine participants' memory for their respective interventions (T. D. Cook & Flay, 1978; Petty, 1977).

As participants repeatedly self-reported dietary behaviour over the ten-week follow-up period, some of the benefits of 'self-monitoring' as a proven behaviour-change technique may have been conveyed (Michie et al., 2009; Michie et al., 2011).

Despite this, no dietary improvement was seen in the information-only control group (Figure 9.3). Also, no improvement was seen relative to the equivalent 'information-only' group in the previous trial (Chapter 6) in which participants did not self-monitor dietary intake as intensively did not require participants to self-monitor dietary intake as intensively, with only two post-baseline dietary reports. These findings further suggest that information provision alone is not sufficient to motivate behavioural improvement.

Although the results of the present study are encouraging, we cannot yet definitively assert that this particular strategy will be useful in motivating diet change at a population level. We do not propose that our approach will be a one-fits-all solution, however the characteristics of our sample prevent us from drawing conclusions about the demographic determinants of intervention success. We investigated the efficacy of this strategy predominantly in a sample of young adults. This group may harbour greater concern over appearance than other age groups (S. J. Chung et al., 2006; Harris & Carr, 2001). As such, it may be the case that our strategy is less effective in other age groups. Our approach may, though, be well placed as a tool to establish life-long beneficial dietary habits from an early age when appearance is most important and motivational.

Our sample was also largely Caucasian. The impact of carotenoid pigments on skin colour is perceived as healthy in a variety of cultures (Chapter 5; Stephen et al., 2011; Stephen et al., 2012), suggesting that our approach could be effective across different populations. It may, however, be the case that individuals with darker or yellower skin (caused by higher levels of melanin) are less motivated by diet-induced skin colour changes than lighter-skinned individuals; hence further empirical work is required here.

The size of our sample was potentially limited by the labour-intensive nature of the personalised stimulus creation. This prevented us from investigating the above factors and other potentially important determinants of intervention efficacy such as gender and socioeconomic status. In order to recruit a larger and more diverse sample it is necessary to develop pre-existing face detection and demarcation tools (Burt & Perrett, 1995) which are necessary to facilitate the automatic creation of the own-face intervention stimuli. Though this process was laborious in the present study, each of the steps between image capture and final stimulus display (see method) has now been fully automated (Tiddeman, Perrett, & Hancock, 2012). Ultimately such technology could be available at minimal expense to practitioners, nutritionists and home users using web-camera or cell-phone camera technology. Further work is required to ensure that the delivery of these intervention materials is comparable to or better than existing dietary interventions in terms of the cost-effectiveness of any DALY gains achieved (Cobiac, Vos, & Veerman, 2010).

As part of the appearance-based interventions that we administered, participants received a take-home photographic leaflet (Appendix B). We did not determine the frequency with which participants referred to these resources after the completion of their initial session. It is necessary for further studies to investigate the impact of formal reminder stimuli, which reintroduce the appropriate intervention materials to participants at set intervals, for instance via email. We expect that such periodic reminders will serve to increase intervention efficacy.

Although this preliminary randomised controlled trial of an appearance-based dietary intervention has been promising, further work is required to determine whether it will be a useful public health tool.

10. General Discussion

10.1. Summary

This programme of work set out to identify, develop and test a behavioural intervention capable of reducing the significant burden of lifestyle-precipitated chronic disease in industrialised nations. Chapter 1 reviewed an extensive epidemiological evidence base which reveals that diet is a strong determinant of chronic disease prevalence. This literature reveals that increased fruit and vegetable consumption is broadly beneficial for health. Intake of this food group is likely to improve wellbeing by precluding excessive consumption of unhealthy foodstuffs and also by providing actively beneficial phytochemicals, which prevent chronic disease in part via complex antioxidant pathways.

Chapter 2 discussed recent estimates of fruit and vegetable consumption in economically developed nations, finding that intake of this food group is very frequently inadequate according to international guidelines set out over 20 years ago (WHO, 1990). In some particularly concerning cases, a sizeable proportion of the population are consuming no fruit or vegetables whatsoever (Scottish Government, 2008, 2010). This literature makes it clear that dramatic increases in consumption of this food group are required at a population level. Chapter 2 also reviewed contemporary dietary intervention methods, finding that the most common approaches have significant drawbacks which are likely to prevent the achievement of their ultimate goals. Population-level health campaigns have not yet adequately focused on the use of evidence-based methodology and smaller-scale, empirically-informed investigations of intervention techniques have lacked focus on scalability.

Chapter 3 identified a behavioural intervention paradigm which involves illustrating the consequences of behaviour for physical appearance. This chapter concludes that because of the commonly high value placed on outward appearance, such a strategy could represent a valuable incentive to change dietary behaviour. To date, this paradigm has chiefly illustrated the *negative* consequences of behaviour on facial appearance (e.g., Mahler et al., 1997; Semer et al., 2005) though a strategy which highlights the ways in which health-promoting behaviours can *benefit* appearance is also likely to be valuable. In line with this, a number of evidence-based behaviour-change frameworks (Chapter 2) consider the establishment of an incentivising target to be a critical step in successful behaviour change (c.f. Carver & Scheier, 1990; Schwarzer, 2008). The use of graphical intervention stimuli represents an accessible and potentially economical means of disseminating important health messages, which can also provide a vehicle for combining the information-provision approach of current public health campaigns with more effective motivation methods.

Chapter 4 identified one way in which fruit and vegetable consumption benefits physical appearance, and set out a practical means of motivating increased consumption of this food group. Before the work conducted as part of this thesis, it was known that carotenoid pigments, abundant in many fruits and vegetables, impart yellow colouration to human skin (Alaluf, Atkins, et al., 2002). Cross-sectional research had also recently found that individuals consuming greater amounts of fruit and vegetables exhibit yellower skin than those consuming smaller amounts (Stephen et al., 2011), and perceptual research had revealed that increases in yellowness (Stephen, Law-Smith, et al., 2009) and carotenoid pigmentation (Stephen et al., 2011) improve the perception of healthiness and attractiveness of human skin. These findings were replicated and extended in Chapter 5, which examined Asian and

Caucasian participants’ preferences for skin yellowness upon African, Asian and Caucasian facial images, suggesting that carotenoid pigmentation is cross-culturally perceived as healthy and attractive. These findings are in keeping with a large body of animal literature (Chapter 4) which finds similar mate-choice preferences for carotenoid-based ornamentation in species endowed with appropriate colour vision (largely birds and fish). These literatures together suggested that illustrating the effects of diet on skin pigmentation may be a valuable means of improving human fruit and vegetable consumption.

An exploratory *pseudo-randomised* controlled trial was conducted in Chapter 6 to investigate the potential efficacy of an appearance-centred intervention based on this effect. This first involved empirical quantification of the impact of fruit and vegetable consumption on human skin colour via spectrophotometry, replicating previous findings (Stephen et al., 2011). These results were used to create on-screen and printed intervention materials, illustrating varying degrees of fruit and vegetable consumption. This study showed for the first time that participants viewing such materials perceived the colouration associated with increased fruit and vegetable consumption as healthy and attractive upon images of their *own faces*. These participants exhibited dietary improvement relative to individuals receiving no intervention or information on the health benefits of a fruit and vegetable rich diet.

Whilst the results of this trial were encouraging, a number of important questions remained. Behavioural economics research indicates that humans discount the value of temporally distal rewards, and favour those that are achieved sooner (Chapter 3; Frederick et al., 2002). It was therefore deemed essential to investigate the short-term impact of fruit and vegetable consumption on skin carotenoid pigmentation *within-subject*, rather than rely on potentially long-term and confounded between-subjects effects. Advertising effects which are achieved relatively rapidly is likely to optimise the efficacy of an appearance-centred intervention strategy by appealing to participants' proximal preferences. Chapter 2 highlighted the additional importance of participants' self-efficacy in achieving behavioural change. A necessary step in this regard was to establish the minimum dietary change required to confer perceptible benefits to skin colouration. This was investigated with two experiments in Chapter 7. A significant within-subject relationship was found between changes in fruit and vegetable consumption and skin redness and yellowness changes over a six week period. Analyses of dermal spectral reflectance supported the claim that the observed effects were driven by common carotenoid pigments, particularly lycopene which is abundant in tomatoes and red peppers. Moreover, perceptible benefits to apparent healthiness were conferred with relatively modest increases in fruit and vegetable consumption (of approximately three additional portions per day) over the six-week period. It was concluded that these findings could potentially improve the effectiveness of the appearance-based intervention implemented in Chapter 6, by increasing participants' perceived ability to achieve illustrated goals and by providing temporally proximal rewards.

A further drawback of the initial dietary intervention trial in Chapter 6 was that it could not highlight the important perceptual distinction between skin pigmentation induced by fruit and vegetable consumption and that conveyed by sun exposure. Although perceptual investigations conducted before this trial had revealed that skin colouration associated with β-carotene supplementation is perceived as healthier than that associated with sun exposure (Stephen et al., 2011), this relationship had not been extended to dietary intake of fruit and vegetables. Hence Chapter 8 examined the relative preferences of skin pigmentation caused by diet and sun exposure, finding that observers add significantly more fruit-and-vegetable linked colouration to optimise skin appearance than they do melanin colouration, in line with preferences for β-carotene colouration (Stephen et al., 2011). This chapter also found that participants increase fruit- and vegetable-linked colouration despite being able to simultaneously able to manipulate β-carotene colouration. Hence, the skin colour benefits associated with the consumption of a range of carotenoid pigments via fruit and vegetable consumption is most likely to be perceived as healthy-looking.

The empirical results of Chapters 7 and 8 were explicitly advertised to appropriate participants in a randomised controlled trial (Chapter 9) designed to test a further iteration of the appearance-based dietary intervention. Before this trial, appearance-based behavioural interventions had predominantly illustrated the consequences of behaviour for appearance upon images of participants' *own faces* (e.g., Stock et al., 2009). Personalisation of intervention materials is likely to optimise the efficacy of an appearance-centred intervention by increasing the salience and perceived relevance of conveyed messages. Such illustrations are more effortful to prepare than their non-personalised counterparts, which are more conducive to wide-scale dissemination via visual media streams (including internet, television and point-of-sale advertisement). This trial, therefore, attempted to investigate the relative efficacy of a personalised (*own-face*) appearance-based intervention and a non-personalised (*generic)* intervention in which the impact of increased fruit and vegetable consumption upon facial appearance was illustrated upon gender- and (for the most part) age-congruent generic images.

This trial again found that witnessing the impact of fruit and vegetable consumption on images of one's own skin confers dietary improvement relative to an information-only control intervention. This trial, however, yielded a statistically intermediary effect regarding the impact of the non-personalised appearance intervention. Participants in this generic group did not exhibit significantly different fruit and vegetable intake to those in the personalised intervention group, whom significantly increased their intake on average. However, the generic appearance intervention group also did not exhibit significantly different consumption to those in an information-only control group, limiting the conclusions can be drawn regarding appearance-based intervention with generic materials.

10.2. Necessary Further Work

As observed in Chapter 6, and to some extent in Chapter 9, university students' diets are likely to be in a state of decline over the studied periods because of dwindling finances and the presence of exam pressures (see no-intervention control group, Figure 6.3). Given this declining baseline, it is encouraging that the generic appearance-based intervention was able to prevent dietary decline and perhaps motivate small improvements (that were statistically unresolvable, potentially due to a small sample size; Figure 9.3) over this period. Even if a non-personalised intervention were to produce a relatively small beneficial effect, this could be valuable at a population level given the ease with which such stimuli could be disseminated. Formal economic analyses are necessary to investigate whether the likely gains in healthy years of life (DALYs) precipitated by this technique would justify the expense of marketing such a strategy at a population level, or whether further investment is required to develop a personalised approach. In this respect, technology required to automatically create personalised intervention stimuli has been recently created (Tiddeman et al., 2012), potentially reducing the temporal and fiscal costs associated with delivery of this technique upon participants' own faces.

Although the personalised intervention trials reported in Chapters 6 and 9 yielded encouraging results, much further work is required to fully justify the use of this approach at a population level. It is particularly necessary to increase trial sample size to investigate the relative efficacy of personalised and generic intervention materials. It is additionally important to increase sample heterogeneity to enable investigation into the impact of several intrapersonal and demographic factors. For instance, it is necessary to examine the impact of baseline diet quality on intervention efficacy, specifically targeting individuals most at risk of developing chronic disease due to very low fruit and vegetable consumption (particularly young Scottish adults; Scottish Government, 2008, 2010). As discussed in Chapter 9, the intervention strategy employed here could act as a strong motivator amongst these individuals as they stand the most to gain in terms of skin appearance benefits according to recent empirical work (Chapters 5 & 7; Stephen et al., 2011; Stephen, Law-Smith, et al., 2009).

It is also important to investigate the impact of this intervention approach across age groups as the trials reported in Chapters 6 and 9 predominantly investigated effects in university-age students. It is particularly necessary to investigate the impact of this intervention in younger participants as such an approach could represent a valuable means of establishing life-long beneficial dietary trajectories. Perceptual investigations are necessary to determine the earliest point at which preferences for skin carotenoid pigmentation concur with those seen amongst adults. In addition to recommending appropriate target groups for further appearance-based interventions, this research would also valuably contribute to developmental and evolutionary psychology literatures by elucidating the processes by which humans become sensitive to this skin colour-based cue of health.

An individuals' dietary behaviour often occurs within the context of other individuals who have partial or full influence over what that person eats. The diet of children and young adults may be particularly strongly determined by exogenous factors, therefore it may be necessary to deliver dietary intervention at a familial, rather than individual level.

The potential efficacy of appearance-based intervention remains to be observed in this context, but it is known that adult perception of skin carotenoid pigmentation on infant faces is similar to that for adult faces (Hahn, Stephen & Perrett, unpublished data).

Gender is also likely to be a determinant of intervention efficacy that could not be investigated in the studies reported here. Dietary habits are strongly shaped by appearance concerns in young women (S. J. Chung et al., 2006) and females more frequently report appearance-related concerns (Harris & Carr, 2001), hence an appearance-based dietary intervention may be most effective amongst this sex.

Socioeconomic status is a key correlate of fruit and vegetable intake. Individuals in low socioeconomic groups often cite cost as a factor prohibiting increased fruit and vegetable intake (A. S. Anderson et al., 1998; Williams, Ball, & Crawford, 2010; Wolf et al., 2008). It is critical to determine whether demonstration of appearance benefits is sufficient to overcome this significant barrier to healthy nutrition or whether beneficial effects are restricted to those with more financial freedom. It may be necessary to combine such an approach with methods that highlight cost-effective ways of achieving a healthy diet (e.g., NHS, 2011a). Further, it may be appropriate in the context of socioeconomic status to investigate the relative contribution of fruits and separately vegetables on skin pigmentation and health, as the latter group are commonly relatively inexpensive (Reed & Frazao, 2004).

It is also important to investigate the impact of this intervention across ethnic groups, as Chapter 5 revealed that individuals with very highly melanised skin may not benefit from the skin colour shifts associated with appearance improvement in less melanised individuals. The relative homogeneity of the samples in Chapters 6 and 9 prevented examination of ethnicity as a determinant of intervention efficacy, this was also the case in Chapter 7, hence further work is also required to determine the unknown physiological effects of diet on skin appearance in non-Caucasian participants.

In line with the Medical Research Council's complex intervention guidance (Medical Research Council, 2008), it is necessary to formally investigate the cognitive pathways through which an appearance-based dietary intervention is held to operate (Chapters 3 & 4). This critical next step will guide the development of the intervention and could involve measurement of; a) changes in dietary intentions pre- and post-intervention; b) the relative value placed on one's appearance and health; c) participants perception of the relative immediacy of health and appearance endpoints; d) participants' confidence in achieving the illustrated appearance-related outcomes; e) and determining participants' readiness to change their behaviour at various points over the lifetime of a randomised controlled trial. It is also important to establish the public acceptability and perception of this approach relative to existing methods as this will be a strong determinant of whether the messages conveyed in an intervention are heeded (Blankenship, Bray, & Merson, 2000; Weinrech, 1999). In this regard, the empirical data presented as part of this thesis has already indicated a largely positive public perception through strong worldwide media interest.

The results of the trials conducted in Chapters 6 and 9 are potentially open to biases in reporting because of their unblinded nature and potential social desirability effects (Orne, 2009). A vital next step, therefore, is to evaluate the effects of an appearance-based dietary intervention with objective measures of fruit and vegetable intake instead of relying on self-reported intake. Quantification of urinary or blood plasma phytochemical concentrations may give some indication of dietary improvements and given the relationships found between fruit and vegetable intake and skin colour in Chapter 7, dermal spectrophotometry may be a further useful means of validating self-report. A more direct method may also involve the use of Raman Spectroscopy to precisely detect the presence and quantity of dermal carotenoid pigments (Darvin et al., 2008; Lademann et al., 2011). It could also be valuable to explore the validity of self-report methods that do not involve the use of food-frequency questionnaires. Given the ubiquity of portable devices able to capture photographs, participants could be encouraged to photograph all food that they consume, for later analysis. There also exists software which can automatically identify and provide nutritional information on foodstuffs present in a given photograph (e.g., Daily Burn, 2012), which could be valuable in eliminating participant-induced bias (particularly relating to portion-size estimation), although the accuracy of such methods require empirical investigation.

Further work in this area must also go beyond the impact of fruit and vegetable consumption and consider other food groups. This is a particularly important line of future research as examining fruit and vegetable intake in terms of a percentage of total caloric intake is likely to strengthen the relationships found between fruit and vegetable consumption and skin colour. Other foodstuffs contribute to systemic antioxidant capacity (e.g., nuts and eggs; Health Canada, 2010), which maintains skin carotenoid levels by buffering against their expenditure (Vinkler & Albrecht, 2010). It is also necessary to investigate the intake of lipids, as carotenoids are transported to the skin via lipoprotein assemblies. It is likely to be the case that the relationship between fruit and vegetable consumption and skin carotenoid pigmentation is contingent on a quadratic relationship with habitual fat intake, which could be assessed either via food frequency questionnaire or blood cholesterol assays. The intake of too little fat may prevent carotenoid absorption (Furr & Clark, 1997), and consumption above a threshold value may detract from appearance via a number of pathways, which are not necessarily mutually exclusive. Namely a) increased fat consumption may increase amounts of visceral adipose tissue, which may actively induce oxidative stress (Furukawa et al., 2004), leading to the increased expenditure of carotenoid pigments; b) increased adipose tissue reserves are likely to act as a ‘sink’ for fat-soluble carotenoids (H. Y. Chung et al., 2009); and c) increased body mass may, through a dose-response style relationship, detract from diet-linked skin carotenoid colouration as one unit of fruit and vegetables represents a smaller proportion of an individual’s total mass. Elucidating such relationships could have important ramifications for dietary recommendations as a number of other beneficial lipid-soluble phytochemicals (e.g., Vitamins D and E) are transported via lipoproteins. The quality and quantity of carbohydrate intake is also potentially an important determinant of skin pigmentation as glucose, a major source of free-radicals (Catherwood et al., 2002; Russell et al., 2002), may necessitate the expenditure of antioxidants including carotenoids, ultimately impacting skin appearance.

Chapter 4 reviewed an extensive non-human animal literature which indicates that carotenoid-based ornament colouration is a reliable ‘health certificate’ which honestly reflects an individual’s general condition via oxidative stress pathways (Vinkler & Albrecht, 2010). There are potentially important clinical applications in this area. Reduced skin-carotenoid pigmentation is likely to be predictive of chronic disease in humans via the impact of diet (Barbosa, Bressan, Zulet, & Martinez, 2008) and other lifestyle factors (namely adiposity, smoking, exercise and alcohol consumption Furukawa et al., 2004; Halliwell & Poulsen, 2006; Sen, 1995; Soardo et al., 2005) on oxidative stress. Effects on skin carotenoid levels could potentially be utilised as a rapid, non-invasive diagnostic tool to provide health professionals with an objective (or indeed subjective) measure on which to base formal lifestyle advice. To investigate this possibility further, it is important to examine cross-sectional and within-subjects relationships between systemic oxidative stress, pro-oxidative lifestyle factors and carotenoid-based skin pigmentation. Oxidative stress is quantifiable via urinary assays of 8-Oxo-2’deoxyguanosine, the production of which occurs via the oxidative breakdown of DNA molecules and malondialdehyde, an oxidative product of lipids). Longer-term prospective cohort studies are also necessary to determine the extent to which carotenoid-based skin pigmentation can predict disease occurrence and trajectory.

The discussed effects of diet and other lifestyle factors on skin pigmentation may also have commercial applications. Given the widespread value placed on appearance, individuals are likely to want to objectively track the impact of their health and lifestyle on appearance. This could be achieved by incorporating skin-pigment measuring equipment into marketable products such as bathroom weighing sales. This would also likely optimise the efficacy of an appearance-based intervention, as self-monitoring and objective feedback are key aspects of effective intervention trials (Michie et al., 2011).

10.3. Conclusions

The work conducted as part of this thesis has further developed the field of appearance-based behavioural interventions, and for the first time has indicated that advertising the beneficial impact of a healthy diet on photographs of participants' own faces represents a valuable dietary intervention tool. Lasting behavioural changes were instigated after very brief one-on-one contact with an experimenter. Further economy is now possible due to the development of software which will facilitate the online demonstration and dissemination of such an intervention. The empirical research conducted here has been encouraging, but further work is needed to establish the feasibility, effectiveness and acceptability of this approach as a population-level health campaign.

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Appendix

A. Leaflet distributed to participants receiving an appearance-based intervention (Chapter 6).

How your skin colour may change if you eat more fruit and vegetables

5 less portions fruit and veg per day | You now | 5 more portions fruit and veg per day

How?

Carotenoids are contained in fruit and vegetables and when we eat foods containing these pigments, they get laid down in our skin, giving a slightly yellowed appearance.

Our group has revealed that ***slightly yellowing a face increases the appearance of healthiness***, and this is perhaps because people use the level of carotenoids in skin as an indicator of health.

Carotenoids are antioxidants, meaning they help prevent tissue damage caused by infection and the sun. Someone that is less yellow will have either obtained less carotenoids from their diet, or used more carotenoids up fighting infection. Humans may have evolved or learned to be sensitive to this.

The photographic manipulation that you took part in today illustrated the consequences of a healthy diet on your skin colour, and we hope that these photographs will serve as a reminder of this.

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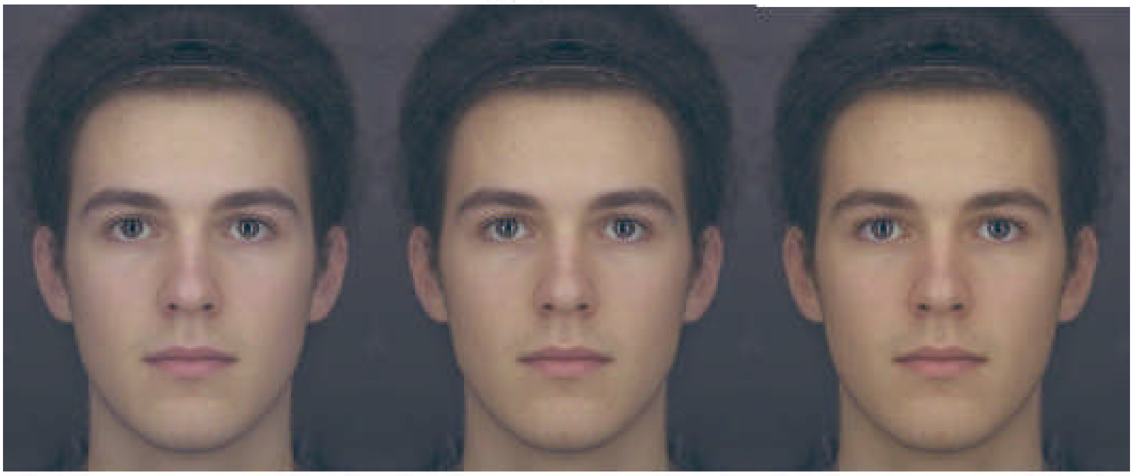
B. Leaflet distributed to participants receiving an appearance-based intervention (Chapter 9). For participants in the 'own-face' appearance intervention group, illustrations were performed upon images of the individual's own face.

Fruit and vegetable consumption can benefit facial appearance



How?

Carotenoids are contained in fruit and vegetables and when we eat foods containing these pigments, they get laid down in our skin, giving a golden colouration.

Our group has revealed that this colouration **improves attractiveness** and the appearance of healthiness, and this is perhaps because people use the level of carotenoids in skin as an indicator of someone's health.

Carotenoids are antioxidants, meaning they help prevent tissue damage caused by infection and the sun. A person that has reduced carotenoid pigmentation may have either obtained less carotenoids from their diet, or used more carotenoids up fighting infection. Humans may have evolved or learned to detect this colouration in order to judge someone's healthiness.

These skin colour changes **can occur within 6 weeks** and may occur sooner, depending on factors such as illness and alcohol intake.

Our research also shows that as little as **1.8 extra portions** can lead to a noticeable difference in skin appearance.

This skin colouration has been shown to improve appearance to a greater extent than melanisation, which is associated with sun-tanning.

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