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MURIEL BELL MEMORIAL LECTURE 2009

Omega-3 fatty acids and human health – more than a fishy tale?

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PREAMBLE: MURIEL BELL

Muriel Bell (1889-1974) was a nutritionist, medical researcher and educator. She received her academic training at the University of Otago, being awarded an MB ChB in 1922 and an MD in 1926; she was the first woman to be awarded the latter degree by the University of Otago. She held academic appointments at the University of Otago from 1922 until her retirement in 1964, and spent a period in the 1930s at University College, London working on vitamins. Muriel Bell was a founder member of the New Zealand Medical Research Council, being appointed in 1937, and chaired the Council's nutrition and dental research committees. In 1940 she was appointed the first nutrition officer in the Department of Health and from then until her retirement she simultaneously held the posts of director of nutrition research at the University of Otago Medical School and state nutritionist. Much of her research was of a practical nature designed to improve human health and well-being: she recommended fluoridation to prevent tooth decay, and was responsible for the introduction of iodised salt to prevent goitre, high extraction flour as a source of vitamin B12, and the delivery of milk in covered lorries to stop it being exposed to sunlight. She advised on rationing during the Second World War, developed rose-hip syrup to provide vitamin C for babies when oranges became unavailable, and found a source of vitamin D in local fish oils. Muriel Bell advised on the food rations for men and dogs involved in the Trans-Antarctic Expedition of 1956-1957. In the 1950s she conducted research into cholesterol and heart disease and she tried to persuade insurance companies to collect statistics on obesity. In that same period she undertook the first nutritional surveys in Fiji and Western Samoa. She experimented with culturing yoghurt, updated feeding tables for bottle-fed babies, and devised new mixtures for babies with milk allergies. She was an enthusiastic educator on nutritional issues, focusing her efforts on nurses, home scientists and dietitians and producing several standard texts. Muriel Bell received numerous honours and accolades during her lifetime: she was made an honorary life member of the Nutrition Society of New Zealand in 1966, a life member of the New Zealand Dietitians Association and of the New Zealand Dental Association, a Fellow of the New Zealand Institute of Chemistry (1941), a Fellow of the Royal Society of New Zealand (1952), and a Fellow of the Royal Australasian College of Physicians (1959). She was awarded a CBE in 1959.

Structure, naming and metabolic relationships of ω -3 fatty acids

Omega-3 (ω -3 or n-3) is a structural descriptor for a family of polyunsaturated fatty acids (PUFAs). The term ω -3 denotes the position of double bond that is closest to the methyl terminus of the acyl chain: in all ω -3 fatty acids this double bond is on carbon 3, counting the methyl carbon as carbon 1 (Figure 1). As with all fatty acids, ω -3 fatty acids have systematic and common names (Table 1); they are also referred to by a shorthand nomenclature that denotes the number of carbon atoms in the chain, the number of double bonds, and the position of the first double bond relative to the methyl carbon (Table 1). The simplest ω -3 fatty acid is α -linolenic acid (18:3 ω -3). α -Linolenic acid is synthesised from linoleic acid (18:2 ω -6) by desaturation, catalysed by delta-15 desaturase (confusingly the desaturase enzymes are named according to the first carbon carrying the newly inserted double bond and counting the carboxyl carbon as carbon number one). Animals, including humans, do not possess the delta-15 desaturase enzyme and so cannot synthesise α -linolenic acid. Thus α -linolenic acid is a classically essential fatty acid, along with linoleic acid. Plants possess delta-15 desaturase and so are able to synthesise α -linolenic acid. Although animals cannot synthesise α -linolenic acid, they can metabolise it by further desaturation and elongation; desaturation occurs at carbon atoms below carbon number 9 (counting from the carboxyl carbon) and mainly occurs in the liver. α -Linolenic acid can be converted to stearidonic acid

(18:4 ω -3) by delta-6 desaturase and then stearidonic acid can be elongated to eicosatetraenoic acid (20:4 ω -3) (Figure 2). This fatty acid can be further desaturated by delta-5 desaturase to yield eicosapentaenoic acid (20:5 ω -3; known as EPA) (Figure 2). Conversion of α -linolenic acid to EPA is in competition with the conversion of linoleic acid to arachidonic acid (20:4 ω -6) since the same enzymes are used. Delta-6 desaturase reaction is rate limiting in this pathway. Although the preferred substrate for delta-6 desaturase is α -linolenic acid, because linoleic acid is much more prevalent in most human diets than α -linolenic acid, metabolism of ω -6 fatty acids is quantitatively the more important. The activities of delta-6 and delta-5 desaturases are regulated by nutritional status, hormones and by feedback inhibition by end products.

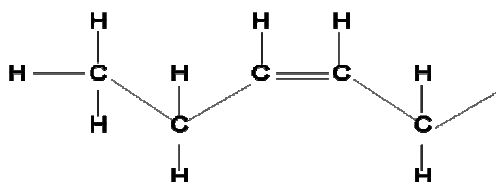


Figure 1: Generic structure of ω -3 fatty acids.

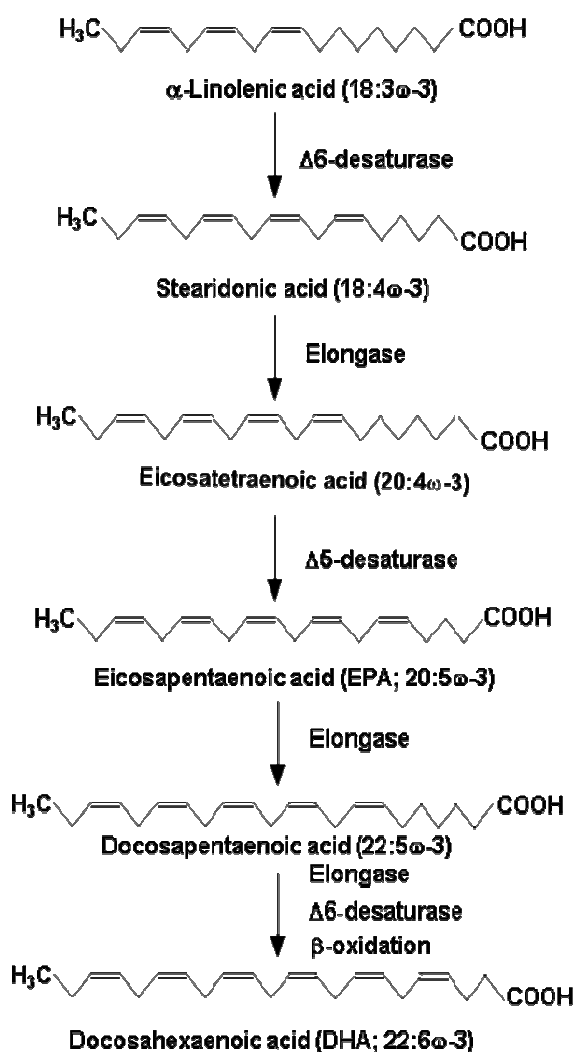


Figure 2: Pathway of conversion of α -linolenic acid to longer chain, more unsaturated ω -3 fatty acids.

Table 1: The ω -3 polyunsaturated fatty acid family.

Systematic name	Common name	Shorthand nomenclature
All <i>cis</i> 9, 12, 15-Octadecatrienoic acid	α -Linolenic acid	18:3 ω -3
All <i>cis</i> 6, 9, 12, 15-Octadecatetraenoic acid	Stearidonic acid	18:4 ω -3
All <i>cis</i> 8, 11, 14, 17-Eicosatetraenoic acid	Eicosatetraenoic acid	20:4 ω -3
All <i>cis</i> 5, 8, 11, 14, 17-Eicosapentaenoic acid	Eicosapentaenoic acid	20:5 ω -3
All <i>cis</i> 7, 10, 13, 16, 19-Docosapentaenoic acid	Docosapentaenoic acid; also Clupanodonic acid	22:5 ω -3
All <i>cis</i> 4, 7, 10, 13, 16, 19-Docosaheptaenoic acid	Docosaheptaenoic acid	22:6 ω -3

A pathway for further conversion of EPA to docosaheptaenoic acid (22:6 ω -3; known as DHA) exists: this pathway involves addition of 2 carbons to form docosapentaenoic acid (22:5 ω -3; known as DPA), addition of 2 further carbons to produce 24:5 ω -3, desaturation at the delta-6 position to form 24:6 ω -3, translocation of 24:6 ω -3 from the endoplasmic reticulum to peroxisomes where 2 carbons are removed by limited β -oxidation to yield DHA. It seems likely that the complex series of fatty acid translocation and β -oxidation steps may act as loci of metabolic control facilitating regulation of DHA synthesis independent from the up-stream activity of the pathway. Short term studies with isotopically-labelled α -linolenic acid and long term studies using significantly increased intakes of α -linolenic acid have demonstrated that the conversion to EPA, DPA and DHA is generally poor in humans, with very limited conversion all the way to DHA being observed (Burdge & Calder, 2006; Arterburn et al., 2006). EPA, DPA and DHA are referred to as very long chain ω -3 PUFAs.

Dietary sources and typical intakes of ω -3 fatty acids

α -Linolenic acid from plant sources

Green leaves contain a significant proportion (typically over 50%) of their fatty acids as α -linolenic acid; however since green leaves are not rich sources of fat these are not major dietary sources of fatty acids including α -linolenic acid. Several seeds and seed oils and some nuts contain significant amounts of α -linolenic acid. Linseeds (flaxseeds) and their oil typically contain 45 to 55% of fatty acids as α -linolenic acid, while soybean oil typically contains 5 to 10% of fatty acids as α -linolenic acid. Rapeseed oil and walnuts also contain α -linolenic acid. Corn oil, sunflower oil and safflower oil are rich in linoleic acid but contain very little α -linolenic acid. Typical intakes of α -linolenic acid among Western adults are 0.5 to 2 g/d (Burdge & Calder, 2006; British Nutrition Foundation, 1999). The main PUFA in most Western diets is the ω -6 fatty acid linoleic acid which is typically consumed in 5 to 20-fold greater amounts than α -linolenic acid (Burdge & Calder, 2006; British Nutrition Foundation, 1999).

EPA, DPA and DHA from seafood

Seafoods are a source of the longer chain, more unsaturated ω -3 PUFAs. Fish can be classified into lean fish that store lipid in the liver (e.g. cod) or "fatty" ("oily") fish that store lipid in the flesh (e.g. mackerel, herring, salmon, tuna, sardines). Compared with other foodstuffs, fish and other seafood are good sources of the very long chain ω -3 fatty acids EPA, DPA and DHA (British Nutrition Foundation, 1999). However different types of fish contain different amounts of these fatty acids and different ratios of EPA to DHA. This is partly dependent upon the metabolic characteristics of the fish and also upon their diet, water temperature, season etc. Nevertheless, it is clear that a single lean fish meal (e.g. one serving of cod) could provide about 0.2 to 0.3 g very long chain ω -3 fatty acids, while a single oily fish meal (e.g. one serving of salmon or mackerel) could provide 1.5 to 3.0 g of these fatty acids. The latest estimate for fish consumption among adults in the United Kingdom is approximately 100 g lean fish and approximately 50 g oily fish per week (SACN/COT, 2004); similar (and in some countries even lower) intakes are expected in other Northern and in Eastern European, North American and Australasian countries. Lean fish intake is higher than

this in Southern European countries and lean and oily fish intake is higher than this in Japan. Average (mean) intakes of very long chain ω -3 fatty acids among adults in the United Kingdom, in other Northern and in Eastern European, North American and Australasian countries are approx. 0.15 to 0.25 g/day (SACN/COT, 2004). However the distribution of intakes is bimodal due to the presence of oily fish consumers and non-consumers and a fairly recent estimate of very long chain ω -3 fatty acid intake among Australian adults gave a median intake of about 0.03 g/day, compared with a mean intake of about 0.19 mg/day (Meyer et al., 2003). Intakes would be rather higher in those populations, such as the Japanese, who consume oily fish in greater amounts and with greater regularity than seen in Europe, North America and Australasia.

Fish oils

The oil obtained from oily fish flesh or lean fish livers (e.g. cod liver) is termed "fish oil" and it has the distinctive characteristic of being rich in very long chain ω -3 fatty acids. EPA and DHA comprise about 30% of the fatty acids in a typical preparation of fish oil, which means that a one gram fish oil capsule can provide about 0.3 g of EPA plus DHA. However, the amount of ω -3 fatty acids that can vary between fish and fish oils, and so can the relative proportions of the individual very long chain ω -3 PUFAs (EPA, DPA and DHA); for example cod liver oil is richer in EPA than DHA while tuna oil is richer in DHA than EPA. Fish liver oils contain significant amounts of fat soluble vitamins, especially vitamins A and D. Encapsulated oil preparations that contain ω -3 fatty acids in higher amounts than found in standard fish oils are available. In fish oil capsules the fatty acids are usually present in the form of triacylglycerols, although ω -3 fatty acids are also available in the phospholipid form (e.g. as krill oil) and as ethyl esters (e.g. in the highly concentrated pharmaceutical preparation Omacor). Clearly capsules could make a significant contribution to very long chain ω -3 fatty acid intake. For example, an individual who consumes little or no fish could increase their daily very long chain ω -3 fatty acid intake 5-fold (or more) by taking a single standard fish oil capsule per day.

Algal oils

Certain algal oils are particularly rich in DHA which may comprise as much as 45% of total fatty acids. These oils may be useful where provision of DHA, but not EPA, is particularly desired, for example in infant formulas.

Increased intake of very long chain ω -3 fatty acids alters the fatty acid composition of plasma, cells and tissues in humans

Different plasma lipid pools, cells and tissues have different, characteristic, fatty acid compositions. These compositions are influenced by the availability of different fatty acids but also by the metabolic characteristics of the particular pool, cell or tissue. Modification of fatty acid profiles has been widely reported after supplementation of the diet with fish oil capsules; studies report that such supplementation results in appearance of EPA and DHA in plasma lipids, platelets, erythrocytes, leukocytes, colonic tissue, cardiac tissue and most likely in many other cell and tissue types. The incorporation of EPA and DHA from fish oil capsules is partly of the expense of ω -6 PUFAs, like arachidonic acid, and occurs in a dose-response fashion. For example, studies using a range of EPA+DHA intakes from 1 to 6 g/day report near linear relationships between EPA and DHA intake and the EPA and DHA contents of plasma phospholipids (Blonk et al., 1990; Harris et al., 1991; Marsen et al., 1992) and of platelet phospholipids (Sanders & Roshanai, 1983). In other studies incorporation of EPA and DHA into blood neutrophils (Healy et al., 2000) and of EPA into plasma phospholipids and blood mononuclear cells (Rees et al., 2006) occurred in a linear dose response manner (Figure 3). In an elegant study combining dose-response and time-course over 12 months in older male subjects, Katan et al. (1997) reported the fatty acid compositions of serum cholesteryl esters, erythrocytes and adipose tissue. This study confirmed that EPA and DHA are incorporated into circulating lipid pools and into erythrocytes when their intakes are increased. It also demonstrated EPA and DHA incorporation into adipose tissue, a storage pool, when their intakes are increased. However this study also clearly showed that incorporation into different pools occurs at different rates and to differing extents (i.e. with different efficiencies) and may not be related to intake in a strictly linear fashion, at least over

the intakes studied. Katan et al. (1997) showed that near-maximal incorporation of EPA and DHA into serum cholesteryl esters occurs within 30 days of beginning supplementation, whereas maximal incorporation into erythrocytes does not occur until sometime between 56 and 182 days. Yaqoob et al. (2000) reported the time dependent incorporation of EPA and DHA into blood mononuclear cells; incorporation of both fatty acids was near-maximal after 4 weeks of supplementation (Figure 4). Upon cessation of supplementation EPA in mononuclear cells returned to starting levels within 8 weeks, while the cells appeared to retain DHA. The same observations of loss of EPA and selective retention of DHA upon cessation of fish oil supplementation have been made for erythrocytes (Popp-Snijders et al., 1986) and platelets (von Schacky et al., 1985). Thus, a significant body of literature reports that EPA and DHA are incorporated into blood, cell and tissue lipids when their intake is increased.

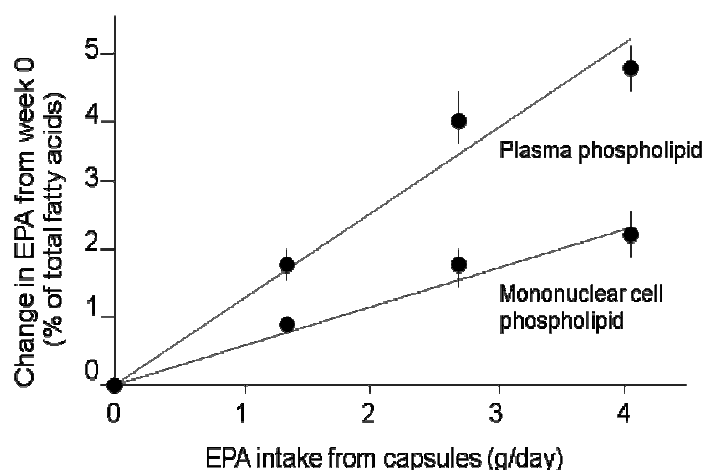


Figure 3: Dose-dependent incorporation of EPA into human plasma phospholipids and blood mononuclear cells. Healthy young males supplemented their diet with differing amounts of an EPA-rich oil for a period of 12 weeks. Plasma and blood mononuclear cell phospholipids were isolated and their fatty acid composition determined by gas chromatography. Data are mean \pm SEM from 23 or 24 subjects per group and are expressed as change in EPA from week 0 (study entry). Data are from Rees et al., 2006.

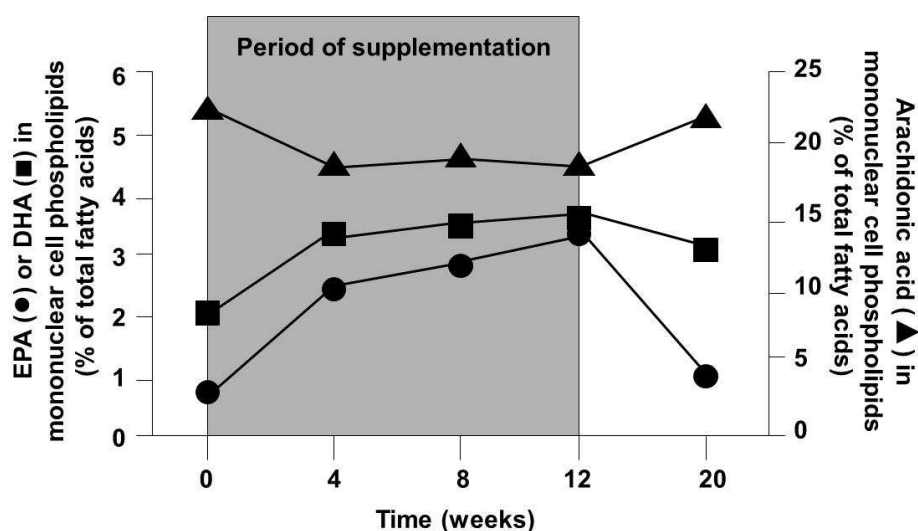


Figure 4: Time course of changes in EPA, DHA and arachidonic acid contents of human blood mononuclear cells in subjects consuming fish oil. Healthy subjects supplemented their diet with fish oil capsules providing 2.1 g EPA plus 1.1 g DHA per day for a period of 12 weeks (indicated by the grey area). Blood mononuclear cell phospholipids were isolated at 0, 4, 8, 12 and 20 weeks and their fatty acid composition determined by gas chromatography. Data from Yaqoob et al., 2000 are mean from 8 subjects (error bars omitted for clarity).

Mechanisms by which very long chain ω -3 fatty acids can influence cell function

Increased cell and tissue ω -3 fatty acid content can influence cell function through a variety of mechanisms as shown in Figure 5.

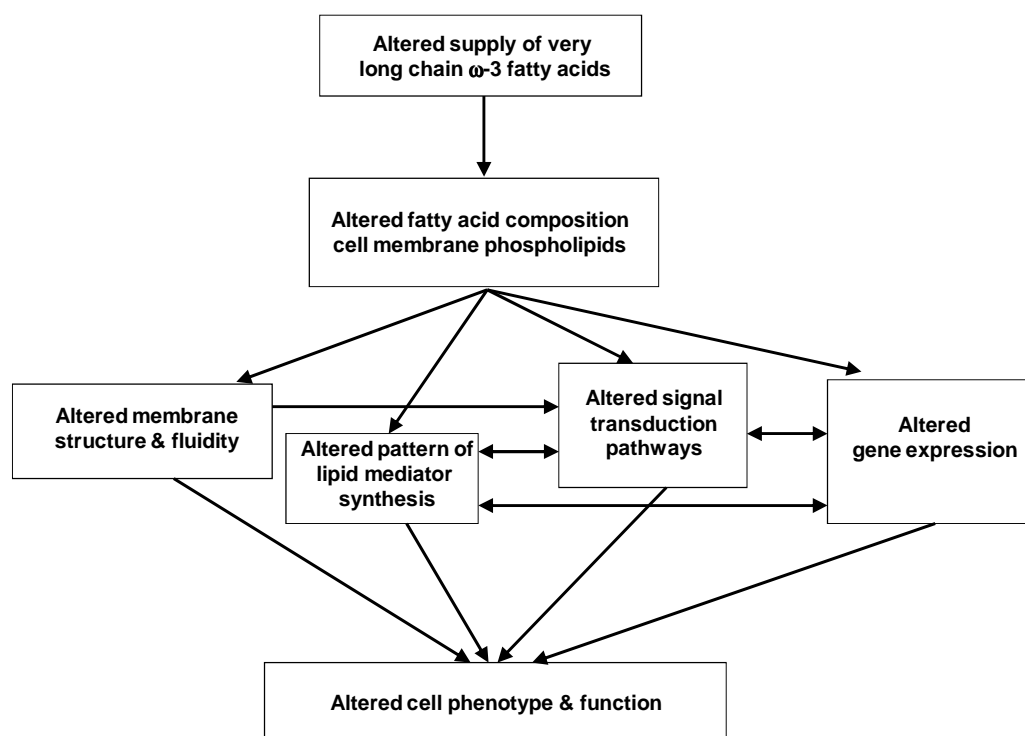


Figure 5: General scheme of the interacting mechanisms whereby very long chain ω -3 fatty acids might influence cell function.

Alterations in membrane structure and function

Increased very long chain ω -3 PUFA content of membrane phospholipids can lead to modifications of the physical properties of the membrane such as membrane order ("fluidity") and raft structure (rafts are membrane microdomains with a particular lipid and fatty acid makeup and which play a role as platforms for receptor action and for the initiation of intracellular signaling pathways) which in turn influence the activity of membrane proteins including receptors, transporters, ion channels, and signaling enzymes (Yaqoob, 2009).

Effects on cell signalling pathways

Very long chain ω -3 PUFAs can affect cell signalling pathways, either through modifying the expression, activity or avidity of membrane receptors or modifying intracellular signal transduction mechanisms (Miles & Calder, 1998). As a result of these effects, transcription factor activation is altered and gene expression modified. Transcription factors reported to be modified by the presence of very long chain ω -3 PUFAs include nuclear factor κ B, peroxisome proliferator activated receptor- α and γ , and the sterol regulatory element binding proteins (Clarke, 2004; Lapillonne et al., 2004; Jump, 2002, 2008; Deckelbaum et al., 2006). Thus, very long chain ω -3 PUFAs can alter patterns of gene expression.

Effects on lipid mediators

Eicosanoids produced from the ω -6 PUFA arachidonic acid, including various prostaglandins, thromboxanes and leukotrienes, have well-established roles in regulation of inflammation, immunity, platelet aggregation, smooth muscle contraction and renal function (Nicolau & Kafatos, 2004). Excess or inappropriate production of these eicosanoids is associated with disease processes. For example cysteinyl-leukotrienes play an important role

in asthma. A range of drugs of varying specificity are used clinically to suppress the production of eicosanoids from arachidonic acid. Very long chain ω -3 PUFAs decrease the production of arachidonic acid derived eicosanoids and so can impact on the actions regulated by those mediators (Calder, 2008a). Furthermore, EPA is a substrate for the synthesis of alternative eicosanoids which are typically less potent than those produced from arachidonic acid (Calder, 2008a). Relatively recently a new family of lipid mediators, termed resolvins, synthesised from both EPA (E-series resolvins) and DHA (D-series resolvins) have been described. These mediators have been demonstrated in cell culture and animal feeding studies to be potently anti-inflammatory, inflammation resolving and immunomodulatory (Serhan et al., 2000a; Serhan et al., 2000b). Protectin D1, produced from DHA, appears to have an important role in protecting tissue, including neuronal tissue, from excessive damage in a variety of experimental situations (Serhan et al., 2002).

An increased intake of very long chain ω -3 fatty acids is beneficial to health

Through the mechanisms of action outlined above and the resulting modifications of cell and tissue function, very long chain ω -3 fatty acids exert physiological actions. These are summarised in Table 2 where they are linked to certain health or clinical benefits. A number of risk factors for cardiovascular disease are modified in a beneficial way by increased intake of very long chain ω -3 fatty acids: these include blood pressure (Geleijnse et al., 2002), platelet reactivity and thrombosis (British Nutrition Foundation, 1992), plasma triglyceride concentrations (Harris, 1996), vascular function (Nestel et al., 2002), cardiac arrhythmias (von Schacky, 2008), heart rate variability (von Schacky, 2008), and inflammation (Calder, 2006). As a result increased very long chain ω -3 fatty acid intake is associated with a reduced risk of cardiovascular morbidity and mortality (Calder, 2004). Indeed supplementation studies with very long chain ω -3 fatty acids have demonstrated reduced mortality from cardiovascular causes (Anonymous, 1999; Marchioli et al., 2002; Bucher et al., 2002; Studer et al., 2005; Yokoyama et al., 2007). A number of other, non-cardiovascular, actions of these fatty acids have also been documented (Table 2), suggesting that increased intake of these fatty acids could be of benefit in protecting from or treating many conditions. For example, they have been used successfully in rheumatoid arthritis (Calder, 2008b) and, in some studies, in inflammatory bowel diseases (Calder, 2008c), and may be useful in other inflammatory conditions (Calder, 2006). DHA has an important structural role in the eye and brain, and its supply early in life when these tissues are developing is known to be of vital importance in terms of optimizing visual and neurological development (SanGiovanni et al., 2000a; SanGiovanni et al., 2000b). Recent studies have highlighted the potential for very long chain ω -3 fatty acids to contribute to enhanced mental development (Helland et al., 2003) and improved childhood learning and behaviour (Richardson, 2004) and to reduce the burden of psychiatric illnesses in adults (Freeman et al., 2006), although these remain controversial areas of possible action which require more robust scientific support. There may also be a role for very long chain ω -3 PUFAs, DHA in particular, in preventing neurodegenerative disease of ageing (Solfrizzi et al., 2009) and the production of protectins, especially protectin D1 (formerly called neuroprotectin D1), appears to be crucial for this effect (Lukiw et al., 2005). The effects of very long chain ω -3 PUFAs on health outcomes are likely to be dose-dependent, but clear dose response data have not been identified in most cases.

Table 2: Summary of the physiological roles and potential clinical benefits of very long chain ω -3 fatty acids

Physiological role of very long chain ω -3 fatty acids	Potential clinical benefit	Target
Regulation of blood pressure	Decreased blood pressure	Hypertension; CVD
Regulation of platelet function	Decreased likelihood of thrombosis	Thrombosis; CVD
Regulation of blood coagulation	Decreased likelihood of thrombosis	Thrombosis; CVD
Regulation of plasma triglyceride concentrations	Decreased plasma triglyceride concentrations	Hypertriglyceridemia; CVD
Regulation of vascular function	Improved vascular reactivity	CVD
Regulation of cardiac rhythm	Decreased arrhythmias	CVD
Regulation of heart rate	Increased heart rate variability	CVD
Regulation of inflammation	Decreased inflammation	Inflammatory diseases (arthritis, inflammatory bowel diseases, psoriasis, lupus, asthma, cystic fibrosis, dermatitis, neurodegeneration); CVD
Regulation of immune function	Improved immune function	Compromised immunity
Regulation of fatty acid and triglyceride metabolism	Decreased triglyceride synthesis and storage	Weight gain; Weight loss; Obesity
Regulation of bone turnover	Maintained bone mass	Osteoporosis
Regulation of insulin sensitivity	Improved insulin sensitivity	Type-2 diabetes
Regulation of tumour cell growth	Decreased tumour cell growth & survival	Some cancers
Regulation of visual signalling (via rhodopsin)	Optimised visual signalling	Poor infant visual development (especially pre-term)
Structural component of brain and central nervous system	Optimised brain development – cognitive and learning processes	Poor infant and childhood cognitive processes and learning

CVD = cardiovascular disease.

Dietary recommendations for very long chain ω -3 fatty acids

The recognition of the benefits of very long chain ω -3 fatty acids has resulted in a series of recommendations to increase the intake of fish and more specifically of very long chain ω -3 fatty acids by various government, non-government and professional bodies. Typical recommendations to maintain general good health are an intake of at least two fish meals per week including at least one of oily fish (SACN/COT, 2004). Such recommendations are based mainly upon the epidemiological evidence for decreased cardiovascular morbidity and mortality with increased consumption of fish and upon supplementation studies using fish oils investigating impact on cardiovascular risk factors (British Nutrition Foundation, 1999; SACN/COT, 2004; Calder, 2004). In terms of the very long chain ω -3 fatty acids, recommendations that have been made include a minimal intake of 0.2 to 0.65 g/day for general good health (de Deckere et al., 1998; Simopolous et al., 1999; SACN/COT, 2004), 1.5 g/day for general good health (British Nutrition Foundation, 1999), 1 g/day for secondary prevention of myocardial infarction (Kris-Etherton et al., 2002; JBS2, 2005; Van der Werf et al., 2008), and 2 to 4 g/day for blood triglyceride lowering (Kris-Etherton et al., 2002). In those individuals not regularly consuming oily fish, the intake of these fatty acids is likely to be < 0.2

g/day and perhaps even much lower than this (Meyer et al., 2003). Strategies to increase intake of very long chain ω -3 PUFAs include eating oily fish, consuming fish oil capsules or liquid, and eating foods specifically enriched in these fatty acids.

Health effects of α -linolenic acid

The foregoing discussion has centred upon the very long chain ω -3 PUFAs for which there is much evidence for human health benefit and an increasing understanding of the multiple mechanisms involved, and for which a number of recommendations for increased intake have been made. The major plant ω -3 PUFA, α -linolenic acid, is an essential fatty acid and may have human health benefits either in its own right or by acting as a precursor for synthesis of the longer chain more unsaturated derivatives using the pathway shown in Figure 2. These possibilities have been reviewed in some detail fairly recently (Burdge & Calder, 2006; Arterburn et al., 2006). Studies in humans using acute ingestion of stable isotopically-labelled α -linolenic acid have demonstrated some conversion to EPA and to DPA, but much more limited conversion to DHA, although this may be greater in young adult women than in men (Burdge et al., 2002; Burdge & Wootton, 2002), possibly because of upregulation of the delta-6 desaturase by female sex hormones. Little is known about the extent of α -linolenic acid conversion to EPA and DHA in infancy and childhood, in the elderly or during pregnancy and lactation, times when synthesis of very long chain ω -3 PUFAs might be important or desirable. A number of studies have examined the effect of chronic (i.e. weeks to months) consumption of increased amounts of α -linolenic acid. These studies confirm that increasing α -linolenic acid intake increases the EPA (and DPA) content of plasma lipids, platelets, leukocytes and erythrocytes but that DHA content does not increase (Burdge & Calder, 2006; Arterburn et al., 2006); clearly these findings are in agreement with the stable isotope studies. Such studies with α -linolenic acid have demonstrated some effects on cardiovascular risk factors and on inflammatory markers, but where these are reported they are typically weaker than the effects achieved from increasing consumption of EPA+DHA, and may be due to the increased appearance of EPA (Caughey et al., 1996; Zhao et al., 2004).

CONCLUSION

Current intakes of very long chain ω -3 fatty acids EPA and DHA are low in most individuals living in Western countries. A good natural source of these fatty acids is seafood, especially oily fish. Fish oil capsules contain these fatty acids too, with a standard 1 g capsule providing about 0.3 g of EPA plus DHA; more concentrated forms are also available in capsules. Very long chain ω -3 fatty acids are readily incorporated from capsules into transport (blood lipids), functional (cell and tissue) and storage (adipose) pools in humans. This incorporation is dose-dependent and follows a kinetic pattern that is characteristic for each pool. Incorporation is most rapid into blood lipids, followed by platelets and white cells, followed by erythrocytes. At sufficient levels of incorporation into cells, EPA and DHA influence the physical nature of cell membranes and membrane protein-mediated responses, lipid mediator generation, cell signaling and gene expression in many different cell types. Through these mechanisms EPA and DHA influence cell and tissue physiology and the way cells and tissues respond to external signals. In most cases the effects seen are compatible with improvements in disease biomarker profiles or in health-related outcomes. An important aspect of this is the requirement for very long chain ω -3 fatty acids, especially DHA, in early growth and development of the brain and visual system, meaning that adequate provision to the foetus and to the newborn infant is essential. As a result of their effects on cell and tissue physiology, very long chain ω -3 fatty acids play a role in achieving optimal health and in protection against disease. Long chain ω -3 fatty acids not only protect against cardiovascular morbidity but also against mortality. In some situations, for example rheumatoid arthritis, they may be beneficial as therapeutic agents although a high intake is required. On the basis of the recognised health improvements brought about by long chain ω -3 fatty acids, recommendations have been made to increase their intake. This can be achieved through increased consumption of oily fish or fish oil capsules. The plant ω -3 fatty acid, α -linolenic acid, can be converted to EPA but in humans conversion to DHA appears to be poor. Effects of α -linolenic acid on human health-related outcomes appear to be due to conversion to the

EPA. It is abundantly clear that the health effects of ω -3 fatty acids amount to rather more than a "fishy tale".

Abbreviations used: CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

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Iodine status during pregnancy and lactation in Palmerston North, New Zealand

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ABSTRACT

Background: Recent studies have reported that iodine deficiency is re-emerging within the New Zealand population. Iodine requirements increase during pregnancy and lactation, increasing the risk of deficiency during these periods. Inadequate iodine status during pregnancy can affect fetal mental development and can lead to psychomotor, speech and hearing defects, and also mental retardation. Iodine deficiency during infancy can impair both mental and physical development.

Objectives: To explore the current iodine status during pregnancy and lactation in a self-selecting population within the Palmerston North locality.

Design: Pregnant and breastfeeding women were recruited from the Palmerston North area. Twenty-four hour urine samples were obtained from pregnant women (n=24) after 26 weeks gestation, and breastfeeding women (n=28) at least three weeks after delivery. Breast milk samples were also collected (n=27). Iodine concentration was determined in urine and milk samples using inductively-coupled plasma mass spectrometry.

Outcomes: During pregnancy, median urinary iodine concentration was 45 µg/l (range 13-121 µg/l), and during lactation the median iodine concentration was 36 µg/l (range 9-89 µg/l). None of the participants met the urinary iodine levels recommended by the World Health Organisation for adequacy of 150-249 µg/l during pregnancy and ≥100 µg/L during lactation. The median iodine for the breast milk samples was 41µg/l (range 16-288 µg/l) below the concentration considered to be adequate of 75 µg/l.

Conclusion: This study indicates iodine deficiency is a problem within this population of pregnant and breastfeeding women, having potential adverse consequences for the mothers and their infants. From September 2009, mandatory fortification of bread with iodised salt will come into effect throughout New Zealand. This research provides base line data to assess the extent to which mandatory fortification improves iodine status, as well as raising the awareness of the need for pregnant and lactating women to increase their iodine intake through supplementation.

INTRODUCTION

Iodine is a critical trace element for the production of thyroid hormones. Increased iodine requirement during pregnancy is due to the need to maintain maternal euthyroidism, and to be transferred to the fetus for fetal thyroid hormone production, particularly in later gestation (Zimmermann, 2009) and to cover the iodine needs for increased renal iodine losses (Dunn and Delange, 2001). The World Health Organisation recommends pregnant women achieve an iodine intake of 250µg/day but not more than 500µg/day; during lactation, to ensure the infants achieve adequate iodine intake from breast milk; lactating women should consume 250µg/day of iodine (Anderson et al., 2007). Previous research has shown it is essential for both pregnant and lactating women to have adequate iodine intake to prevent the fetus and infant from impaired physical and neurological development (Andersson et al., 2007; Dunn and Delange, 2001; Glinioer, 2007; Semba and Delange, 2001; Zimmermann and Delange, 2004; Zimmermann, 2009)

A number of studies in New Zealand suggest that mild iodine deficiency exists within the New Zealand adult population (Thomson et al., 2008; Thomson et al., 2001; Thomson et al., 2009), schoolchildren (Skeaff et al., 2002), and pregnant and breastfeeding women (Mulrine et al., 2005; Thomson et al., 2001) and that breastfed infants are at high risk of

moderate iodine deficiency (Skeaff et al., 2005). The re-emergence of iodine deficiency in NZ is of particular concern to pregnant and lactating women and their infants.

The objective of this study is to assess the current iodine status among pregnant and lactating women in Palmerston North in the North Island of New Zealand prior to the commencement of mandatory fortification of bread with iodised salt from September 2009, to obtain baseline data.

METHODS

Between January and July 2009, women were recruited from the Palmerston North area in New Zealand through local newspapers, Massey University website, and fliers/posters placed in local maternity service providers. Volunteers were aged 16 years and older, who were in their third trimester of pregnancy (greater than 26 weeks gestation) or who were lactating at least three weeks after giving birth. Women who had medical complications during their pregnancy were excluded. Ethical approval was obtained from the Massey University Ethical Committee. Written consent forms were signed by all the participants.

All the participants completed a face-to-face interview. Oral and written instructions for sample collection were provided to the participants during the interview. All participants provided twenty-four hour urine samples and breast milk samples (30 ml) were collected from lactating women. Samples were stored without preservative at -20°C prior to analysis. Iodine concentration in both urine and breast milk samples was determined by use of inductively-coupled plasma mass spectrometry (Fecher et al., 1998). The total volume of urine was measured, which allowed 24 hour urine iodine excretion values to be calculated. Iodine intake was estimated by extrapolation of 24 hour urinary iodine excretion based on the assumption of 90% excretion rate for women of childbearing age and pregnant women (Bath et al., 2008; Andersson et al., 2007; Delange, 2007). Data was analysed using SPSS (Statistics Package for the Social Science) version 14.

RESULTS

Twenty-four pregnant and twenty-eight lactating women took part in the study. The mean age was 31.6 ± 5.6 years for pregnant and 32.0 ± 4.2 years for breastfeeding women. The majority of the participants (88% pregnant and 79% lactating) achieved education at tertiary level or higher, and most (88% pregnant and 93% lactating) were Caucasians.

The World Health Organisation (WHO), International Council for the Control of Iodine Deficiency Disorders (ICCIDD), and the United Nations Children's Fund (UNICEF) (2007) suggest the median value of urinary iodine for the sampled population is the most commonly assessed indicator. The median urinary iodine concentration was 45.5 (26.5, 51.3) µg/l in pregnant participants (n=24) and 36.4 (24.5, 60.1) µg/l in lactating participants (n=28). Urinary iodine values of all participants were below the WHO insufficient cut-off points (pregnancy: 150 µg/l and lactating: 100 µg/l µg/l).

Iodine excretion in urine can be assumed to be 90% during pregnancy (Andersson et al., 2007) allowing daily iodine intake to be estimated from 24 hour urinary iodine excretion. Median daily iodine intake for pregnant participants was estimated at 96.6 (66.7, 153.1) µg/d (distribution shown in Figure 1). The median iodine value in breastmilk samples (n=27) from lactating participants was 42 (32, 66) µg/l. Only three out of twenty-four pregnant participants (12.5%) met the Recommended Dietary Intake of iodine in New Zealand (220 µg/day), and four out of 27 (14.8%) lactating participants had their breast milk iodine concentration higher than 75 µg/l - an index of sufficient iodine intake (Azizi & Smyth, 2009).

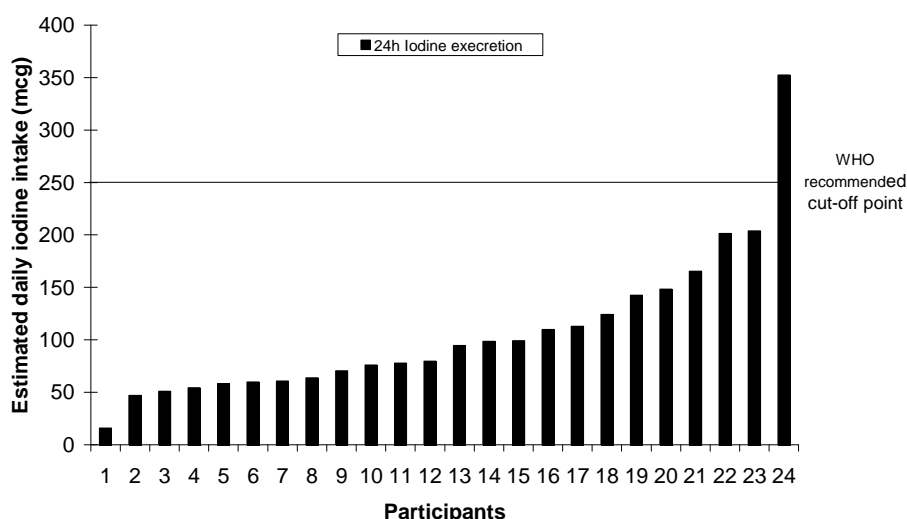


Figure 1: Pregnant participants in ascending order of estimated daily iodine intake (mcg)

DISCUSSION

Iodine deficiency was demonstrated in this sample of pregnant and lactating women in Palmerston North, New Zealand. The median urinary iodine concentration (MUIC) of pregnant participants in this study (45.5 µg/l) was less than one third of the minimum WHO recommendation (150-249 µg/l) (WHO et al., 2007). This result was similar to that reported in the TRIP survey of 170 pregnant women study in 2005, 38 µg/l (MUIC) with 70% less than 50 µg/l (Pettigrew Porter et al., 2006) and a study of 57 Dunedin mothers, 43 µg/l (MUIC) (Mulrine et al., 2005). Both previous studies and this study indicated a moderate iodine deficiency among pregnant women in New Zealand.

The estimated daily iodine intake for pregnant participants showed only one out of twenty-four (4.2%) achieved the latest Recommended Daily Intake (RDI) for iodine, 250 µg/d (Andersson et al., 2007). The majority of pregnant participants in this study did not achieve the recommendation; Thomson et al. (2001) also reported estimated median iodine daily intake was in the range of 60-70 µg, which is lower than the value of 96.6 µg/d for pregnant participants in the present study.

Both urinary iodine concentration and the estimated daily iodine intake for pregnant participants indicated moderate iodine deficiency in the current study. Furthermore, the median urinary iodine concentration of lactating participants (36.4 µg/l) did not meet the WHO recommendation (100 µg/l) (Andersson et al., 2007). Semba and Delange (2001) suggest that the monitoring of milk iodine concentration provides a simple and non-invasive measurement to evaluate whether lactating mothers and their infants meet the dietary requirements. Values above 75 µg/l of milk may be considered as an index of sufficient iodine intake, even though the exact cut-off for concentration of iodine in human milk has not been specified (Azizi and Smyth, 2009). In this study, only 15% of lactating participants met this recommendation. The median iodine concentration in breast milk samples was 42 µg/l, which is similar to the findings reported from a 1990 study of Wellington lactating women with infants over 3 months old (50 µg/l) (Johnson et al., 1990). However, another iodine study of breastfeeding mothers conducted in the South Island between May 1998 and March 1999, found a much lower mean iodine concentration in their breast milk samples (22 µg/l) (Skeaff et al., 2005).

According to the 2003-4 New Zealand Total Diet Survey, dietary iodine exposure for all age-sex groups were well below recommended levels and have steadily decreased since 1982 (Thomson et al., 2008).

CONCLUSIONS

This study provides baseline data for iodine status in pregnant and breastfeeding women in Palmerston North in the North Island of New Zealand, prior to the mandatory fortification of bread with iodised salt throughout the country; most previous studies were

carried out in the South Island. This study indicates moderate iodine deficiency among women at a critical period of their lifecycle. Achieving the WHO recommendations for both pregnant and lactating women is essential in preventing fetus and breastfed infants from suffering impaired physical and neurological development.

The limitation of this study is its small sample size which makes it impossible to extrapolate the data to a larger population within New Zealand. However, such baseline data will be useful for a later study investigating the effect of mandatory fortification of bread. Further research should be conducted among a larger sample of pregnant and lactating women throughout New Zealand.

This study highlights the need for ongoing surveillance of iodine status among pregnant and lactating women in New Zealand. Alternative strategies to prevent deficiency amongst this vulnerable group need to be investigated.

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Accuracy in determining glycaemic impact values for meals by adding individual food values requires allowance for homeostasis

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ABSTRACT

Background: The glucose dose-blood glucose response relationship is non-linear, due to homeostasis, so simple addition of glycaemic impact values of foods to obtain the glycaemic impact value for a meal may be inaccurate.

Objectives: To theoretically determine the inaccuracy that results from homeostasis when the relative glycaemic impact of meals is obtained by adding glycaemic glucose equivalent (GGE) values of the individual foods in the meal.

Design: The glucose dose-blood glucose response relationship was determined from seven published studies, all normalised to 50 GGE at a dose of 50 g glucose. The equation of the relationship was used to determine the discrepancy between the glucose weight that would give a response equivalent to that of the food (its GGE content (g)), and the glucose equivalent calculated off a straight line connecting the reference response on the dose-response curve to zero.

Outcomes: The blood glucose dose-glycaemic response relationship was quadratic and lead to accumulation of inaccuracy when GGE values were added to obtain a meal glycaemic impact value. The error depended on the size of the reference used, the number of carbohydrate foods involved, and the total GGE content of the meal.

Conclusion: Accurate determination of the relative glycaemic impact of meals by adding GGE values of individual foods is limited by the non-linearity of the GGE dose-blood glucose response relationship, unless an adjustment for non-linearity is made.

INTRODUCTION

The relative glycaemic impact (RGI) of a specified amount of food is defined as the weight of glucose that would give a response equal to that of the food quantity (Miller-Jones, 2007). In other words it is the number of glycaemic glucose equivalents (GGE) in the specified amount of food (Monro and Shaw, 2008). The most practical way to use GGE values in dietary management of glycaemic impact would be to treat them as a linear function of food intake, and to simply add the GGE values of different foods to determine a meal RGI value. However, as GGE reflects physiological changes it will be subject to homeostasis, so will not be a linear function of food intake. It is important to know how much this non-linearity affects both the accuracy with which GGE values are determined, and the accuracy with which they may be applied.

This paper reports the results of a theoretical examination of the effect of homeostasis on measurement and use of GGE. In particular, we have determined the effect of the non-linearity of response that it causes on the accuracy with which GGE values for individual foods in a meal may be simply added to determine the glycaemic impact (RGI) of the meal. A theoretical analysis is justified, firstly because the inter and intra subject variability in measurement of blood glucose responses is so great (CV 20-40%) that the number of comparisons required for the study to be conducted clinically would not be feasible, and secondly, because the glucose dose-blood glucose response relationship on which the study is based is robust, having been replicated in a number of published clinical trials.

METHODS

Effect of homeostasis on blood glucose response to dietary glucose

A glucose dose-glycaemic response curve, reflecting the net homeostatic response to increasing glucose dose, was established by combining published results from seven independent studies of the relationship between glucose dose and glycaemic response (Gannon, 1988; Jenkins et al., 1981; Lee and Wolever, 1998; Wallace et al., 2006; Wolever et al., 1994; Wolever and Bolognesi, 1996; Venn et al., 2006). The results of each study were normalised by expressing them relative to the response to 50 g glucose within each study, which was assigned a value of 50 GGE, and were plotted against glucose dose (Monro and Shaw, 2008) (Figure 1). Trend lines were fitted to show the equation which best represented the effects of homeostasis (Figure 1) and revealed that a quadratic equation gave the best fit.

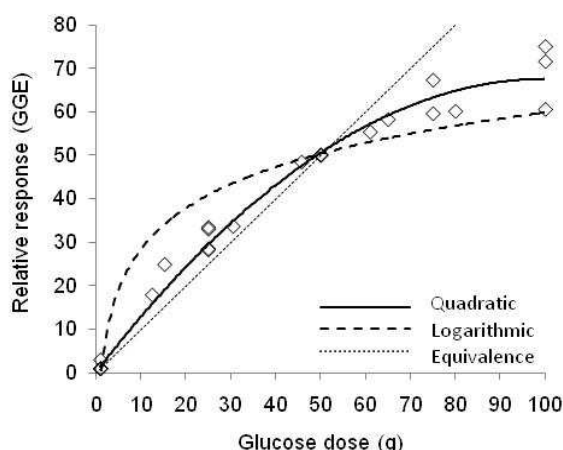


Figure 1: Relationship between glucose dose and glycaemic response. Equations of trendlines:

Quadratic: $y = -0.006x^2 + 1.346x$; $R^2 = 0.981$

Logarithmic: $y = 13.69\ln(x) - 3.227$; $R^2 = 0.894$

Linear: $y = 0.692x + 9.0053$; $R^2 = 0.9138$ (line not shown)

Disparity between actual response and response estimated from responses to single references.

The quadratic equation of the polynomial trendline was used to determine responses on the glucose response curve to glucose doses (references) of 10, 20, 30, 40, 50 and 60 g glucose (Figure 2). For each reference, a straight line extrapolation to the origin (zero) from its response on the quadratic curve was made and the equation of the straight line determined. The difference between the linear extrapolations and the quadratic curve showed the inaccuracies which would result, using each of the above reference doses, if the GGE content of a food was determined from the response to a reference at a location on the dose-response curve separated from the location of the response to the food. The difference between the linear and polynomial trendlines in Figure 2 shows this discrepancy for a reference of 50 g glucose.

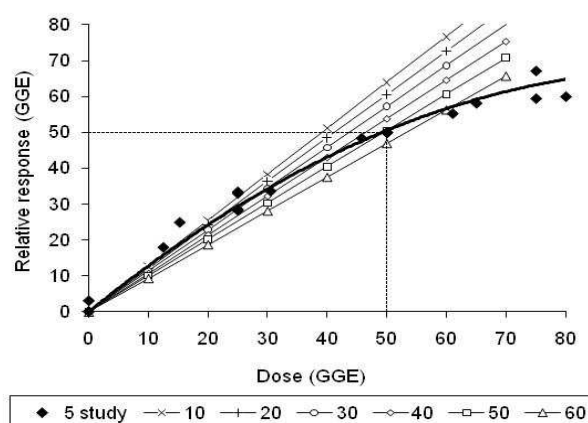


Figure 2: Straight line extrapolations from reference responses to glucose on the quadratic dose-response curve. The equations for the straight lines are in Table 1. The difference between the linear and quadratic is the inaccuracy incurred when a single reference is used to calculate the GGE dose inducing a response separated from the reference, by direct proportion.

Disparity between sum of individual food GGE values and whole meal GGE using different references

A set of realistic breakfast meals based on foods listed in the *International table of glycaemic index and glycaemic load values: 2002* (Foster-Powell et al., 2002), were identified in a recent publication (Wolever et al., 2006), and their glycemic loads (GI x available carbohydrate) were calculated as estimates of the GGE values for the foods. The inaccuracy associated with simple within-meal summation of GGE values of individual foods, based on glucose references of 10, 20, 30, 40, 50 and 60 g, was determined by entering the GGE estimates for the individual foods into the straight line equations for each reference in Table 1, adding the linear GGE estimates for each food obtained from each separate equation, and subtracting them from the quadratic (true) value for the whole meal, calculated by entering its GL value into Equation 1 (Figure 1):

$$\text{GGE (g)} = -0.006\text{GL}^2 + 1.346\text{GL} \quad \text{Equation 1}$$

In this way the discrepancy between the “true” whole meal relative glycaemic impact (GGE quantity) and the sum of the GGE values of the individual foods in the meal was obtained for each meal and each reference (Table 2).

Table 1: Equations of straight lines linking reference glucose responses to the origin (zero)

Glucose reference (g)	Linear equations linking response to reference with zero
10	$y = 1.279x$
20	$y = 1.212x$
30	$y = 1.145x$
40	$y = 1.078x$
50	$y = 1.011x$
60	$y = 0.940x$

Table 2: Accumulation of disparities between true and linear estimates of GGE when adding estimated GGE values for individual foods in a meal. Calculation shown is for one of 13 breakfast meals published by Wolever et al.(2006)

Diet	*GL	†GGE ₅₀	Linear estimates of GGE ₅₀					
			Glucose reference (g)					
			10	20	30	40	50	60
15 g Whole-wheat cereal	6.60	8.6	8.4	8.0	7.6	7.1	6.7	6.2
120 mL 1.4%-fat milk	1.90	2.5	2.4	2.3	2.2	2.0	1.9	1.8
4 g Brown sugar	1.90	2.5	2.4	2.3	2.2	2.0	1.9	1.8
90 g Banana	9.40	12.1	12.0	11.4	10.8	10.1	9.5	8.8
100 mL Orange juice	4.90	6.4	6.3	5.9	5.6	5.3	5.0	4.6
A Sum of food values	24.70	32.2	31.6	29.9	28.3	26.6	25.0	23.2
B Whole meal GE ₅₀		29.16						
A-B (inaccuracy)		3.0	2.4	0.8	-0.9	-2.5	-4.2	-5.9

* GL calculated from carbohydrate x glycaemic index values in International tables of glycaemic index and glycaemic load values: 2002 (Foster-Powell et al., 2002).

† Calculated by entering the GL values into Equation 1.

B "Whole meal" GGE is calculated by entering the GL sum for the meal into Equation 1. This represents the actual response to the meal.

Dependence of inaccuracy on number of foods and GGE total

The theoretical dependence of cumulative inaccuracy on the GGE content of a meal, and on the number of contributing foods involved was tested for 4 meals with total GLs of 20, 30, 40 and 50, with the totals distributed equally between 1, 2, 3 or 4 foods within each meal. True GGE for the whole meal was calculated using Equation 1, as well as the GGE for each food, then for each meal the sum of the GGE values for the foods was added, and the sum total subtracted from the GGE for the whole meal to obtain a value for the total of the inaccuracies of all of the foods within the meal (Figure 4).

RESULTS AND DISCUSSION

The present paper has shown that homeostasis, which may include both blood glucose clearance and gastric emptying components, may have to be allowed for to accurately determine the glycaemic impact of meals.

Trendlines fitted to the normalised glucose dose-glycaemic response relationship (Figure 1) revealed that the relationship was most adequately represented by a quadratic equation up to a dose of at least 100 GGE. As the carbohydrate in most foods has a glycaemic index of less than 100, for most foods a GGE of 100 would require an intake of much more than 100 g carbohydrate.

For the 13 breakfast meals analysed, the greatest accuracy, on average, would have been achieved using a glucose reference dose of 31.6 g glucose, which is the point of true glucose equivalence (Figure 3). In contrast, by using a reference such as 50 g glucose, which is almost 20 GGE units removed from the GGE content of the food, there would have been a loss of accuracy. Glycaemic load can be an inaccurate estimate of glucose equivalence because it is based on response relative to a 50 g glucose dose, because that is the usual reference used in GI determination. However, if a glucose standard curve is used it is possible to align the responses to reference and food to achieve accuracy (Wallace et al, 2008).

Just as greatest accuracy in determining GGE values for individual foods is achieved by aligning the reference and food responses, determination of the glycaemic impact of meals consisting of several foods is most accurate if based on the effect of the whole meal. In direct clinical measurement a glucose reference curve can be used, but if the meal GGE content is to be determined by summation of the GGE values for individual foods account must be taken

of the accumulation of disparity between the added responses and the response to the meal as a whole.

When accurate GGE values for individual foods are added the inaccuracy of the value for the whole meal soon grows to exceed tolerable limits (Figure 4). However, when the total GGE dose in a meal is reasonably low, and the number of carbohydrate foods involved is small, the accumulated error is also small.

To calculate the glycaemic impact of large carbohydrate intakes derived from several foods the problem of inaccuracy may be overcome by converting the GGE value of each food to its linear equivalent, i.e. to the value that would have been determined from the same single reference, adding these linear values, and then reconverting the total linear sum to its corresponding quadratic value.

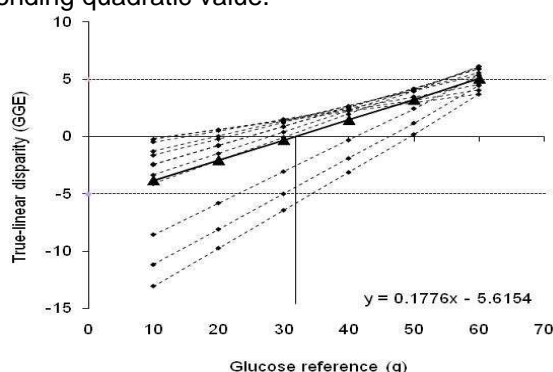


Figure 3: Differences between true relative effects of meals (whole meal GGE) and the meal GGE estimated by adding the GGE values of the individual foods, as a function of reference dose used, for 13 breakfasts given in Wolever et al. (2006). The equation of the curve of mean disparity (solid triangles) shows that a greatest accuracy for the 13 meals would be achieved using a reference of 31.6 g glucose.

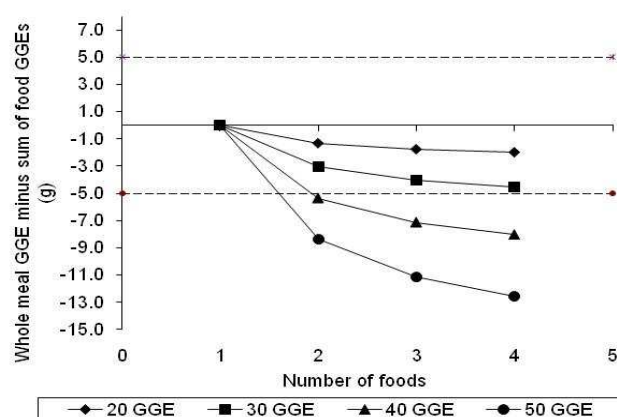


Figure 4: The dependence of disparity between the sum of the GGE values of individual foods in a meal, and the GGE value for the meal as a whole, on the number of foods in a meal and on the total GGE content of the meal, shown for four meals of 20, 30, 40 and 50 GGE. The foods in each meal were assumed to be of equal GGE content.

CONCLUSIONS

The intrinsic non-linearity of the glucose dose-blood glucose response relationship imposes limits on the linear summation of the GGE values of individual foods to determine the glycaemic impact of a meal, requiring caution in dietary management and epidemiological interpretation. The results have shown that the effects of homeostasis may need to be allowed for when using food values for the efficacy of functional foods to predict the efficacy of meals in which the foods are combined.

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The effect of dietary vegetable and fruit fibres on gut health in healthy rats

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ABSTRACT

Background: Undigested dietary fibre from the small intestine enters the large intestine and becomes a fermentation substrate for colonic microbiota. The main fermentation products of dietary fibre, short chain fatty acids such as acetic, propionic and butyric, are rapidly absorbed in the colonic mucosa and have positive physiological effects on gut health.

Objective: To measure the effect of dietary fibre from apple and broccoli on gut health parameters in healthy rats.

Design: Sixty-four male Sprague-Dawley rats (6 weeks of age) were fed four experimental dietary treatments (16 rats per treatment) for 6 weeks. The dietary treatments were low fibre (2.5% cellulose), mixed fibre (3.75% cellulose + 3.75% pectin), broccoli fibre (5% + 2.5% cellulose), and apple fibre (5% + 2.5% cellulose).

Outcomes: There was a significant increase in caecum butyric acid ($p=0.048$) concentration in rats fed broccoli and apple fibre. Caecum lactic acid concentrations were significantly ($p=0.020$) increased only in rats fed broccoli fibre. There was no significant effect on specific bacteria in the caecum of rats fed experimental diets. Colon goblet cell numbers were significantly ($p=0.076$) increased only in rats fed the broccoli fibre diet. There was no significant effect of diet on colon crypt depth in the rats fed the experimental diets.

Conclusion: There was evidence of positive gut health benefits when rats were fed broccoli and apple fibres.

INTRODUCTION

Several studies have reported the beneficial effects of increased dietary fibre intake in promoting positive health effects in patients with obesity and diabetes. Weickert et al. (2006) reported the significance of dietary fibre supplementation in improving carbohydrate metabolism and insulin sensitivity in overweight and obese women. Furthermore, improved glycemic control, decrease in hyperinsulinemia and lower plasma lipid levels were detected in type 2 diabetes mellitus patients after high intake of soluble dietary fibre (Chandalia et al. 2000). Fermentable dietary fibres are also capable of modulating epithelial crypt cell proliferation and enhancing goblet cells in the large intestine, particularly in the colon. The mucus layer covering the gut epithelium is an important part of the innate immune system and is the first physical line of defence against pathogens and harmful foreign antigens passing through the gastrointestinal tract. The present study aimed to determine the effect of dietary fibre prepared from New Zealand-grown apples and broccoli on gut health parameters in healthy rats.

METHODS

Sixty-four weaned male Sprague-Dawley rats (21–23 days, 45–50 g) were housed in hanging cages and fed a commercial pelleted feed for 21 days. At 6 weeks of age, rats were randomly assigned (16 rats per treatment) to the experimental diets. The study was carried out with ethics approval (Application number 11572) from the AgResearch Grasslands Animal Ethics Committee. The four experimental diets were low fibre (2.5% cellulose), mixed fibre (3.75% cellulose + 3.75% pectin), broccoli fibre (5% + 2.5% cellulose) and apple fibre (5% + 2.5% cellulose). The compositions of the experimental diets are given in Table 1.

Table 1: Ingredient composition (%) of experimental diets

Ingredient	Low fibre	Mixed fibre	Broccoli fibre	Apple fibre
Lactic casein ¹	12.0	12.0	12.0	12.0
Starch ²	65.0	65.0	65.0	65.0
Sugar ³	4.0	4.0	4.0	4.0
Corn oil ⁴	6.5	6.5	6.5	6.5
Vitamin mix ⁵	5.0	5.0	5.0	5.0
Salt mix ⁶	5.0	5.0	5.0	5.0
Cellulose ⁷	1.25	3.75	1.25	1.25
Pectin ⁸	1.25	3.75	1.25	1.25
Broccoli fibre ⁹			5.0	
Apple fibre ¹⁰				5.0

¹Alacid 80 mesh, New Zealand Milk Products, Wellington, New Zealand

²Wheaten cornflour, Golden Harvest, Primary Foods Ltd, Auckland, New Zealand

³Caster sugar, Chelsea Sugar Company, Auckland, New Zealand

⁴Davis Trading Company, Palmerston North, New Zealand

⁵A mixture prepared at Plant & Food Research that supplied (mg/kg diet): retinol acetate 5.0, dl- α -tocopheryl acetate 100.0, menadione 3.0, thiamin hydrochloride 5.0, riboflavin 7.0, pyridoxine hydrochloride 8.0, d-pantothenic acid 20.0, folic acid 2.0, nicotinic acid 20.0, d-biotin 1.0, myo-inositol 200.0, choline chloride 1500; (μ g/kg diet): ergocalciferol 25.0, cyanocobalamin 50.0.

⁶A mixture prepared at Plant & Food Research that supplied (g/kg diet); Ca 6.29, Cl 7.79, Mg 1.06, P 4.86, K 5.24, Na 1.97; (mg/kg diet): Cr 1.97, Cu 10.7, Fe 424, Mn 78.0, Zn 48.2; (μ g/kg diet): Co 29.0, I 151, Mo 152, Se 151.

⁷Ceolus PH102, Commercial Minerals Ltd, Auckland, New Zealand

⁸Sigma, St Louis, MO, USA

⁹Prepared at Plant & Food Research, Auckland, New Zealand

¹⁰Prepared at Plant & Food Research, Palmerston North, New Zealand

The rats were fed the experiment diets for 6 weeks. Rat live weights and food intakes were recorded every 7 days. At the end of the 6-week feeding period, the rats were euthanized and caecum digesta and colon tissues were taken for analysis. The microbiota and short-chain fatty acid analyses in the caecum digesta, and colon morphological changes were performed according to Paturi et al. (2010). The results were statistically analysed using one-way analysis of variance (ANOVA), followed by post-hoc analysis by least significant difference (LSD) test. A value of $p < 0.05$ was considered statistically significant. All analyses were carried out using GenStat 11th edition (VSN International, Hemel Hempstead, UK).

RESULTS

Rats remained healthy and gained weight during the 42-day trial. There were no significant differences ($p > 0.05$) in live weight or food intake for rats fed the experimental diets (Figures 1 and 2). Short-chain fatty acid concentrations in the caecum of rats were similar across the diets (Figure 3) except for butyric and lactic acids. The rats fed the low fibre diet had significantly lower concentrations of butyric acid ($p = 0.048$) than those fed broccoli and apple fibres. Lactic acid concentrations in the caecum of the rats fed broccoli fibre was significantly higher ($p = 0.020$) than in the rats fed the other dietary treatments.

There were no significant differences ($p > 0.05$) in bacterial numbers measured in the caecum of the rats fed the four experimental diets (Figure 4). Goblet cell numbers in the colon were significantly ($p = 0.076$) increased only in rats fed the broccoli fibre diet (Figure 5). Colon crypt depths were not affected ($p = 0.237$) by dietary treatment (Figure 6).

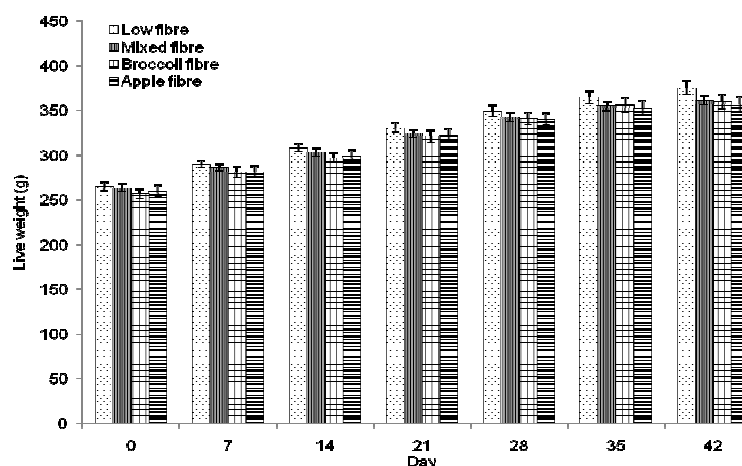


Figure 1: Effect of dietary fibres on live weight (g) in rats during a 42-day experimental feeding period. Values are means \pm SEM, $n=16$.

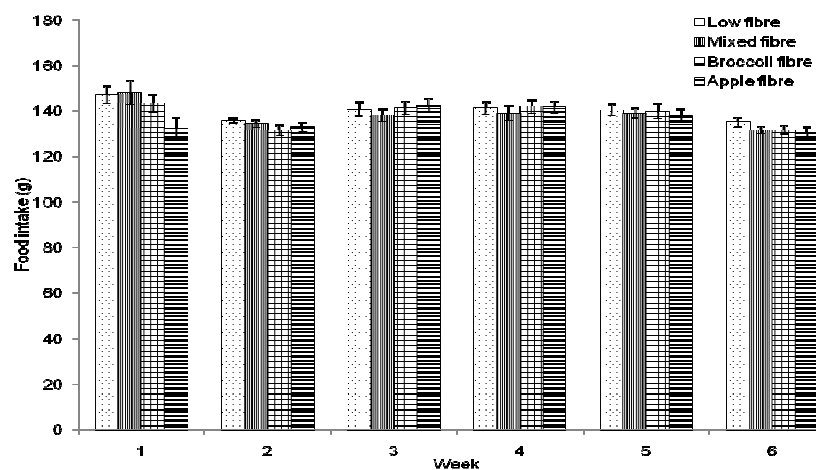


Figure 2: Effect of dietary fibres on food intake (g/7 days) in rats during a 42-day experimental feeding period. Values are means \pm SEM, $n=16$.

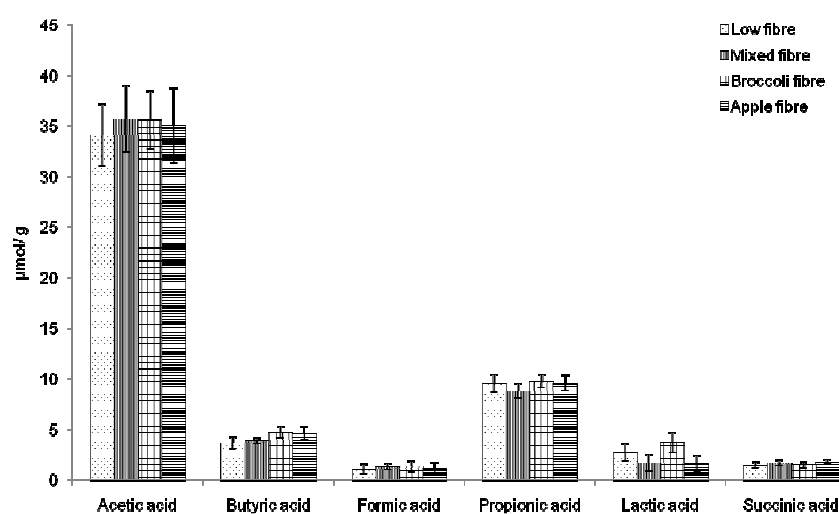


Figure 3: Effect of dietary fibres on caecum short-chain fatty acid concentration ($\mu\text{mol/g}$) in rats during a 42-day experimental feeding period. Values are means \pm SEM, $n=16$.

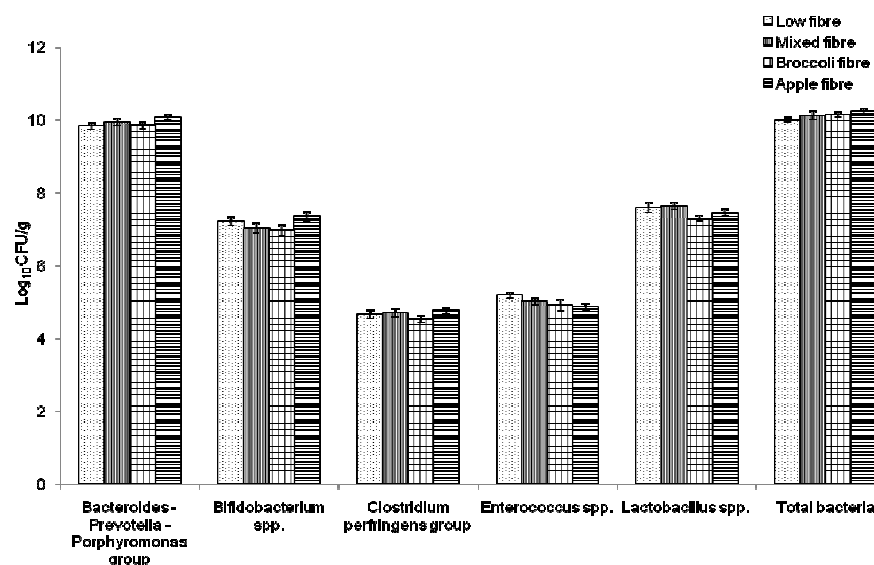


Figure 4: Effect of dietary fibres on caecum microbiota (\log_{10} CFU/g) in rats during a 42-day experimental feeding period. Values are means \pm SEM, $n=16$.

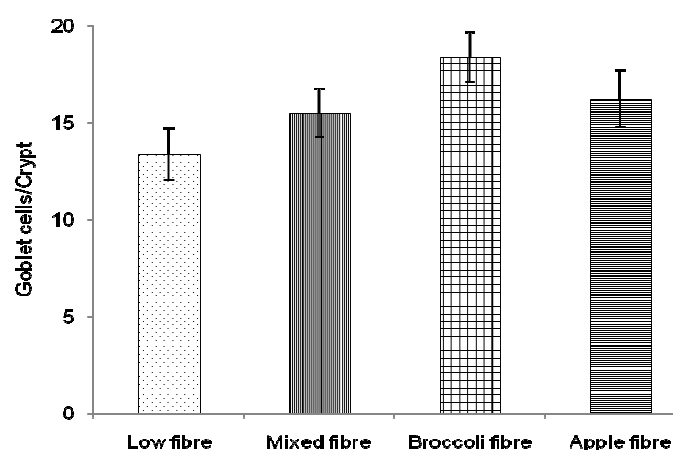


Figure 5: Effect of dietary fibres on colon goblet cell count (cells/crypt) in rats during a 42-day experimental feeding period. Values are means \pm SEM, $n=16$.

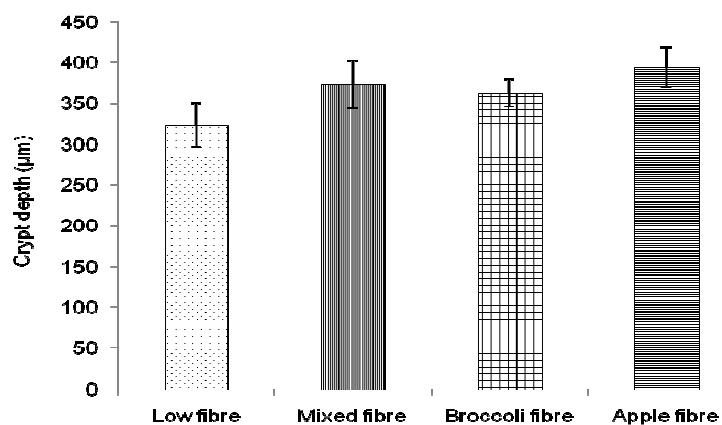


Figure 6: Effect of dietary fibres on colon crypt depth (μ m) in rats during a 42-day experimental feeding period. Values are means \pm SEM, $n=16$.

DISCUSSION

Rats fed diets supplemented with broccoli and apple fibres had increased levels of butyric acid in the caecum, and rats fed the broccoli fibre diet has increased levels of caecal lactic acid and numbers of goblet cells in the colon. Butyric acid is a preferred energy source for colonocytes (Pryde et al. 2002), and the bacterial groups that produce butyric acid are thus interesting because they confer health benefits to the host. This increase in butyric acid could be a result of metabolic cross-feeding between bacteria (Duncan et al. 2002) or of bacteria possessing CoA-transferase being stimulated by the presence of acetate to produce butyrate (Duncan et al. 2002). In the present study there was no change in bacterial numbers. This result is supported by the study of Humblot et al. (2005) who found no increase in *Lactobacillus* spp. numbers when they fed rats 10% freeze-dried Brussels sprouts, but is in contrast to a study investigating faecal bacterial composition in healthy humans following the consumption of cruciferous vegetables (a mixture of broccoli, cauliflower, green and red cabbage, and radish sprouts) where there were changes in the bacterial community composition (Li et al. 2009).

Dietary fibre has been shown previously to increase colon goblet cells, crypts per circumference and crypt branching in rats (McCullough et al. 1998, Paturi et al. 2010). The fermentation of the broccoli fibre in the large intestine produces short-chain fatty acids that stimulate intestinal cell proliferation and mucosal growth (Sakata 1987), and the release of glucagon-like peptide-2 (Tappenden et al. 2003), an intestinal hormone that stimulates cell proliferation and increases villus height and crypt depth (Drucker 2003). In addition, the presence of probiotic bacteria such as *Lactobacillus casei* or *Clostridium butyricum* has been shown to increase gut epithelial cell proliferation (Ichikawa et al. 1999).

CONCLUSIONS

We found evidence that the consumption of apple and broccoli fibres improves some gut health parameters. In particular, consuming apple fibre increased caecal butyric acid levels while consuming broccoli fibre increased the levels of lactic and butyric acids in the caecum as well as the number of goblet cells (mucin-producing cells) in the colon.

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The glycaemic response of concept snack bars made from starches with different digestibility

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ABSTRACT

Background: There is a market for reduced blood glucose response foods with high consumer appeal and validated nutrition and health advantages.

Objectives: To determine the blood glucose response in humans of snack bars made with: a) a vegetable starch-gluten mixture replacing white flour and b) a wheat starch-gluten mixture replacing white wheat flour, in order to determine the effects of the different starches in a background of approximately equivalent protein, starch, lipid and fibre. Blood glucose responses were compared with those from a bar made with standard white wheat flour.

Design: Snack bars developed at The New Zealand Institute for Plant & Food Research Limited, Lincoln, were tested in 13 free-living individuals. Bars were consumed in fifty-gram serve sizes, and oral glucose loads of 5, 12.5, and 25 g in 320 ml carbonated water were also measured on two occasions. Subjects attended the clinic after an overnight fast and capillary blood samples were taken fasting (two) and every 15 minutes for the first hour and every half hour for the second hour.

Outcomes: The vegetable starch-gluten bar elicited a lower blood glucose response than the standard white bar (13.0 glycaemic glucose equivalents (GGE) compared with 23.4 GGE, approximately a 44% reduction, $p=0.035$). The vegetable starch-gluten bars also elicited a lower blood glucose response than the wheat starch-gluten bars made on an equal starch, protein and fibre basis (13.0 GGE cf. 19.2 GGE), but the effect was not statistically significant ($p=0.206$).

Conclusion: The vegetable starch bars showed an ability to modulate glycaemic response favourably in this setting; however, more work is needed to determine whether a significant difference in blood glucose response will result between a vegetable starch bar and a wheat starch bar of equivalent protein, starch, lipid and fibre.

INTRODUCTION

Reducing the glycaemic response of foods is a useful element of dietary management that may provide significant health benefits to consumers (Livesey et al., 2008). Substantial evidence exists to show that glycaemic response is linked to several pathological conditions of metabolism and health such as insulin resistance (IR) and its associated co-morbidities (metabolic syndrome, a combination of IR, hyperinsulinaemia, obesity, hypertension, elevated low density lipoprotein cholesterol (LDL) and triglycerides and reduced high density lipoprotein cholesterol (HDL), all of which are frequently associated with type 2 diabetes and cardiovascular disease (Cordain et al., 2005)). The glycaemic response may also affect body weight, which provides a further potential benefit of low glycaemic foods in the area of weight management, by reducing energy intake and affecting feelings of satiety (Niwano et al., 2009).

Manipulation of the blood glucose response in food is commonly achieved through the replacement of the available carbohydrate source with a carbohydrate source that is less readily available and more slowly digested, to provide a sustained release of glucose into the body, resulting in less extreme glucose fluctuations, which extremes are known to contribute to disrupted glucose metabolism and its detrimental health consequences. Several vegetable and arable products have been identified (e.g. lentils, oats, rye) that can be used to produce

flour with a chemical composition that renders glucose less readily available for digestion. These can then be used to design functional foods with a specific glycaemic impact that may ultimately offer consumers more appropriate food selections. The objective of this randomised controlled human clinical study was to determine the glycaemic glucose equivalents (GGE) of a new concept snack bar made from starch derived from garden peas, which had been previously identified as producing a lower blood glucose response. Pea flour may be an inexpensive substitute for wheat flour to create healthier, low glycaemic index versions of typically high glycaemic index foods, such as cookies, breads, and pasta. The GGE of this bar was compared with those of a bar derived from wheat starch and one derived from standard wheat flour. *In vivo* GGE values were compared with *in vitro* GGE values, as comparisons have shown that the *in vivo* and *in vitro* methods correlate well. Such *in vitro* testing may provide a useful tool for measurement of glycaemic response in situations where *in vivo* measurement is not possible.

METHODS

Thirteen participants (nine women and four men) were recruited. The mean age was 45 years (range 21-68 years) and the mean body mass index (BMI) was 26 kg/m² (range 20-34 kg/m²). Participants were healthy and non-smokers. They were required to have normal glucose tolerance with fasting blood glucose levels of ≤ 6.1 mmol/L as classified by the World Health Organisation (WHO, 2006). Other exclusion criteria included renal, hepatic, cardiovascular, endocrine, gastrointestinal disorders and pregnancy. All participants gave informed consent and the study was approved by the Canterbury Ethical Committee.

To produce glucose reference curves, glucose solutions of 5 g, 12.5 g and 25 g in 320 ml carbonated water were measured on two occasions to obtain a standard curve. The test foods were: Standard white flour snack bar, Wheat flour concept snack bar and Pea flour concept snack bar. All bars were provided without filling. 50 g portions of each bar were fed to the participants along with 250 ml water on two separate occasions, and testing was completed over a period of six weeks.

Snack bars were formulated from pea starch (cultivar 'Sonata'), wheat starch (commercial brand) and standard white flour (wheat cultivar 'Epic') from a recipe designed at The New Zealand Institute for Plant & Food Research Limited. Adjustments were made so the resulting products would be as close as possible equivalents in terms of protein, starch, fibre and moisture content.

Subjects attended the clinic after an overnight fast and capillary blood samples were taken fasting (two) and every 15 minutes for the first hour and every half hour for the second hour. At 120 minutes, if the glucose measurement was still raised by greater than 0.2 mmol/L above baseline, further blood samples were collected every half hour until baseline was reached, up to a maximum of three hours. Blood samples were analysed using a Hemocue® Glucose 201 Analyser (Helsingborg, Sweden). *In vitro* GGE were determined using the method of Monro et al. (2010).

Incremental areas under the curve (iAUC) were calculated for test foods and reference drinks. Glycaemic Load (GL) values were calculated as the average of the responses of 13 participants, with each individual GL calculated by comparing the iAUC of the bars with the iAUC of the glucose reference curve (Wolever et al., 1994). Paired t-tests were used for statistical comparisons.

RESULTS AND DISCUSSION

The results of the *in vivo* testing, illustrated in Table 1, indicate that the standard white flour bar had a high GL rating, whilst both pea starch-gluten and wheat starch-gluten bars had a medium rating, although the pea starch-gluten bars elicited a considerably lower glycaemic response than the "equi-starch" wheat starch-gluten bars. There was good agreement between the *in vivo* and *in vitro* tests in terms of measuring GGE.

Furthermore, replacing the standard flour with the pea starch-gluten combination reduced the *in vivo* response by 44% compared with that from the standard white flour bar ($p=0.035$), and replacing standard flour with the wheat starch-gluten combination reduced the *in vivo* response by 18% ($p=0.032$). Comparing the wheat starch-gluten bars with the pea-starch gluten bars, the pea starch gluten bars elicited a reduced GGE (13 GGE v. 19 GGE,

~32%); however, this was not statistically significant ($p=0.206$), probably because of the small numbers of participants in this study.

Table 1: GGE values/serve of snack bars as provided by in vivo and in vitro tests.

Glycaemic Load results.				
Food tested	GL/50 g serve (GGE)* <i>In vivo</i>	GL rating	GL/50 g serve (GGE)* <i>In vitro</i>	GL rating
Standard white flour bar	23.4	high	22.5	high
Pea starch – gluten bar	13.0	medium	14.0	medium
Wheat starch – gluten bar	19.2	medium	18.5	medium
*Low GL = 0–10 per serve, Medium GL = 11–19 per serve, High GL = greater than 20 per serve.				

Throughout the trial, participants described the bars as having an acceptable taste but (generally) they found them to have a slightly dry mouth feel.

This study shows that in comparison with the standard white flour snack bar, which had a high glycaemic load rating (23 GGE), both the pea starch-gluten and the wheat starch-gluten snack bars had a reduced glycaemic load value (13 GGE and 19 GGE respectively), providing them with a medium GL rating. However, upon further analysis of the composition of the bars, it was shown that although the standard bar was matched for sugar with the pea and the wheat starch bar, it did contain more starch (16.1 g v. 10.9 g), which reduces the validity of the comparison. It does, however, demonstrate that manipulating the bars to contain starches of different digestibility and fibre content may reduce the GGE. Table 2 provides the starch and sugar content of the bars.

Table 2: Starch and sugar contents of the snack bars.

	Control	Pea starch – gluten	Wheat starch – gluten
Sugar content (g) per 50-g bar	7.9	7.7	8.1
Starch content (g) per 50-g bar	16.1	10.9	10.9
Sugar + starch content (g) per 50-g bar	24.0	18.6	19.0

The starch/sugar contents of the pea starch-gluten bar and wheat starch-gluten bar were similar. The ability of pea starch to reduce glycaemic load more effectively than wheat starch may be related to the composition of the starch, which contained higher amounts of amylose (65.9%) than amylopectin (34.1%), in addition to high amounts of insoluble fibre (31.68%). Amylose is a linear polymer of glucose linked by α (1→4) bonds, which has a tightly packed structure much less available to hydrolysis by digestive enzymes than amylopectin, which is a highly branched glucose polymer. Comparatively, the wheat starch contained 30% amylose and 70% amylopectin.

CONCLUSION

The pea starch bars showed an ability to modulate glycaemic response favourably in this experiment. However, more work is needed to determine whether a significant difference in blood glucose response is elicited between a pea starch-gluten bar and a wheat starch-gluten bar of equivalent protein, starch, lipid and fibre. Pea starch may be a valuable ingredient in the production of functional foods. This readily available ingredient is useful not

only for its impact on lowering blood glucose but also for its favourable sensory characteristics when incorporated into food products, including appearance, taste, smell, texture and overall acceptability. Pea flour could then be used in combination with other modifications, such as replacement of glucose and sucrose with fructose, to achieve larger reductions in blood glucose response.

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Total phenolic content of Tommy Atkins mangoes imported into New Zealand

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ABSTRACT

Background: Mango (*Mangifera indica* L.) is an important tropical fruit and has widespread consumer appeal. The major countries producing mangoes are India, Mexico, Brazil, China, Thailand, Indonesia, Philippines, Vietnam, Egypt and Nigeria. Mango is recognised as a rich source of antioxidants, carotenoids, tocopherol, Vitamin C and phenolic compounds. Phenolic compounds, in particular, have received a lot of publicity as natural antioxidants due to their protective effects against cancer and heart diseases and their contribution to the total antioxidant capacity of foods.

Objectives: The total phenol content of the mango cultivar, Tommy Atkins, imported into New Zealand, is reported. The phenol profile of extracts from the peel, flesh and kernel was determined in order to provide information as to whether these mango components would be viable source of phenols for human consumption.

Design: Trays of mangoes from a major fruit importer were purchased and twelve mangoes were randomly selected. Each mango was photographed, colour on two sides recorded using a Hunter Colorimeter and then weighed. Physicochemical characteristics including firmness, total soluble solids (TSS), titratable acidity (TA) and moisture content of Tommy Atkins mangoes were analysed. The total phenolic content of the peel, flesh and kernel was estimated using the Folin-Ciocalteu method. A similar procedure was used to determine the total phenolic content of extracts obtained in Vietnam from freeze-dried Vietnamese mango varieties.

Outcome: The total phenolic content of the flesh, peel and kernel of the cultivar, Tommy Atkins, imported into New Zealand were 532 ± 83 , 4858 ± 974 and 10749 ± 1479 mg GaE/100g of dry matter, respectively. Freeze-dried samples of Vietnamese mangoes show a similar distribution of total phenols in flesh, peel and kernel, ranging from 253 ± 95 to 699 ± 206 , 822 ± 164 to 4120 ± 1172 and from 6286 ± 50 to 10664 ± 2077 mg GaE/100g of dry matter, respectively.

Conclusion: The mango flesh of Tommy Atkins cultivar imported into New Zealand is a good source of total phenols. The peel and kernel yield even greater amount of phenols and are considered as a potential commercial source of total phenols and antioxidants.

INTRODUCTION

There is growing consumer interest in the consumption of functional and nutraceutical foods particularly fruits and vegetables, since epidemiological studies have shown there is a strong correlation between an increase in the dietary intake of fruits and vegetables and a reduction in the incidence of chronic/degenerative diseases. The beneficial health effects have been attributed to the presence of antioxidants that scavenge free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), both in food and in the body (Locatelli et al., 2009). Indeed, an overproduction of ROS and RNS, which are secondary metabolites in normal physiological functions, can induce destructive and lethal cellular effects. Synthetic antioxidants used as food additives may have carcinogenic side effects if included in diets at high levels (Tappel, 1995). Antioxidants from fruit and vegetables offer food processors the opportunity to replace synthetic antioxidants with a natural alternative source (Gulcin et al., 2007).

Mango (*Mangifera indica* L.) is an important tropical fruit with a global production in 2005 of 28 million tons (Azoubel & Silva, 2008). The fruit has widespread consumer appeal due to its excellent eating qualities (bright colour, sweet taste and luscious flavour) and nutritional attributes. Mango flesh is a rich source of the antioxidants carotenoids, tocopherol, vitamin C and phenolic compounds (Aguilar et al., 2007). Recently, researchers suggested

that mango seed kernels and peel are also potential sources of antioxidants (Puravankara et al., 2000; Arogba, 2002; Berardini et al., 2005; Soong & Barlow, 2006; Abdalla et al., 2007; Ribeiro et al., 2008; Pitchaon & Gordon, 2009). The seed constitutes 10-25 % of the whole fruit (Hemavathy et al., 1988) and peel 15-20% (Ajila & Rao, 2007) depending on the variety. The seed and peel of mangoes may, therefore, be a new natural dietary source of antioxidants and should have no undesirable health effects.

Tommy Atkins is the most important mango cultivar in the global trade and the most popular mango imported into New Zealand. However, Tommy Atkins contains low to medium levels of phenolics compared to cultivars such as Uba, Palmer (Ribeiro et al., 2007), Keitt, Kent, Haden and Ataulfo (Gustavo, 2007; Manthey & Veazie, 2009). Recent studies showed that the flesh, peel and kernel of Tommy Atkins were potential sources of antioxidants (Ribeiro, 2008). There is no information on the antioxidant capacity of Tommy Atkins mangoes imported into New Zealand or the changes that might occur from postharvest treatments. Phenolic compounds, in particular, have received a lot of publicity as natural antioxidants due to their protective effects against cancer and heart diseases and their contribution to the total antioxidant capacity of foods (Zulueta et al., 2007).

The aim of this study is to determine whether the peel, flesh and kernel of Tommy Atkins mangoes imported into New Zealand are viable sources of phenolic compounds for human consumption. Also examined was the relationship between the concentration of phenolic compounds and the physicochemical characteristics of the Tommy Atkins mangoes. The total phenolic content of Tommy Atkins mangoes was also compared to the total phenolic concentrations of peel, flesh and kernel from mango cultivars sourced and sampled in Vietnam.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and solvents were analytical grade. Acetone and sodium carbonate anhydrous were obtained from BioLab. Folin Ciocalteu phenol reagent and gallic acid were purchased from Sigma Chemical Co.

Mango and sample preparation

In New Zealand, trays of Tommy Atkins mangoes imported from Mexico were purchased from a major fruit importer and twelve mangoes were randomly selected at the ready-to-eat stage. Each mango was photographed and colour parameters determined using a Hunter Lab Colorimeter. The peel (outer skin) was removed with a fine blade then the flesh and seed were separated. The kernel inside the seed was removed manually using a hammer or knife. The whole mango, peel, flesh, seed and kernel were weighed. Flesh was homogenised in a Grindomix fruit processor (model GM 200) at 10,000 rpm and kept at 4°C prior to solvent extraction. The total soluble solids (TSS), titratable acidity (TA) and moisture content of the mango flesh were determined using standard procedures. Peel was cut into small pieces with a scalpel, immediately frozen in liquid nitrogen and then ground in a coffee grinder for 20 seconds. The frozen finely ground peel was kept at -40°C before extraction. The mango kernel was finely ground under the same conditions as the peel. Four mango cultivars (Ghep, Cat Chu, Cat Hoa Loc and Nam Dok Mai) grown and harvested in Vietnam were selected from Vietnamese retail markets. Flesh, peel and kernels of the Vietnamese mangoes were freeze-dried, finely ground and then transported to New Zealand for analysis of total phenolics.

Mango physicochemical characteristics

Twelve Tommy Atkins mangoes were analysed for their physicochemical characteristics. Firmness was determined using a Fruit Pressure Tester (FT 327, Italy) equipped with an 6 or 10mm-diameter plunger tip. Mango colour on two sides was recorded using a Hunter Colorimeter. Moisture content was determined gravimetrically by oven drying at 105°C. Total soluble solids (TSS) was determined using a hand refractometer (model WZ103) and the results were expressed as °Brix. Titratable acidity (TA) was determined by the AOAC method 942.15 (AOAC, 1990). TSS/TA ratio was calculated.

Extraction of mango fractions

Approximately 2 g of peel, flesh and kernel were weighed into 50 ml volumetric flasks followed by 20 ml of 80% (v/v) acetone. The flasks were rotated on a rotary shaker in a dark cold room (4°C) for 2 hours. The volume of the mixture was then adjusted to 50 ml and the contents transferred to 50 ml plastic centrifuge tubes which were centrifuged at 3500 rpm for 10 minutes. The resulting supernatant was transferred to glass tubes and kept at -40°C prior to analysis.

Determination of total phenolics

The total phenolic content of the peel, flesh and kernel were estimated using the Folin-Ciocalteu method as described by Singleton (1999) with some minor modifications. Gallic acid standard (75 µg/ml) was prepared and a standard curve established by aliquoting 0-0.5ml of the gallic acid standard and diluting to 0.5 ml with 80% acetone. Supernatants of the peel and kernel extracts were diluted 10 and 20 times, respectively with 80% acetone prior to the phenolic assay. The flesh extracts required no dilutions. For the assays, an aliquot (0.5 ml) of the extracts were mixed with 2.5 ml of the Folin–Ciocalteu reagent (0.2 N) and after 8 minutes at room temperature, 2 ml of 7.5% (v/v) sodium carbonate was added. The mixture was vortexed, incubated at 50°C in a water bath for 5 minutes and then cooled in an ice bath. The absorbance was measured at 765 nm with a spectrophotometer (Unicam UV/Visible). Results were expressed as mg of gallic acid/100 g dry weight sample. A similar procedure was used to determine the total phenolic content of the peel, flesh and kernel extracts obtained from the freeze-dried Vietnamese mango samples.

Statistical analysis

Each analysis was performed in triplicate. The differences between the total phenolic content of the Tommy Atkins and varieties of Vietnamese mangoes were determined using a one-way analysis of variance (ANOVA) at $p < 0.05$. Significant differences between means were determined using the Fisher's LSD multiple comparison tests. The relationship between the total phenolics in the mango fractions and peel colour was investigated, using the L, a^* , b^* , Hue and Chroma values in a multiple linear regression model (Jha et al., 2007). Pearson's correlation coefficients were calculated with $p < 0.05$ to determine whether there was a relationship between the physicochemical characteristics and total phenolic content of the Tommy Atkins mangoes. Minitab 15 was used to analyse the data.

RESULTS AND DISCUSSION

Mango physicochemical characteristics

Table 1: Physicochemical characteristics of Tommy Atkins mangoes

Cultivar	Colour parameters								
	Firmness (Kg/cm ²)	TSS (°Brix)	TA (%)	TSS/ TA	L	a^*	b^*	Hue	Chroma
Tommy Atkins	25.3 ± 10.6	6.7 ± 2.0	0.9 ± 0.2	8.3 ± 3.8	32.1 ± 4.1	18.8± 6.1	16.6± 5.4	1.5 ± 0.8	27.3 ± 5.5

Note. Values expressed as means ± standard deviation, n=12

The measured parameters of Tommy Atkins mangoes are shown in Table 1. The mean weight of the randomly selected Tommy Atkins mangoes was 365.55 ± 0.28 g and the percentages of flesh, peel and kernel were 77.57 ± 2.95 , 9.07 ± 1.84 and $5.48 \pm 1.25\%$, respectively. The mean moisture content of mango flesh was $82.92 \pm 2.11\%$. The colour of Tommy Atkins mangoes was green with dark red blushes. The colour did not change with maturity, which suggests that full colour development of the peel had occurred early during

transportation to New Zealand or in storage. Thus, colour in this instance was not a good indicator of mango ripeness or potential shelf life.

The Tommy Atkins mangoes contained $0.88 \pm 0.21\%$ TA (wt/wt basis) and $6.72 \pm 2.0\%$ °Brix. The Brix value is lower than 12.0, which is considered by Ribeiro et al. (2007) to be the ideal value that mangoes should attain for consumption. Firmness is another parameter used to test the ripeness of mangoes. The firmness was 25.32 ± 10.65 kg/cm², which is relatively high indicating that the retail mangoes are presented to consumers in a 'green' condition.

Total phenolic content in the peel, flesh and kernels

Table 2: Total phenolic content of flesh, peel and kernel of mangoes sourced from New Zealand and Vietnam

Cultivars	Total phenolics (mg GAE/100g DM)		
	Flesh	Peel	Kernel
Tommy Atkins (n=12)	532 ± 83a	4858 ± 974a	10749 ± 1479a
Cat Chu (n=3)	699 ± 206d	4120 ± 1173ac	10664 ± 2077a
Nam Dok Mai (n=3)	379 ± 145ab	8089 ± 1775d	6987 ± 577b
Ghep (n=3)	253 ± 95bc	822 ± 164b	6286 ± 50b
Hoa Loc (n=3)	397 ± 118ac	2886 ± 463c	7973 ± 764b

GAE, gallic acid equivalents; DM, dry matter. In each column, different superscripts a,b,c,d mean significant differences ($p < 0.05$)

The flesh of Cat Chu had the highest ($p < 0.05$) total phenolic content of any of the five cultivars tested, followed by Tommy Atkins then Hoa Loc and Nam Dok Mai which had similar concentrations (Table 2). The peel of Nam Dok Mai had significantly higher concentrations of phenolics than any other cultivar followed by Tommy Atkins and Cat Chu. The total phenolic concentrations in the Tommy Atkins and Cat Chu kernels were significantly higher than the other three cultivars. In all the selected cultivars, the total phenolics were significantly higher in the kernel than in the peel or flesh, except for Nam Dok Mai, which had the higher levels of total phenolics in the peel.

The total phenolic contents of the peel and kernel of Tommy Atkins imported into New Zealand were similar to values reported elsewhere for Tommy Atkins (Ribeiro et al, 2007; Ribeiro et al, 2008; Manthey & Veazie, 2009). Total phenolics in the flesh, however, were lower than other reported figures. This may be due to differences in the maturity of the mangoes or variations in the experimental procedures such as the extraction techniques, extraction solvents or assay protocols used by researchers.

The absolute amounts of total phenolics in the flesh, peel and kernel of Tommy Atkins were 204 ± 38 , 358 ± 64 and 831 ± 252 mgGAE, respectively when the weights of flesh, peel and kernel were taken into account. The yield of total phenolics in the kernel was at least double that from the peel and four times that from the flesh whether expressed in concentration (Figure 1a) or amount (Figure 1b).

Figure 1a

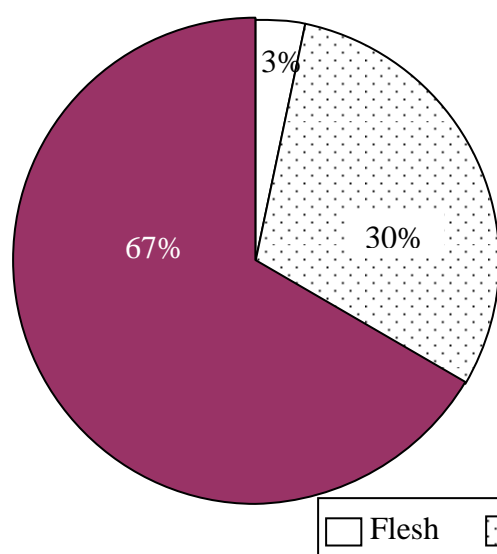


Figure 1b

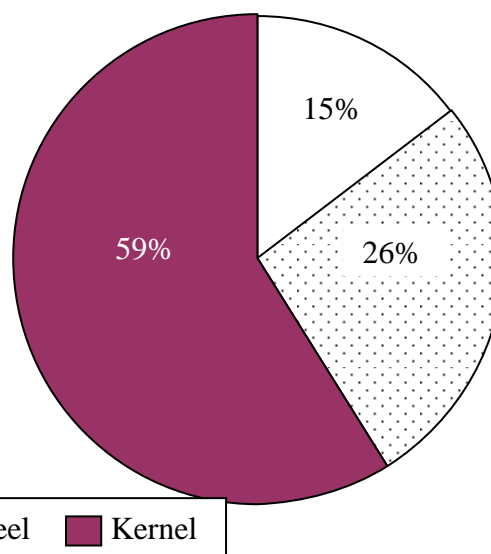


Figure 1: Yield (%) of total phenolics from flesh, peel and kernel: (a) Based on Phenolic Concentrations, (b) Based on Phenolic Amounts

Thus, mango peel and kernel are excellent sources of phenolics. However, to determine whether the levels are sufficient to provide health benefits requires additional research.

Correlations of total phenolics and physicochemical characteristics of mangoes

There was no relationship between total phenolics and any measured colour parameter. There was also no correlation between the phenolic contents and any other measured physicochemical parameter. However, there were correlations between the measured maturity parameters such as TSS, TA, firmness and moisture. Firmness was inversely proportional to TSS/TA ratio (Figure 2) illustrating that firmness decreases as ripening occurs. The TSS increases will be due to the conversion of starch to sugars and the acidity reduction to the conversion of acids into soluble solids (Ribeiro et al., 2007; Nair & Singh, 2003).

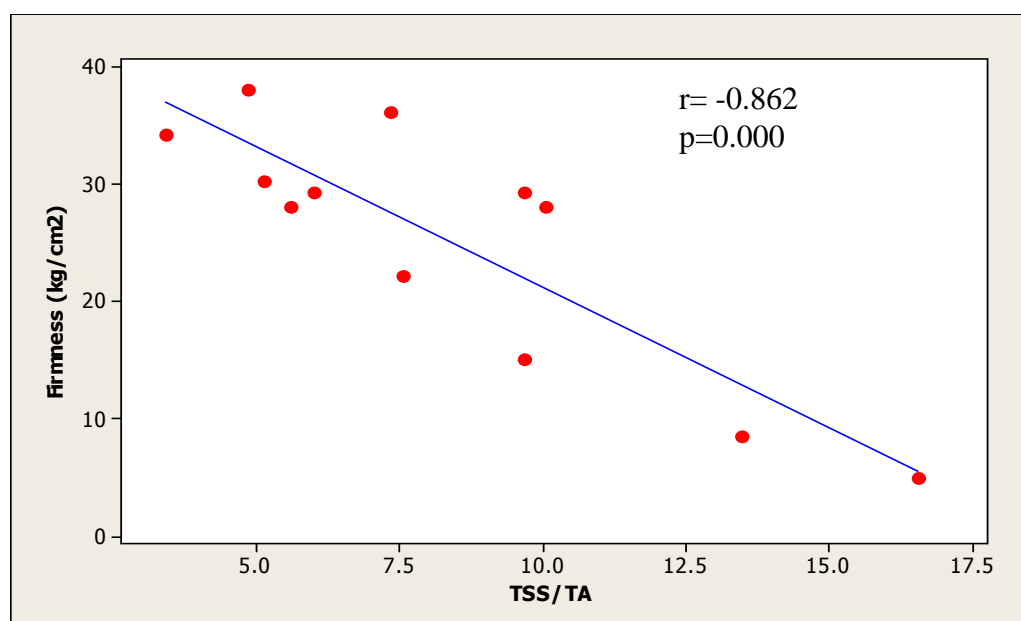


Figure 2: Correlation of firmness and TSS/TA ratio of Tommy Atkins mango (n=12)

The relationship between the total phenolic contents of the individual flesh, peel and kernel fractions was examined. The phenolic content in the peel was directly proportional with that in the kernel but inversely proportional to that in the flesh ($p < 0.05$).

CONCLUSION

This study shows that the levels of total phenolics in flesh, peel and kernel of Tommy Atkins mangoes imported from Mexico into New Zealand were similar to the levels in Vietnamese grown cultivars. In all the cultivars examined except for Nam Dok Mai, seed kernels contained the highest concentration of total phenolics. It is apparent that irrespective of the country of origin, there is an opportunity to use mangos as a source of phenols, which are powerful and effective antioxidants. Furthermore, the research shows that mango kernel and peel, which are often treated as waste products and discarded, should now be considered as desirable dietary ingredients that can protect food from oxidative damage and thus humans from oxidative stress.

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MURIEL BELL MEMORIAL LECTURE 2010

Vitamin D: silver bullet or fool's gold?

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ABSTRACT

Interest in the potential health benefits of vitamin D is increasing. Since 1950, the annual number of scientific publications in PubMed has increased 17-fold, more than twice the increase in all scientific publications over the same period. In the 1950s and 1960s, the focus of vitamin D publications was on its therapeutic effects, including its harm from too high doses.

Since the 1980s, the focus has turned increasingly to the potential preventive effects of vitamin D against a wide range of diseases. For example, the research on vitamin D and cardiovascular disease shows how scientific opinion has turned 180 degrees from believing that excess intake of dietary vitamin D was a cause of cardiovascular disease, to the current prevailing opinion that vitamin D, given in physiological doses, may protect against this group of diseases. Other diseases now thought to be prevented by vitamin D include various cancers, particularly colorectal cancer, diabetes mellitus, osteoporosis and fractures, and more recently, a renewed interest in infectious diseases, such as TB, and related effects of vitamin D on immune function. The annual number of publications since 1950 for each of these disease groups shows a different pattern, with initial interest in infection and cardiovascular disease, which then waned in the 1970s, to be overtaken by rapid increases in the number of publications of cancer and bone disease.

International research is now at a tipping point with recent cohort studies showing that people with low 25-hydroxyvitamin D levels, measured in blood samples collected at the beginning of the study, have increased risk of cardiovascular disease and colorectal cancer during the follow-up period. Because most of this evidence is from observational studies, uncertainty remains about whether 25-hydroxyvitamin D measured in these studies is the true protective factor, or whether there is some other variable associated with it, such as physical activity, that is protecting against disease. To provide complete certainty, randomised controlled trials are required to determine if vitamin D supplementation protects against these chronic diseases – and also to show that it is safe.

INTRODUCTION

Interest is increasing among the general population in vitamin D and its potential health benefits. Hardly a week goes by without an article in the popular press of some new scientific report linking low vitamin D status with increased risk of disease. This reflects increasing interest in the topic by researchers around the world. Since 1950, the annual number of scientific publications in PubMed with the MeSH key-word 'Vitamin D' has increased 17-fold, more than twice the increase in all scientific publications over the same period (Figure 1).

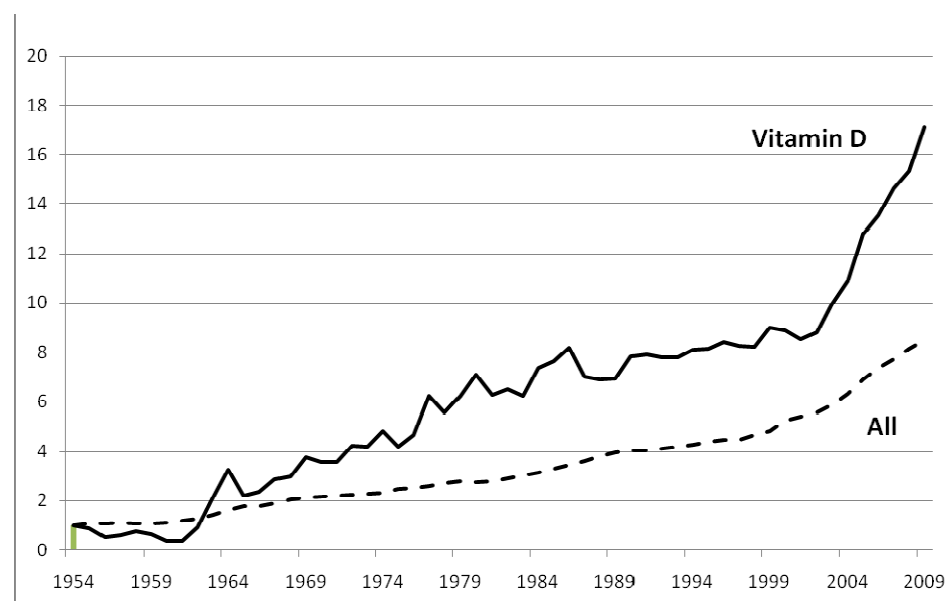


Figure 1: Ratio of Pubmed publications by year, relative to the average of 1950-54: all publications versus those with keyword 'Vitamin D'.

In the 1950s and 1960s, the focus of vitamin D publications was on its therapeutic effects, including its harm from too high doses. Since the 1980s, the focus has turned increasingly to the potential preventive effects of vitamin D against a wide range of diseases. The diseases currently linked to low vitamin D levels (Table 1, Holick, 2007) are so wide-ranging that vitamin D has taken on the aura of a silver bullet in both medical and lay circles. Yet, the recent report from the US Institute of Medicine considers much of this evidence to be "fool's gold" and has poured cold water on it by concluding that convincing evidence of beneficial effects from vitamin D is very limited and does not justify increasing vitamin D daily intakes by much more than those previously recommended (Institute of Medicine, 2011).

Table 1: Diseases linked to low vitamin D status

Disease group or Organ	Specific diseases
Bone & muscle	Rickets
	Osteoporosis
	Fractures
	Muscle disease (weakness, pain)
Cancer	Colorectal
	Breast
	Prostate
Cardiovascular	Coronary heart disease
	Heart failure
	Hypertension
	Type 2 diabetes
Altered Immune Function	
Autoimmune disease	Multiple sclerosis
	Type 1 diabetes
Innate immunity	Tuberculosis
	Respiratory infections (eg. pneumonia)

This review focuses on the diseases that explain most of the increase in the number of vitamin D publications since 1950, which are cancer, cardiovascular disease, infection (including immunity) and bone disease (Figure 2). The number of publications for 5-year time-periods since 1950 for each of these disease groups shows a different pattern, with initial interest in infection and cardiovascular disease, which then waned in the 1970s, to be overtaken by rapid increases in the number of publications of vitamin D and cancer and bone disease. The evidence linking vitamin D to each of these disease groups is discussed.

Vitamin D and Cancer

The earliest evidence suggesting that vitamin D may protect against a range of cancers came from US epidemiological studies of sunlight exposure and latitude published around 1940 by Peller and Apperly (Peller & Stephenson, 1937; Apperly, 1941). The latter was an Australian pathologist who originally worked at the University of Melbourne. It took another four decades until the paper which has stimulated the current body of research on vitamin D and cancer was published. This was an ecological study showing an inverse association between latitude and colorectal cancer mortality in the US, by the Garland brothers from San Diego who were unaware of the earlier research (Garland & Garland, 1980). Since then, further ecological studies have stimulated interest in the possible causative role for low vitamin D status in other cancers by showing inverse associations between solar radiation and cancers of the breast (Garland, *et al.*, 1990), prostate (Schwartz & Hulka, 1990), ovary (Lefkowitz & Garland, 1994) and lymphoma (Newton, 1995).

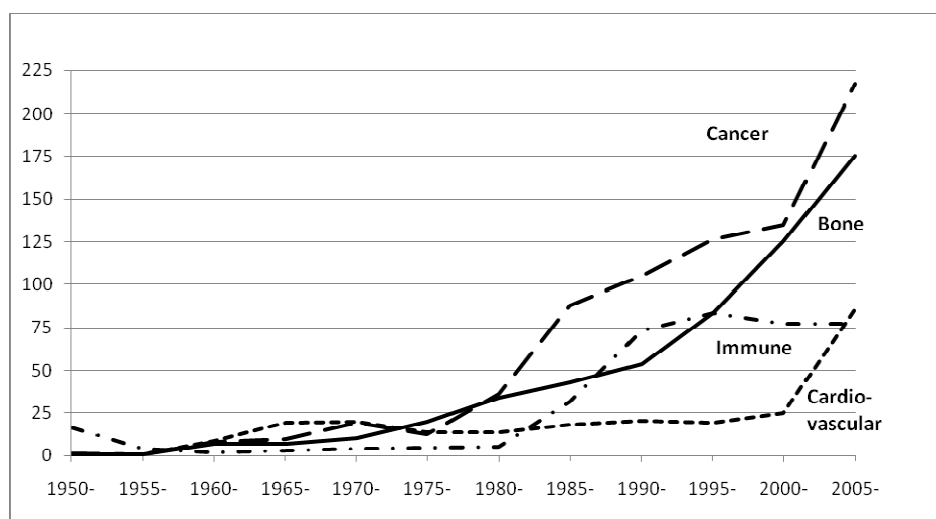


Figure 2 Annual number of vitamin D publications in PubMed by 5 year periods, for cancer, bone disease (fractures & osteoporosis), immune function (infections & cytokines) and cardiovascular disease.

However, ecological studies only generate hypotheses that have to be tested by analytical studies which correlate exposure (in this case, vitamin D status) with the presence of disease at the individual level. A large number of observational studies have been carried out, mainly of colorectal, prostate and breast cancer. Studies of dietary vitamin D provide weak evidence since diet contributes less than 20% of vitamin D entering our bodies, as most is dermally produced from sun exposure (Holick, 2004). The observational studies which provide the strongest evidence of causation are cohort studies (including case control studies nested within cohort studies). These have analysed the association between vitamin D status from measurements of 25-hydroxyvitamin D [25(OH)D] concentrations (which reflect vitamin D from both sun and dietary sources), in blood samples collected at baseline, with subsequent risk of cancer during follow-up. These studies have been reviewed in a recent authoritative report by the International Agency for Research on Cancer (IARC) (IARC, 2008), and since updated by the authors of the IARC report (Gandini, *et al.*, 2011). There have been eleven cohort studies of 25(OH)D and prostate cancer up until December 2009 (Gandini, *et al.*, 2011), with enough cases ($n=3,956$) to detect any effect; but meta-analyses of these studies show no association between 25(OH)D and prostate cancer risk (IARC, 2008, Gandini, *et al.*, 2011). The evidence is slightly stronger for breast cancer with five cohort

studies published up until December 2009, but the pooled relative risk was not statistically significant (Gandini, *et al.*, 2011). Thus, the IARC report concluded that the overall evidence suggesting an inverse association was “weak” and required further cohort studies to determine whether-or-not vitamin D protects against breast cancer (IARC, 2008).

In contrast, the evidence for colorectal cancer is much more supportive of a beneficial effect. The IARC report concluded that “The results show evidence for an increased risk of colorectal cancer and colorectal adenoma with low serum 25-hydroxyvitamin D levels” (page 2), but considered this to be “only limited evidence of a causal link due to confounding by other dietary or lifestyle factors” (page 305). The main possible confounder that researchers consider could possibly explain the inverse association between 25(OH)D levels and risk of colorectal cancer is physical activity, since this also is inversely associated with colorectal cancer risk (Wolin, *et al.*, 2009). The well-known difficulty in accurately measuring physical activity with questionnaires may have resulted in residual confounding and failure to control fully for its effects in multivariate analyses. On the other hand, it is equally possible that any beneficial effect from physical activity could be from increased 25(OH)D levels resulting from activity (Scragg & Camargo, 2008). Thus, only clinical trials will resolve this uncertainty.

A randomised controlled trial (RCT) has been carried out of vitamin D supplementation and colorectal cancer. This is the Women’s Health Initiative which recruited 36,282 women aged 50-79 years into the vitamin D/calcium part of the wider study (Wactawski-Wende, *et al.*, 2006). Women in the treatment arm of the study received 400 IU of vitamin D and 1000 mg of calcium daily. After an average 7 years follow-up, there was no difference in the incidence of colorectal cancer between treated and placebo arms. This study has some well-recognised limitations which may explain the failure to detect a beneficial effect from vitamin D. These are too low a dose of vitamin D (only able to increase 25(OH)D levels by about 7 nmol/L) (Heaney, *et al.*, 2003), low compliance with only 70% of participants taking the study capsules 50% or more of the time, and contamination in the control group with many of them continuing to take vitamin D supplements during the follow-up period (Newmark & Heaney, 2006). The only other RCT of vitamin D supplementation and cancer is from Nebraska (Lappe, *et al.*, 2007). This study followed 1179 women for 4 years, and found that women who received both vitamin D (1100 IU per day) and calcium (1400-1500 mg per day) had about half the risk of any type of cancer during the follow-up period, compared with the placebo group. This is an unexpected finding for such a small trial, and could be a false-positive result that needs confirmation by larger studies.

Vitamin D and Cardiovascular Disease

Views by physicians that sunlight could improve outcomes for people with heart disease are documented from the early part of the 20th century (Christensen, 1923). However, shortly after the discovery of vitamin D in the 1920s (Chick, 1975), experimental studies published soon after found that extremely high doses of vitamin D in a range of animals – up to 10 mg (400,000 IU) per day – resulted in decreased weight and heart rate, increased mortality and wide-spread calcification of arteries, heart, kidneys and other organs (Pfannenstiel, 1928; Harris & Moore, 1929). Subsequently, an animal model of research was developed which combined high dose intakes of both vitamin D and cholesterol to produce a similar arteriosclerotic lesion as seen in humans with hypercholesterolaemia (Seelig, 1969). This animal model was used extensively during the following decades and led to the prevailing viewpoint in the 1960s that vitamin D was a cause of cardiovascular disease (Taussig, 1966). Observational studies published in the 1970s, which showed a positive correlation between dietary vitamin D and coronary heart disease confirmed this position (Knox, 1973; Linden, 1974). It was during this period that cardiovascular disease was the disease most commonly reported in vitamin D publications (Figure 2).

A major development in the 1970s, which eventually led to a re-evaluation of the role of vitamin D in causing cardiovascular disease, was the introduction of competitive protein-binding assays for measuring 25(OH)D concentrations in blood (Hollis & Horst, 2007). The application of these methods to small case control studies of coronary heart disease showed, surprisingly at the time, that cases had similar or lower levels of 25(OH)D compared to matched controls (Schmidt-Gayk, *et al.*, 1977; Lund, *et al.*, 1978; Vik, *et al.*, 1979). Thus, the pendulum of scientific opinion started to swing back the other way towards a position that vitamin D may protect against cardiovascular disease. In 1981, the author, ignorant of the above history, and primarily influenced by the descriptive epidemiology of cardiovascular disease mortality, which is increased in winter and with latitude, but decreased with altitude,

published the hypothesis that ultraviolet radiation and vitamin D may protect against cardiovascular disease (Scragg, 1981). A further key development was the identification of the vitamin D receptor in cardiac muscle (Simpson, 1983), indicating that vitamin D was likely to have an effect on cardiac function.

The opportunity to test the hypothesis that vitamin D may reduce the risk of cardiovascular disease was provided by a population-based case control study carried out in Auckland during 1985-97 (Scragg, *et al.*, 1990). Cases came from a register of coronary heart disease and controls were sampled from the electoral roll, matched by age, sex and date of blood collection. The unit of measurement for 25(OH)D in this study was actually ng/mL, rather than nmol/L as reported. Cases were restricted to those providing blood samples within 12 hours of onset of symptoms, since a pilot study showed that 25(OH)D concentrations were unchanged during this period (Scragg, *et al.*, 1989). A significant inverse association was observed between 25(OH)D level and risk of myocardial infarction, with the odds ratio for those in the highest 25(OH)D quartile (≥ 106 nmol/L) being 0.30 (95% CI: 0.15, 0.61) compared with the lowest quartile (< 62 nmol/L) (Figure 3).

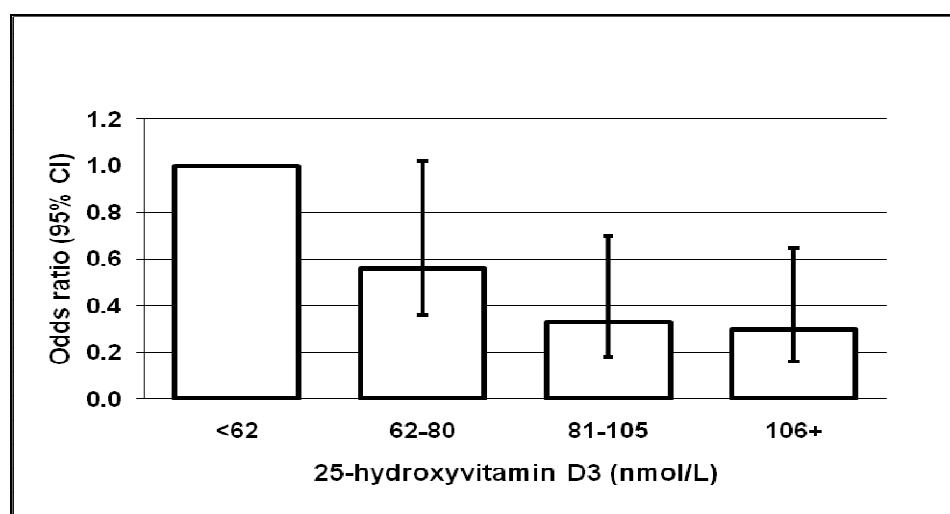


Figure 3: Odds ratio of coronary heart disease associated with plasma 25-hydroxyvitamin D (nmol/L) – compared to reference category < 62 nmol/L (Scragg, *et al.*, 1990).

Evidence from animal studies that vitamin D might be linked to diabetes started to emerge in the early 1980s. These studies identified a pancreatic receptor to the active metabolite of vitamin D (1,25-dihydroxyvitamin D) (Christakos, *et al.*, 1979) and showed that vitamin D deficiency decreased insulin secretion (Norman, *et al.*, 1980). To investigate this possible link further in humans, a large cross-sectional diabetes survey, in which nearly 6000 adults were administered oral glucose tolerance tests, was carried out in Auckland and Tokoroa during 1988-90. When blood samples from cases of newly detected diabetes mellitus (mainly type 2) or impaired glucose tolerance were compared with those of controls matched by age, sex, ethnicity and date of collection, the odds ratio for participants with 25(OH)D levels > 82 nmol/L was 0.36 (95% CI: 0.19, 0.71) compared with the lowest tertile (≤ 60 nmol/L) (Figure 4 – Scragg, *et al.*, 1995). Thus, by the mid-1990s there was evidence from New Zealand studies that low vitamin D levels were associated with increased risk of coronary heart disease and type 2 diabetes.

However, as shown by Figure 2, in contrast to the pattern for the other disease groups, there was little change in the annual number of publications on vitamin D and cardiovascular disease from the 1980s until the middle of the last decade. But since 2008, the number of publications for the latter disease group has increased nearly three-fold. This flood of papers was started with a publication from the Framingham cohort study which showed that participants with baseline 25(OH)D levels < 10 ng/mL (25 nmol/L) had nearly double the risk of having a cardiovascular event during the 5-year follow-up period compared to those with 25(OH)D levels ≥ 15 ng/mL (37.5 nmol/L) (Wang, *et al.*, 2008). This paper has been followed by the publication of several other cohort studies, most of which have reported inverse associations between baseline 25(OH)D levels and subsequent risk of cardiovascular disease (Grandi, *et al.*, 2010). The only RCT large enough to determine whether vitamin D supplementation reduces risk of cardiovascular disease is the Women's Health Initiative,

which found no difference in risk of cardiovascular disease during the follow-up period between the vitamin D/calcium arm and the placebo arm (Hsia, *et al.*, 2007). However, as discussed above for colorectal cancer, this study has major limitations and is not considered an adequate test of the vitamin D hypothesis (Michos & Blumenthal, 2007).

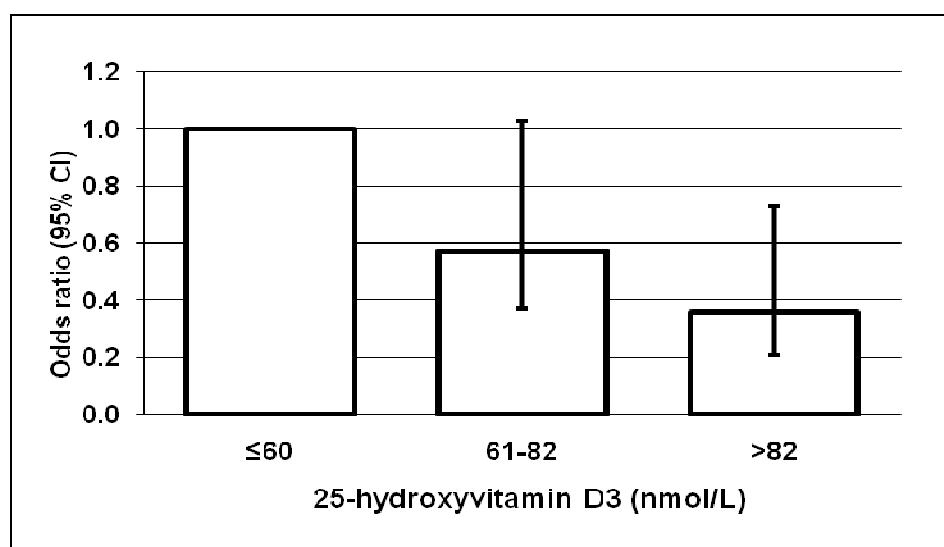


Figure 4: Odds ratio of diabetes and impaired glucose tolerance associated with serum 25-hydroxyvitamin D (nmol/l) – compared to reference category ≤60 nmol/L (Scragg, *et al.*, 1995).

Vitamin D and Infection

Research on the beneficial effect of ultraviolet therapy and infection goes back to the beginning of the 20th century when the Danish doctor Niels Finsen was awarded the Nobel prize in 1903 for the treatment he developed to cure lupus vulgaris (tuberculosis (TB) of the skin), which is a very disfiguring condition often affecting the face (Anonymous, 1904). He developed an eponymous lamp to shine ultraviolet rays directly onto the skin lesion, which often resulted in cure. The treatment was taken up around the world, and extended to the sanatoria in the Swiss Alps which used exposure to natural sunlight to cure TB (Rollier, 1929). By the 1940s, doctors eventually started to treat lupus vulgaris with vitamin D (Dowling & Prosser Thomas, 1946). Manuscripts on the treatment of TB with vitamin D account for most of the publications on vitamin D and infection during the period 1950-54 (Figure 2). However, these almost stopped during the following period up until about 1980, most likely because the introduction of antibiotics in the mid-1950s provided a much more effective treatment for TB. But since 1980 the number of publications on vitamin D and infection has increased rapidly (Figure 2).

Initially, most of this increase was due to research showing that vitamin D affected markers of acquired immunity, such as cytokines, and autoimmune response to infection (Eikelenboom, *et al.*, 2009). However, a key paper published in 2006 showed that vitamin D also affected the innate immune system by increasing levels of the antimicrobial peptide, cathelicidin (Liu, *et al.* 2006). This is thought to be the mechanism by which vitamin D protects against TB and other respiratory infections (Gombart, 2009; Bartley, 2010). Observational studies have shown inverse associations between 25(OH)D and risk of upper respiratory tract infection (Ginde, *et al.*, 2009), and that low baseline levels of cathelicidin predict increased risk of mortality from infectious disease in haemodialysis patients. A recent meta-analysis has shown that TB cases have lower 25(OH)D levels compared with controls (Nnoaham & Clarke, 2008). Evidence from RCTs indicates that vitamin D supplementation protects against respiratory infection (Aloia & Li-Ng, 2007; Manaseki-Holland, *et al.*, 2010; Urashima, *et al.*, 2010).

Vitamin D and Bone Disease

The bone disease historically linked to vitamin D deficiency is rickets. However, with the decline in the incidence of this disease through increased vitamin D fortification and supplementation started in the 1930s, the focus of research since the 1950s for bone disease has been on the prevention of osteoporosis and fractures. The increase in the annual number

of publications for bone disease has been second only to that for cancer, with a substantial continuing increase since 1995 (Figure 2). In contrast with the other disease groups above where most of the research comes from observational studies, there have been numerous RCTs (at least 18) to see if vitamin D supplementation reduces the risk of fractures (Autier & Gandini, 2007), with the earliest being a French study published in 1992 (Chapuy, *et al.*, 1992). Another influential study is one from Cambridge (England) which found that 100,000 IU doses of vitamin D₃ taken every 4 months reduced the risk of non-vertebral fractures on people aged ≥65 years living in the community (Trivedi, *et al.*, 2003).

But despite these successes, a state of equipoise currently exists, with expert opinion divided about whether vitamin D supplementation by itself prevents fractures. For example, a recent meta-analysis of RCTs concluded that vitamin D by itself in doses of 10-20 µg/day (400-800 IU/day) did not prevent fractures; but that vitamin D given in combination with calcium was effective (DIPART, 2010). However, another meta-analysis concluded that vitamin D by itself, in doses of >400 IU per day reduced the incidence of fractures by 20% (Bischoff-Ferrari, *et al.*, 2009). The conflicting opinions about whether vitamin D by itself is beneficial are due to the two following reasons. Firstly, many of the RCTs gave vitamin D and calcium together. Thus, when a benefit is seen, it is unclear whether it was due to vitamin D, or calcium, or both. Secondly, the vitamin D doses given in previous studies (most ≤800 IU per day) are now considered too low. Current recommendations are that people need at least 1700 IU per day, and ideally more than 2000 IU per day to boost vitamin D to levels associated with optimum health (Vieth, *et al.*, 2007). A recent RCT from Geelong (Victoria) has further muddled the waters by reporting that an annual 500,000 IU dose of vitamin D₃ actually increased the risk of both fractures and falls in community dwelling women aged ≥70 years (Sanders, *et al.*, 2010).

Silver Bullet or Fool's Gold?

The evidence linking low vitamin D levels with increased risk of colorectal cancer, cardiovascular disease and infection comes primarily from observational studies, while equipoise currently exists as to whether vitamin D by itself reduces fracture risk. Vitamin D may be a silver bullet but to prove it is not fool's gold, further RCTs using vitamin D doses of >2000 IU per day are required. A recent meta-analysis of RCTs initially carried out to determine if vitamin D supplementation prevents fractures has reported that vitamin D supplementation reduced all-cause mortality by 7%, just reaching statistical significance with the upper confidence limit being 1% below the value for those in the placebo arm (Autier & Gandini, 2007). However, because this was a secondary analysis of RCTs carried out for a different purpose, many of which used low-doses of vitamin D, and which just reached statistical significance, further RCTs using higher doses of vitamin D are required to determine the full benefit (if any) of vitamin D. The need for further RCTs is emphasised by the results of RCTs of supplementation with other vitamins (i.e. vitamins A, B, C & E), which have failed to confirm the inverse associations previously found in observational studies between vitamin status and risk of chronic disease; in fact, supplementation may even increase mortality (Bjelakovic, *et al.*, 2007; Byers, 2010). However, vitamin D is a hormone, and may be different to other vitamins (Morabia & Costanza, 2010). But until large well-designed RCTs are done, we will not know whether vitamin D is effective and safe – whether it is a silver bullet or fool's gold.

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Integrating food futures: technology versus nature

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ABSTRACT

Background: In recent years, whilst food technology has been able to provide a wider range of foods to the consumer, there has been a growing interest in environmental, social or community aspects of food supply. This has expressed itself in the regrowth of local food or farmers markets, concern with food miles and the sustainability of the food supply and a degree of rejection of packaged, processed or 'fast' foods. "Medicalisation" of the food supply has also become an issue for many people. How will the two approaches to improvement of the food supply be integrated in the future and what are consumers seeking from their food supply?

Methods: Consumer and food choice surveys.

Results: Many factors influence consumer food choice with the marketplace for foods reflecting the complexity of consumer needs. Whilst there is increasing interest in a 'back to nature' approach to foods and in related environmental issues, consumer surveys indicate that cost of foods delivered in this way to the broader marketplace may be a limiting factor. Health concerns are likely to be a continuing consumer driver even though this may involve some degree of 'medicalisation' of the food supply through fortification or inclusion of novel ingredients.

Conclusion: It is likely that the future of food will involve a mix of the 'back to nature' and 'technological' approaches. It will require an integrated approach across the agricultural, food and educational sector to address the increasingly diverse demands of consumers.

INTRODUCTION

Food is central to human existence, speculation about the future of food and access to food has been a recurring theme in the both the scientific and general literature. Nutrition science is also in a state of continuous flux and refinement. For example, over the past few decades in the area of fats we have moved from consideration of the role of animal versus plant fats, to saturated versus unsaturated fat ratios and, more latterly, to issues such as the role of long chain omega 3 fatty acids, trans fats, conjugated linoleic acid and n6:n3 ratios. In the area of protein nutrition, again research has moved on from consideration of animal versus plant-based proteins, to the role of specific protein sources such as soy or casein and, more latterly, to consideration of the role of individual amino acids and peptides. For carbohydrates research has moved from consideration of the benefits of wholegrain versus refined, to the issue of dietary fibre and subsequently to areas such as resistant starch, glycaemic index and prebiotics. For micronutrients, we have moved from concentrating on traditional vitamins and minerals such as vitamins C and A, iron and zinc to areas such as betacarotene, sodium/potassium ratios and antioxidants and, more recently interest has been renewed in iodine, selenium, folate, vitamin D, other carotenes and phyto-nutrients such as phenols, terpenes, alkaloids and glucosides. So what will the future hold in terms of the food supply?

METHODS

The discussion below is based on a synthesis of the results of consumer surveys and food trend data reported in the literature.

RESULTS AND DISCUSSION

It is likely that the future of food will be dichotomous. On the one hand, the 'back to nature' movement is gaining momentum whilst on the other hand 'technological' progress

proceeds apace. The 'back to nature' movement involves consideration of environmentally friendliness, food miles, grow your own/local food issues, farmers markets, organic foods, vegetarian foods, no food additives, minimal packaging and fresh and a holistic approach with what are perceived as whole 'natural' foods. A technological approach, in contrast, may include the potential use of genetic engineering, nanotechnology; novel processing techniques and fortification or mediatization of the food supply. It is perceived by those who favour the 'back-to-nature' paradigm as a reductionist, food by food, nutrient by nutrient approach.

A purely technological approach to satisfying nutrient needs is possible but, in many cases, is unlikely to be sustainable or able to be used on the scale necessary. For example, as part of a space experiment in 1965, twenty four men volunteered to be fed nothing but a food (syrup) made from pure chemicals for nineteen weeks. The experiment proved quite successful from a medical point of view and everyone who finished was perfectly healthy. However out of the twenty four men who started, only fifteen finished (Zondy, 2010).

Another example of a potential technological versus 'back to nature' approach to solve an emerging problem can be illustrated in relation to meat consumption. In recent years, concern has been expressed by some people about the effect that the trend for increasing consumption of meat may have in relation to water use, methane production and land degradation caused by the additional animals that would be required worldwide to meet this demand. The "natural" approach to solve this potential problem would be to eat less/no meat. The downside to this is that meat, and particularly red meat, is a major source bio available iron, zinc, B12, long chain omega 3. The red meat industry is also a major export industry and major employer in both Australia and New Zealand.

A purely technological approach, such as the production of test-tube meat, might be possible, but it unlikely to provide the complete answer. The concept that you can grow cuts of meat in a laboratory began in 1908. Nobel laureate Dr. Alexi Carrel (1873-1944) bathed a piece of embryonic chicken heart in a nutrient broth. He found that that not only could he keep the chicken heart tissue alive, but that it doubled in size each day. The tissue never seemed to age or die. This went on for weeks, months, and then years. So why isn't there a chain of Kentucky Fried Chicken Hearts restaurants across the land? Since these early experiments, it has proven difficult to repeat the high degree of success achieved by Carrel, although some are still trying. An approach which employs technology to reduce, for example, the environmental impacts of increasing numbers of cattle on methane production, water use and land degradation may be more feasible in the long term. In the Australian context, red meat production has a low impact on water usage compared to fruit or vegetable production and only a low to medium effect on fossil energy or biodiversity compared to a high impact for cereal foods and medium for fruits and vegetables. It does, however, have a major effect on green-house gases compared to the production of plant-based foods. To try to ameliorate this effect, the industry in Australia has initiated a continuous tree planting program to offset emissions and a number of breeding programs to improve feed conversion efficiencies. They have also begun to implement a methane reduction program through the use of gut microbes, vaccines and changes in feed quality. On-farm, best practice, natural resource management is also being encouraged.

Whilst novel technologies or products such as genetic engineering, nanotechnology, phyto-sterols and pro-biotic may play a role in the future of the food industry, evolution rather than revolution is more common. Improvements tend to come through introduction of new flavours/colours, strains or preservatives/additives etc; to added vitamins, minerals, omega fats, fortification; to removal of fat, salt sugar, trans fats; to new packaging/presentation/positioning (labelling, claims); to the import of 'new' products from other cultures – yoghurts, feta cheese, Indian spices, premade sauces or part meals.

So what of the future? The food industry is dependent on the consumer for its success. Consumer food choice can be influenced by a number of factors including, availability and supply, taste, convenience, cost, safety, cooking skills, culture and food philosophy and, increasingly, health concerns. Surveys in the UK and USA assessing future food trends have identified a wide range of food futures or mega trends (IFIC, 2009a and b; Palmer, 2009). These include more comfort food, incorporating retro, nostalgia; feel good foods of the past and treats. They predict more scratch cooking and home baking; more cooking from raw ingredients, cheaper cuts, also more cakes, tray bakes and sponges not just because it saves money but also it makes you feel great. They conclude that there will be a trend to more local foods from specific regions or traditions and greater use of a variety of

breeds and species. There will also be a trend towards more 'whole of animal consumption'; eating more of the fish, meat and vegetables and throwing less away; using new and forgotten recipes to utilise more of the animal - a principle that can be applied to anything. Sustainable meat and fish will be in more demand – including new varieties.

These same surveys predict changing drinking habits - drinking at home rather than out in pubs and restaurants, with beer, cider and cocktails increasing in popularity. Thirst for food skills and knowledge will increase with more entry level cookery schools teaching the basics to consumers. There will be increasing restaurant and farm alliances; more savvy restaurateurs teaming up with farms to bring the consumers food that they know and trust. Miniaturisation of foods with greater choice, less cost, more variety and a greater "cute" factor will increase with greater customisation - more brands and businesses will offer consumers the opportunity to customise or tailor their goods, products or services. Instant or ultra nutrition will be an increasing trend together with foods that enhance inner or outer beauty and raw foods and raw food diets that are perceived to retain all of their natural goodness. They predict that the use of 'free' food is set to increase, incorporating foraging, and foreignism, growing your own and fishing. In contrast, bistronomics is predicted to increase - avant garde cuisine at bistro prices by using what is in season, not throwing anything away and using modern cooking techniques. In terms of food delivery, more food will be ordered by mail and be delivered.

In future years, surveys indicate that community food projects are likely to increase as an expression of power to the people - groups of people sharing land, skills and knowledge to share food within communities. Modernised and interpreted cuisines will be more common and anti (this and that) foods - foods that fight certain conditions and ailments will be more readily available. In contrast, there will also be more fun around food delivery with personality and informality brought into brands and the dining room. Multi-sensory emotional food experiences are set to increase with the use of alternative techniques to cook, serve and present food, delivering an all encompassing food experience that is multi-sensory.

. In the UK a renewed zest for ethical and premium products has been identified with the finest, organics and fair-trade ranges returning to growth, suggesting that the trends seen prior to the global recession are beginning to return.

However, although the notion of going green, buying organic and sourcing free trade products is gathering momentum in around the world, research suggests the added expense remains a significant deterrent.

With an evolving and increasingly complex food supply, there will be some challenges for nutritionists. Foods with added 'health' potential will proliferate and require validation and monitoring (ageing, wealthier population). Labelling will become increasingly important but will have to address opposing needs – more information demanded but at the same time simplified. Fortification will continue to be an issue that needs to be addressed including emerging areas such as vitamin D adequacy. Do we continue to fortify staple foods as 'deficiencies' emerge? When do you fortify; when do you recommend supplementation, when do you recommend a change in food choice or lifestyle? The evolving nature of the food supply results in a system with creeping changes and, in terms of food legislation, with a need for a largely product by product approach. Unless managed within an agreed overarching framework, this can cause problems with the balance of the overall food supply. There may be a need to decide if a line needs to be drawn between protecting and promoting public health – where does/should government's responsibility begin/end? When should personal choice be paramount? How do we integrate environmental and social concerns with health needs? What gets priority?

CONCLUSIONS

It is likely that the future will involve a mixture of the 'back to nature' and 'technological' approaches to the development of a healthy and sustainable food supply. This will occur in the context of an ever increasing diversity in consumer demand which will bring an added complexity to the work of food legislators and nutrition educators as well as those people involved in agricultural, food and nutrition policy setting.

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Nutrition for NZ kids

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ABSTRACT

Background: The Children's Nutrition Survey 2002 report (CNS02) provides the first overview of the nutritional status of New Zealand's school children (5-15 years). It includes descriptions of nutrient intakes, food sources of nutrients, eating patterns, frequently eaten foods, physical activity, dental health, body size, selected biochemical indices and anthropometric measures.

Objectives: To describe results from integrated data derived from the CNS02 data set.

Design: Survey data were available from the Ministry of Health (MOH) to researchers as Confidentialised Unit Record Files (CURFs). Subsequently about 20 peer reviewed papers have been published, and a number of MOH and University of Otago reports further analysed the data. Peer reviewed papers integrating aspects of the data encompass the broad areas: food, nutrients, food choices; specific micronutrients; body weight status.

Outcomes: When the material in the CNS02 report is considered, along with the peer-reviewed published papers, nutrition issues of importance to NZ school children emerge.

Conclusion: Current (2002) issues for NZ children included: the importance of breakfast, low vegetable consumption, rates of overweight and obesity. Key areas for ongoing research include selenium and vitamin D status.

INTRODUCTION

The Children's Nutrition Survey 2002 report (CNS02) (Parnell et al, 2003) concludes that younger children were better nourished than older in terms of their nutrient intakes and body weight status. Intakes of vitamins and minerals were largely satisfactory with the exception of the iron status of menstruating females. Urinary iodine levels (a surrogate for intake) were indicative of 'mild' iodine deficiency. Bread (80% of which was white bread) was the largest single contributor of energy to their diets. Most children were omnivorous with chicken being the most common meat choice; 2 out of 5 children ate at least 2 serves of fruit a day, and 3 out of 5 ate at least 3 serves of vegetables per day. The largest contributor of total fat to the diet was the food group *Potatoes, kumara and taro*. Pacific children, compared to Maori and NZ European and Other (NZEO), derived the most energy from fat. *Milk* was the largest contributor of saturated fat, followed by *Potatoes, kumara and taro, Pies and pasties*. Body weight status showed that 68.9% were normal weight, 21.3% were overweight and 9.8% obese. Overweight and obesity levels were highest for Pacific children, followed by Maori and NZEO (Parnell et al, 2003).

RESULTS AND DISCUSSION OF PUBLISHED PAPERS

Four papers have explored the area of food and nutrient intake, and food choices (Rockell et al, 2010a and b; Wilson et al, 2006; Regan et al, 2008). The question of the influence of the school environment on children's nutrition is addressed in a comparison of food and nutrient intake on school versus non-school days. School day food choices included more frequent consumption of fruit, sandwiches, biscuits and snack bars; higher intakes of sucrose, fructose and dietary fibre. There was no difference in frequency of consumption of pies and sausage rolls and hot chips were more frequently consumed on non-school days.

A study of food consumed before 9 a.m. (breakfast) (Wilson et al, 2006) indicated that the daily nutrient intake of breakfast eaters was better than non-breakfast eaters. Utter et al (2007b) concluded that increasing 'at home' breakfast consumption among older children from more deprived backgrounds was a priority, especially as skipping breakfast at home was associated with a higher Body Mass Index (BMI).

During the afternoon, frequent food choices were fruit, biscuits and crackers, and 'afternoon eating' contributed positively to overall daily nutrient intakes. Older children, compared to younger, consumed a higher proportion of their daily energy intake during the evening. One third only consumed vegetables other than potato in the evening and vegetable eating was more likely among those who also ate from the group *Potatoes, kumara and taro* and who ate meat. The eating of hot chips was associated with a lower likelihood of eating vegetables.

Children who used the school food service more often made choices of high fat, high sugar foods, whether or not these were sourced from the school. However it was concluded that school canteens should offer healthier foods (Utter et al, 2007a).

Correlates of BMI explored both nutrition/food behaviours and physical activity (Parnell, 2007). While Maori and Pacific children reported being more active, they were also the most likely to be overweight, obese or extremely obese (Goulding, 2007). BMI was associated with TV watching, purchasing food from a dairy/takeaway and the consumption of sweet drinks (Utter et al, 2005, 2006a and b, 2007b and c).

The use of BMI either to reflect fat mass or as a useful tool to evaluate cardiovascular risk was questioned by Rush and Goulding (Rush et al, 2003, 2009; Goulding et al, 2010) who variously suggested the use of waist/height ratio, Fat Free Mass (FFM) or Fat Free Mass Index (FFMI), or waist measurement alone. The latter however is difficult to measure on pre-pubertal children.

For zinc and selenium both dietary intake and biochemical measures of status were obtained. Thomson et al (2007) conclude that selenium status was lower among South Island children compared to North and Maori children, compared to Pacific and NZEO. Some improvement in selenium intake from food sources could be beneficial. The risk of zinc deficiency was highest among Pacific children (Gibson et al, 2010) compared to others. Rockell et al (2005) explored 25-hydroxyvitamin D concentrations across seasons and by ethnicity and concluded that not only was season a major determinant but the highest prevalence of insufficiency was among Pacific children.

CONCLUSION

School children in New Zealand rely primarily on food provided from home but should also be able to readily purchase healthy foods, particularly at school. Breakfast eating needs to be encouraged and meals planned to include more vegetables. Further research into the most appropriate indices to assess body fatness among children is important. Monitoring of selenium status and vitamin D status should continue among children and be a research priority.

While nutrition issues have been highlighted, solutions in the area of food choice and food patterns need to move beyond simple prescriptive statements to include considerations of the whole food environment and be culturally appropriate.

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Is Junk Food Promoted Through Sport? 'Here's how we see it...' Capturing Children's and Parents Perspectives of the Food Environment in Sport

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ABSTRACT

Background: Diet is a key determinant of health and a common risk-factor for obesity and dental caries, two diseases of public health importance. The food environment is a probable cause of both, in part due to the widespread availability and marketing of energy-dense, nutrient-poor foods and beverages. For many New Zealanders and young people in particular, the sport setting may constitute an important part of this food environment, and although little research been conducted in this area there is anecdotal evidence that the sport environment is used to promote energy-dense, nutrient-poor (EDNP) foods.

Objectives: The FEAST (Food Environment And SporT) Study is a multi-faceted research project investigating the promotion of food and beverages in sports settings. The aspect presented in this paper aims to explore parents understanding of the food environment with respect to sport and assess parent's attitudes to and opinions of the food environment in sport and its influence on their children's food choices.

Design: Parents whose children were involved in organised sport (rugby, soccer and netball) participated in the qualitative aspect, which utilised the Photovoice method. Each participant was provided with a camera to photograph and record the food and nutrition environment they associated with sport. Using the photographs and a semi-structured questionnaire, eight parent focus groups were conducted.

Outcomes: Many of the foods and beverages available at sporting venues are EDNP and choice is limited. EDNP foods and beverages have become an expectation in many sports settings. Marketing strategies used to promote food and beverages through sport increase purchasing requests and many parents report these strategies and the overall food environment make it difficult for them to provide a healthy nutrition environment for their children.

Conclusion: Preliminary findings indicate the sport setting is not supportive of a healthy food and nutrition environment. Study findings contribute to existing knowledge regarding the nutrition environment within which children live and how it contributes to their development. In addition, the research will inform and influence policy development regarding supportive food environments, and for nutrition professionals, raise awareness of the broader issues which influences nutrition behaviours.

INTRODUCTION

Diet is a key determinant of health and a common risk-factor for obesity and dental caries, two chronic diseases of public health importance (World Health Organization 2003). The high rates of obesity, particularly in Māori and Pacific children and those with higher levels of deprivation, is of concern in New Zealand. One-third of New Zealand children aged 5-14 years are overweight or obese and higher proportionally among Māori and Pacific children (Parnell et al. 2003). Furthermore, despite reductions in incidence in the last two decades, dental caries is still the most prevalent chronic disease in the New Zealand population, and impacting Māori and Pacific peoples disproportionately (Ministry of Health 2009). The short and long-term consequences of these chronic diseases, which have been well documented (Broadbent et al. 2005; Kagihara et al. 2009; Lee 2009; Sattar & Lean 2007), will likely result in significant costs in terms of healthcare and loss of productivity (New Zealand Government 2007).

With respect to causes and prevention strategies, a move away from individually-focussed approaches to an ecological approach has been proposed (Egger & Swinburn 1997; Watt 2007). The current food environment, including New Zealand's, has been described as 'obesogenic' – promoting the dual causes of obesity, overeating of energy dense nutrient poor (EDNP) foods and physical inactivity (Hill & Peters 1998). Though the causal pathways of obesity and dental caries are multi-faceted and complex, recent research has concluded the widespread availability and marketing of EDNP foods and beverages (the environment) are one 'probable' cause of overweight/obesity and dental caries in children (Cairns et al. 2009).

The Ottawa Charter of Health Promotion stresses the critical role of environmental settings in health promotion (World Health Organization 1986). Though there is considerable evidence regarding the marketing and promotion of food and beverages in the school setting (Carter & Swinburn 2004; Maher et al. 2005; Richards et al. 2005), for many New Zealanders, and young people in particular, the sport setting may constitute another important part of the food environment. While expert opinion and anecdotal evidence suggest that EDNP foods and beverages are widely available and extensively and creatively marketed in the sport setting, there is a paucity of research to support this view. One recent study, an internet survey of sports websites in New Zealand, found sponsors of popular New Zealand sports, particularly junior sport, were biased towards EDNP foods (Maher et al. 2006).

The sport setting has a number of features which are advantageous for marketing food and beverage products to children. First, almost all New Zealand children (92%) are involved in organised sport or activities (Sport and Recreation New Zealand 2003), providing a captive market (including their parents/caregivers) for industry and marketers. Second, the sport setting provides an opportunity to utilise a range of marketing strategies including non-broadcast media - sponsorship, celebrity endorsement, free giveaways and incentives, and fundraising as well as the more common and dominant forms of marketing, television and radio. While all marketing strategies are effective independently, they are possibly more successful in combination, collectively supporting and reinforcing marketing messages. When considering influences on chronic disease, it is not only one strategy which is influential, *"...the issue is all commercial food marketing targeting children..."* (White 2007, p.4). A recent review of Australian and international research on food marketing revealed while most studies focused on commercial television advertising; the non-broadcast media remains under-explored (Cairns et al. 2009; Chapman et al. 2009). Finally, the sport setting provides a virtually unregulated environment with regard to food and beverage marketing – currently only broadcast media is regulated, albeit self-regulated, in New Zealand.

Parents appear to support bans on unhealthy food advertising to children (Kelly et al. 2008) and feel that food marketing is a pervasive influence on children's food preferences and purchase requests (Morley et al. 2008). Though previous studies have explored attitudes, opinions and perceptions of children and parents to food advertising (Chapman et al. 2009), and one international study investigated food sold at community sport facilities (Kelly et al. 2008), none have specifically explored the organised sport setting.

The FEAST (Food Environment And Sport) Study is a multi-faceted research project investigating the promotion of food and beverages in sports settings. The aspect presented here aims to explore parents understanding of the food environment with respect to sport and assess parent's attitudes to and opinions of the food environment in sport and its influence on their children's food choices. This paper presents some preliminary findings from the qualitative aspect of the FEAST Study. A cross-sectional survey to quantitatively assess parental attitudes and perceptions is planned for 2011.

MATERIALS AND METHODS

Schools, and rugby, soccer and netball clubs, in Wellington and Porirua, were purposively selected from a list of sports clubs registered with the Wellington regional sporting trust (Sport Wellington). Following initial contact with team coaches and managers, children aged 10-12 years, and their parents, were approached at either team practice or weekend games and invited to participate in the FEAST Study and become 'parent' and 'junior researchers'. The chosen sports have a strong administrative base, a high participation rate amongst New Zealand children and provide a balance with regard to gender; the two cities chosen represent a range in socio-economic status.

Data was collected mid-2010, using the participatory qualitative research method, Photovoice. This method aims to enhance researchers' ability to understand participants'

viewpoint of the world, using photography by participants; it provides *“a means of accessing other people’s worlds and making those worlds accessible to others”* (Booth & Booth 2003, p.431). Photovoice is underpinned by the assumption that people are experts in their own lives and that they hold valuable and valid information about their own needs, preferences and concerns (Booth & Booth 2003; Wang & Burris 1997). The method offers research participants greater agency in the research process, giving the participants ‘a voice’ and shifting the power away from the researcher towards the participant – the research is conducted *with* rather than *on* the participants (Booth & Booth 2003; Wang & Burris 1997).

Each ‘parent researcher’ was provided with a disposable camera for a two week period to photograph and record the food and nutrition environment they associate with sport; and a notebook to document their photographs and a brief statement on their reasoning for taking each photograph. Eight parent focus groups, five in Wellington (2 rugby, 1 soccer, 1 netball, 1 mixed) and three in Porirua (1 each rugby, netball and soccer) were conducted using a semi-structured interview schedule; combined with the participants’ photographs, parents’ discussed their opinions and attitudes to the promotion of food and beverages in sport.

Focus group discussions were recorded and transcribed verbatim. Transcripts were analysed using thematic analysis searching explicitly for supporting and conflicting data. NVivo 8 was used to code and arrange data into categories determined by the interview schedule, the photographs and the line of questioning and direction of the focus group discussions. Analysis is ongoing and findings are preliminary; outcomes currently presented refer to ‘parent researchers’ only.

RESULTS

Food and beverage availability at sports venues was a major topic of conversation in all parent groups; venues included the regions’ international sport stadium and Saturday morning children’s sports venues – playing fields and netball courts. Food and beverage available for purchase at the regional sports stadium was described by all parent groups as EDNP and of poor quality, *“It’s expensive and garbage...it’s the worst ever.”* (Wellington, soccer, male). The contradiction in providing unhealthy food at a sporting event did not go unnoticed by most groups, however, the food and beverages aligned with the ‘fun’ quality of the events attended at the stadium, *“It’s entertainment food, totally inappropriate for sport, but entertaining food.”* (Wellington, rugby, male) (Figure 1). Moreover, the availability of EDNP foods had become an expectation, an attitude which was firmly based on tradition, *“Oh, it’s an expectation, it’s what you want, a cold beer and a hot dog or a pie...my Granddad did it at the rugby at Athletic Park and that’s what they are still doing now.”* (Wellington, rugby, female). Alternative foods and beverages were discussed; most participants, particularly mothers, wanted to see healthier choices available. However, most groups also agreed substitutes were unlikely to be successful due to issues of perishability and convenience, and most crucially, because of the demand created by spectators’ expectations for EDNP foods and beverages.



Figure 1: *“Entertaining food”* (Porirua, rugby, female)

Most groups reported limited choice at weekend sporting venues where food and beverages were predominantly EDNP, *“I was just surprised, it was freezing cold, and it was wet, but on the day you couldn’t buy hot soup or sandwiches. There was hot dogs, pies, chips*

and hamburgers.” (Porirua, netball, female) (Figure 2). Given the number of children present at Saturday morning venues, many parents thought the food unsuitable; furthermore, they believed it to be inappropriate food to consume when playing sport. All groups discussed the availability of ‘traditional’ half-time ‘oranges’; parents were particularly pleased to be able to supply some healthy food for their children and the traditional practice was well received, “...all the boys...eat their oranges...they love them...they gobble them down and they’re always happy, and no kid is turning up their nose and ‘I don’t want to eat an orange – yuck, they’re all right into it.” (Wellington, netball, female) (Figure 3).



Figure 2: Food available at weekend sports venues (Porirua, netball, female)



Figure 3: 'Oranges' on a Saturday morning (Porirua, rugby, female)

According to most groups, food was frequently used as a reward for children at the end of a game; confectionary was mentioned by some groups, but more commonly, commercial ‘Player of the Day’ certificates containing vouchers for free food at local fast food establishments were said to be utilised (Figure 4). Parents agreed this practice established an expectation for children as well as increasing purchase requests and creating family conflicts.



Figure 4: 'Player of the Day' certificate (Porirua, netball, female)

Other marketing strategies parents reported were the use of incentives (free giveaways or competitions) and sports identities to promote specific food and beverage items. All groups agreed these strategies were effective in attracting children to products and encouraging purchasing requests and consumption, “...*what does make an impact is when there’s promotional items.*” (Porirua, rugby, female). Some products used both strategies, “*With the Weetbix, it’s not only the All Blacks thing; it’s the cards they have in them, that is the big thing.*” (Porirua, rugby, female) (Figure 5). Groups often commented they did not realise the extent of the marketing until they became ‘parent researchers’ in the FEAST project, “*What amazed me...was just how much the All Blacks sponsored everything, it was just incredible, the All Blacks name was on everything.*” (Wellington, soccer, male).



Figure 5: Competitions, collectibles and sports personalities (Wellington, rugby, male)

Though many of the foods and beverages promoted using these strategies were EDNP, not all were considered unhealthy; parents welcomed the associations between children’s ‘idols’ and healthy products, “*In a way I’m relieved that they promote that [Weetbix]...with national heroes because it does encourage small children....so it can be quite positive.*” (Wellington, soccer, female). However, because of the perceived high level of influence sporting identities had with children, parents sounded a caution – to ensure the products sports identities were promoting were appropriate in terms of health, “*...sporting identities need to be careful with regards to who their fans are.*” (Wellington, netball, female)

When asked their opinions about the use of marketing strategies which targets children, many parents stated marketing strategies encouraged and heightened children’s requests for products, which in turn potentially created family conflict, Participant A; “*You get sick of saying ‘no’*”; Participant B: “*Yeah don’t you? It’s a battle actually.*” (conversation between two Wellington, rugby participants). Further, they often commented marketing strategies made it difficult for them as parents to provide a healthy nutrition environment for their children, “*I think it’s very hard on the parents...I’d rather it was a little less blatant. I wish they had their facts a little more accurate. I wish it was more fair the way they promote some things...if you look at it it’s actually unfair. I think some of these adverts should not go to air. I think we should be a lot more fussy what we show our kids.*” (Wellington, soccer, female).

DISCUSSION

This is one of the first studies in the New Zealand context, and internationally, to explore the attitudes and perceptions of children and parents to the food environment in the sport setting and evaluate the impact on children’s food and nutrition behaviour. Preliminary findings indicate many of the foods and beverages available and promoted, through the sport setting, locally and nationally, are EDNP. Sport is used to promote food and beverages at local and national levels and marketing strategies entice children to request products, influence preferences and possibly consumption; many foods and beverages eventually become part of children’s normal nutrition environment. Initial results show the food and beverage environment with regard to sport is “*very hard*” on parents and does not always enable, nor support them, in providing a desirable nutrition environment and therefore, behaviours in children.

As a signatory to the United Nations Convention on the Rights of the Child, New Zealand is required to report regularly on progress being made in meeting the requirements and obligations to the convention. The convention contains articles which guide signatories in upholding the universal principles and standards for the status and treatment of children, including the right for children to have good health and nutrition, protection from exploitation

and protection from information and material injurious to their well-being. Further, the Convention acknowledges parents as having primary responsibility for their children's upbringing and the State has an obligation to assist parents in meeting that responsibility. The preliminary findings from this research suggests the State, through a lack of appropriate controls around food and beverage marketing to children, could be failing to provide a nutrition environment conducive to health or which supports parents; moreover, it could be argued the New Zealand Government is not meeting its obligations to the Convention.

CONCLUSION

The majority of foods and beverages available at sport venues, including stadia, weekend sporting arenas and fields are EDNP. Furthermore, food and beverage choices at these venues were limited. Parents reported the marketing strategies used to promote foods and beverages influenced purchase requests from children; this often created family conflict in addition to creating difficulties for parents trying to provide a healthy nutrition environment for their children.

This research contributes to existing knowledge regarding the nutrition environment in which children live, and how that environment contributes to their development. Additionally, this research is useful for informing and influencing development of healthy public policy, locally and centrally, with the view to creating supportive food environments in sports settings. With regard to nutrition professionals specifically, this research raises awareness of the broad determinants of healthy nutrition behaviour which influence their day-to-day practice.

More importantly, nutrition professionals also have a role to play in creating healthy public policy. The findings of this research should be used by nutrition professionals at a 'grass-roots' level to assist sporting clubs in developing policies which promote healthful nutrition behaviours and interventions in children's sport; influence the local government sectors to ensure healthy nutrition is included as part of food and beverage policies at council-run facilities; and at a higher level, to raise awareness in central government for the requirement to develop policies which give consideration to nutrition, particularly where children are impacted. Being cognisant of the broader issues which determine nutrition behaviour not only benefits daily practice but arms nutrition professionals with the knowledge required to contribute proactively to policy development. Creating an environment which ensures the health of our children is improved and maintained will require a comprehensive approach to policy development, an action which nutrition professionals are well positioned to provide.

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Heinz Wattie's: Shaking up on Salt

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ABSTRACT

Heinz Wattie's NZ Ltd (Wattie's) established a sodium reduction programme in 2005 and since this time has worked progressively to reduce use of salt in our recipes. The details of our programme have been reported previously in 2008.

The programme has encouraged product development teams to consider sodium when developing new recipes and reviewing existing recipes. It is most important that the taste of the foods is not compromised as purchase behaviour shows that taste is a higher priority for consumers, than nutrient content.

Now five years into the programme, a significant reduction of 735 tonnes less salt used in manufacturing has been achieved. However, making changes can be challenging and foods need to be considered on an individual basis. It is also important to assess how the company's foods compare to the wider food supply and to understand current consumer food behaviour.

There is also a real need for current new up to date New Zealand data on sodium levels in the food supply and dietary behaviour as it relates to sodium and salt. This would enable a more targeted focus on sodium reduction with direct relevance to health outcomes.

INTRODUCTION

Wattie's has worked for years on the subtle reduction in use of salt, within the many constraints that exist in such a process. While food industry is a key stakeholder in the implementation of government policy (Healthy Eating Healthy Action Implementation Plan. 2004), it relies on the acceptance of consumers to support any changes made for public good.

Striking a balance is important to meet the needs of consumers and achieve health outcomes. Industry must balance a person's proven desire for great tasting food, and their need for affordable and convenient food, with a recognised responsibility to provide nutritious foods that support health.

Wattie's sodium reduction programme was initiated in 2005 and continues to be supported in the company allowing a gradual reduction in the use of salt in recipes.

METHODS

The details of Wattie's sodium reduction programme have been reported previously (Dick, 2008). The programme has been in place now five years and continues to be evaluated and adapted as required.

The key features of the programme are a defined set of targets for sodium for each category of foods in the company's portfolio. The targets are predominantly consistent with the sodium levels used in the 'Tick' programme run by the Heart Foundation. The targets are reviewed annually to ensure they remain appropriate. Each food within that category is classified against the target into one of three groups: 'acceptable' (at or below target), 'borderline' (within 20% above target), or 'high' (greater than 20% above target).

There are two key teams responsible for implementing the programme: Marketing and Product Development. The Marketers include a request to consider sodium in their brief. The Product Development team use their specialist skills in food development to create a great tasting recipe using as little salt as possible. Their constraints include, but are not limited to, time and budget.

Nutrient details of any new recipes, and changes to existing foods, are captured in the company product composition database, throughout the year. In the first quarter of the

calendar year a review of sodium is completed and reports given to all teams working on sodium. The annual review includes recognition of positive results.

Periodically benchmarking data is collected that gives comparative values for sodium in company foods versus the wider the market.

RESULTS

Since 2005 when this programme was initiated, Wattie's has reduced its use of salt in manufacturing by 735 tonnes. This does not capture further reductions in total sodium levels in foods that may have been achieved through reductions in sodium-containing ingredients such as cheese, soy sauce.

Overall, by the end of 2010, there was a 25% increase in the number of savoury foods meeting target for sodium. There has also been a significant reduction in the percentage of foods classified as high in sodium.

There are no set reduction levels (for example, 5%) across the company. People are instead encouraged to 'do the best they can' with each individual recipe. Each year the team that reformulated the product to achieve the greatest reduction overall is recognised.

Table 1 Products with greatest sodium reduction achieved each year

Year	Product	% reduction
2006	Eta creamy Ranch Dressing 250mL	27
2007	Oak Steak & Kidney canned meal 400g	64
2008	Wattie's Very Special Minestrone Soup 430g	36
2009	Wattie's Spaghetti Bolognese frozen meal 250g	46
2010	Wattie's Potato Roasters 700g	62

Many other foods have reduced sodium levels ranging from large to smaller amounts, down to two percent. However, any reduction is considered beneficial as the aim is to make significant change over a long term strategy.

Benchmarking reviews provide interesting data on the wider marketplace. To date the data collected is narrow and not representative of all products in the category, however it gives an indication of how wide the variation in sodium can be between products.

*Table 2. Variation in sodium levels in the market**

Product	Sodium	Product	Sodium
Wattie's Butter Chicken frozen meal	245mg/100g	Takeaway Butter Chicken [^]	364mg/100g
Wattie's frozen meal range	870mg/serve (avg) (430-1500mg/serve)	Supermarket purchased chilled meal (Brand X)	2746mg/serve

**Data collected in store October 2010*

[^] Food Composition Tables 2009

The Wattie's Butter Chicken frozen meal previously had a sodium level of 310mg/100g in 2005 and reduction work resulted in the new level of 245mg/100g. This has moved even further away from other options in the market, with Takeaway variants possibly providing 48% more sodium (364mg/100g). This can have implications on consumer preference.

In the quick meal category (frozen and chilled meals), an initial review indicates significant variation in the market as well with one meal (Brand X) containing 83% more sodium than the highest level in the Wattie's frozen meal range (2746mg and 1500mg per serve respectively).

DISCUSSION

The Wattie's sodium reduction programme is continuing to work successfully with improvements in sodium profiles seen year on year. The success has been made possible by taking a long term stepwise approach with realistic expectations and monitoring of consumer reaction. The programme requires considerable investment in time and budget for reformulation, sensory analysis, reporting, and label changes.

Monitoring and reporting brings challenges. Tracking change is difficult as the landscape changes constantly, both in the company and within the wider marketplace. In the previous six years, there have been many product launches along with deletions. There are also new brands in the Wattie's portfolio which add new categories for addition to the programme.

More information needs to be gathered on sodium levels in the New Zealand food supply. The current data available in the New Zealand Food Composition Database may not capture recent changes made by food manufacturers and food service establishments.

Wattie's also needs to undertake further benchmarking analysis to understand where their products fit alongside competitors. However, it must be noted that using Food Composition data may not provide an accurate comparison and obtaining values directly from food labels can be prohibitively time consuming. While Wattie's has and can continue to demonstrate leadership in sodium reduction it is important that the company's foods do not sit significantly outside the range in the market (in terms of sodium levels) as this is likely to deter consumers.

Going forward the company will continue to focus on sodium when producing new foods and reviewing existing foods. It is well understood that consumer's taste preferences for salt can adapt with time, so it is important that other players (both in the retail and food service sectors) are encouraged to lower sodium in high salt foods also.

In future, more reduction work could focus specifically on those foods that are known to provide significant sodium in the New Zealand diet. However, currently there is insufficient data available to identify these foods. Dietary sources of sodium were not reported in the previous National Nutrition Survey (Russell DG et al, 1999) however the dataset from this survey was modelled using DIAMOND and reported in 2003 (Nutrition and the Burden of Disease). While useful as a guide, this information is limited by use of outdated food composition data and dietary behaviour as surveyed in 1997.

It is hoped that government organisations with responsibilities to food and health may be supported to conduct a more accurate review of: New Zealanders dietary practices in relation to sodium-containing foods and use of salt, and levels of sodium in the food supply including significant dietary sources. This information would assist industry in identifying problem areas as they relate to current consumption and health, and would be useful to direct further reduction work.

CONCLUSION

Heinz Wattie's has a committed focus on minimising use of salt in recipes, as much as possible to produce great tasting and affordable food, every day. Over the previous five years Heinz Wattie's has reduced salt use in manufacturing by 735 tonnes.

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Development of sweet potato-soybean blend, an alternative to maize-legume mix as complementary food for infants in Ghana

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ABSTRACT

Background: The composition of the foods given to infants and young children in Ghana significantly contributes to the prevalence of malnutrition. Currently, a better option may be Weanimix (maize-soybean-groundnut blend) designed to be processed as industrial- or household-level complementary food. Weanimix has adequate protein and energy densities, but is high in phytate an antinutrient which inhibits nutrient bioavailability.

Objective: To formulate a low-phytate complementary food from cream-fleshed sweet potato to contain comparable levels of macronutrients as Weanimix.

Design: A composite blend of sweet potato, defatted soybean and soybean oil was cooked on a stove-top, oven-dried, milled and enriched with fishmeal (referred to as stove-top cooked ComFa).

Outcomes: Stove-top cooked ComFa had a protein level which was higher than that of Weanimix (25.49 ± 0.10 vs. 14.26 ± 0.29 g/100 g; $p=0.001$). However, the energy content was low compared to Weanimix (370 ± 1.70 vs. 431 ± 0.71 kcal/100 g; $p=0.001$). Stove-top cooked ComFa had a 7.5% energy deficit compared to the recommended level of at least 400 kcal/100 g in Codex standard, but met the calcium (105 mg/100 kcal) and zinc (1.6 mg/100 kcal) densities as recommended by WHO for complementary foods.

Conclusion: The sweet potato-soybean blend has the potential to serve as an alternative complementary food if the energy content could be improved.

INTRODUCTION

In 1987, Weanimix, an optimal complementary food which can be processed at either the household-level or at the industrial-level, was introduced in Ghana through collaboration between the Nutrition Unit of the Ministry of Health, Ghana and the United Nations Children's Fund (UNICEF, Ghana) to improve the nutritional status of older infants (Lartey *et al.*, 1999). Weanimix is a blend of maize, soybean and groundnut; it is higher in protein and energy densities compared to *koko* - a household-level complementary food prepared from fermented dough of maize, millet or sorghum only. Weanimix improved growth (height and weight indices) but not micronutrient status unless it was fortified with a vitamin and mineral premix (Lartey *et al.*, 1998, Lartey *et al.*, 1999). Weanimix is also high in phytate (480 mg/100 g) which will limit bioavailability of iron (Hurrell *et al.*, 2003, Hurrell and Egli, 2010), zinc (Gibson and Ferguson, 1998), probably calcium (Perlas and Gibson, 2005) and to some extent protein (Greiner *et al.*, 2006). Therefore, formulation of another complementary food with low phytate and made from available local resources warrants attention.

The objective of this study was to formulate a low-phytate complementary food from cream-fleshed sweetpotato and compare the levels of macro and micronutrients of the sweetpotato-based product with Weanimix. Both formulations were also compared with recommended standards (Codex Alimentarius Commission, 1991, Dewey and Brown, 2003).

METHODS

Ingredients used for processing complementary foods

All ingredients were sourced from New Zealand, unless otherwise stated. *Toka Toka Gold* (cream flesh with orange streaks) sweetpotato (*Ipomoea batatas*) was supplied by Delta Produce Co-op Ltd in Dargaville and processed into flour by peeling, drying and grinding.

Defatted toasted soybean (*Glycine max*) flour was purchased from Oppenheimer, Wellington. Refined white maize meal (Springbok™, South Africa), soybean seed, groundnut (*Arachis hypogea*) paste and soybean oil (SIMPLY™) were obtained from local supermarkets in Palmerston North. Fishmeal prepared by milling smoke-dried anchovies (*Engraulis hepsetus*) with the heads removed was imported from Ghana.

Formulation and processing of sweetpotato-soybean complementary food

The formulation was estimated using published nutrient composition of sweetpotato (FoodWorks, 2009) and the nutritional information on labels of the other ingredients to obtain a product with an energy level of 400 kcal/100 g, fat content between 10 – 25 g/100 g and protein content of at least 15 g/100 g on dry matter basis to meet the specified guidelines for complementary foods for infants and young children (Codex Alimentarius Commission, 1991).

ComFa, a composite blend containing 66% sweetpotato flour, 23% defatted soybean, 10.3% soybean oil, 0.20% lecithin and 0.50% iodised salt, was cooked on a stove to replicate the usual household-level food preparation and then oven-dried (9.31 ± 0.27 g/100 g moisture content). The dried sweetpotato-soybean formulation was broken into smaller chunks, milled and then enriched with fishmeal (20% wt/wt) prepared from anchovies. This is a recommended practice in Ghana to improve the nutrient quality of household-level complementary food (Akor *et al.*, 2001). The complementary food obtained was referred to as stove-top cooked ComFa. Sweetpotato was chosen to replace the maize and groundnut of Weanimix because it is low in phytate (Lukmanji *et al.*, 2008, Gibson *et al.*, 2010), high in vitamin A precursor and available in Ghana (Ofori *et al.*, 2009). It has been confirmed in another study that the calcium content of household-level prepared complementary food increased when this fishmeal was added (Perlas and Gibson, 2005).

Processing of maize-soybean-groundnut blend (Weanimix)

The method described by Lartey *et al.* (1999) was used with a slight modification. Refined (dehulled) maize flour was used instead of flour prepared from roasted white maize grain. This modification was necessary due to the unavailability of white maize grain for human consumption in New Zealand.

Nutrient analysis

The moisture, protein, fat and ash contents of the ComFa formulation and Weanimix were determined using the standard methods described by the AOAC International (AOAC, 2005). Carbohydrate and the energy contents were estimated (FAO, 2003). Calcium, iron and zinc in the samples were determined by atomic absorption spectrophotometry. All the analyses were performed on three independent replicates.

Data analysis

The data were analysed using two-sample t-test procedure in Minitab v15.1™ (Minitab Inc., US). Means were considered to be significantly different at $p < 0.05$. Results are reported as means of triplicate determinations \pm standard error of the mean (SEM).

RESULTS

Nutrient composition

The stove-top cooked ComFa met protein and fat specifications of the Codex Standard (Codex Alimentarius Commission, 1991) for complementary foods for infants and young children (Table 1). However, the ComFa formulation met approximately 70% of the estimated carbohydrate (60 – 75%) and 92% of the specified energy (400 kcal/100 g) in the Codex standard. It is evident that the fat in the ComFa formulation and Weanimix was not significantly different. Although protein content of the stove-top cooked ComFa was higher than Weanimix by about 56% ($p = 0.001$), the estimated energy value of the ComFa formulation was lower than Weanimix by 15% ($p < 0.001$). The estimated carbohydrate content of ComFa was lower than that of Weanimix by 34% ($p < 0.001$). The protein level of Weanimix was slightly lower (by 4.7%) than the protein level stipulated in the Codex standard but met the stipulated fat level. Weanimix met the minimum energy specification of complementary foods stated in the Codex standard.

Table 1: Macronutrient composition of complementary food processed from cream-fleshed sweetpotato, defatted soybean flour, soybean oil enriched with anchovies-fishmeal at 20% wt/wt (stove-top cooked ComFa) and maize-soybean-groundnut (Weanimix)^a

Nutrient(/100 g)	Codex Standard ^b	Stove-top cooked ComFa	Weanimix	p-value ^c
Protein, g	15	25.49 ± 0.10	14.29 ± 0.29	0.001
Fat, g	10 - 25	11.76 ± 0.11	12.02 ± 0.23	0.22
Ash, g		6.07 ± 0.01	1.84 ± 0.01	<0.001
Carbohydrate, g (by difference)	60 – 75 ^d	47.36 ± 0.27	66.52 ± 0.37	<0.001
Energy, kcal	400	370.06 ± 1.70	431.27 ± 0.71	0.001

^a Values (mean ± SEM, n=3) reported on dry matter basis;

^b Source: Codex Alimentarius Commission (1991);

^c ComFa formulation and Weanimix are significantly different at $p < 0.05$;

^d Estimated from data given for protein and fat in the Codex Standard.

The stove-top cooked ComFa containing anchovies met the requirement for calcium and zinc, and about 49% of the recommended level of iron from complementary food for breastfeeding infants (6 – 8 months old) (Dewey and Brown, 2003) (Table 2). Weanimix met only approximately 3.0% of the calcium, 30% of the iron and 94% of the zinc recommended levels. The nutrient densities for calcium and iron but not zinc of the ComFa formulation were significantly higher than that of Weanimix.

Table 2: Calcium, iron and zinc densities of cream-fleshed sweetpotato, defatted soybean flour, soybean oil enriched with anchovies-fishmeal at 20% wt/wt (stove-top cooked ComFa) and maize-soybean-groundnut (Weanimix) complementary foods compared to average desired densities^a

Nutrient density (mg/100 kcal)	Recommended level (WHO 2002) ^b	Stove-top cooked ComFa	Weanimix	p-value ^c
Calcium	105	131.50 ± 8.10	3.59 ± 1.10	0.004
Iron	4.5	2.19 ± 0.02	1.34 ± 0.14	0.03
Zinc	1.6	2.13 ± 0.52	1.50 ± 0.17	0.37

^a Values (mean ± SEM, n=3) reported on dry matter basis;

^b Source: WHO recommended levels as cited by Dewey and Brown (2003);

^c ComFa formulation and Weanimix are significantly different at $p < 0.05$.

DISCUSSION

This research shows it is possible to process a dried complementary food from sweetpotato as reported in another study (Nandutu and Howell, 2009). The low energy and carbohydrate content of the stove-top cooked ComFa could be increased by modifying the formulation. For instance, the use of full-fat soybean flour instead of the defatted flour would increase the energy content. The fishmeal added to the ComFa formulation accounted for the high protein content compared with the level in Weanimix. A similar protein content of 28 g/100 g was obtained when Weanimix was enriched with anchovies-fishmeal at 20% (wt/wt) (Lartey *et al.*, 1999). The pronounced effect of the addition of the fishmeal on the calcium content in ComFa suggests that it could be used as a rich source of calcium in complementary feeding and should be encouraged in other localities where anchovy is available. Our findings support the observation that adding fishmeal prepared from anchovies improved calcium content of plant-based complementary food as reported in another study (Perlas and Gibson, 2005). A further advantage of the sweetpotato-based complementary food containing anchovies is that the overall micronutrient content is increased, and so

combined with its low level of phytate (Gibson *et al.*, 2010), the bioavailability of micronutrients would be enhanced. This would make the sweetpotato-based product more useful for improving micronutrient status of infants than the maize-based complementary food.

CONCLUSIONS

The preliminary results from this study suggest that cream-fleshed sweet potato has the potential for providing the basis of an alternative complementary food for infants in Ghana. However, the energy value of the formulation needs to be increased to make it suitable as complementary food. The addition of anchovies-fishmeal to sweet potato-soybean blend provided the requirement for calcium and zinc densities and about half the recommended level for iron density.

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Fishing for Iron: The Effects of Salmon on Non-Haem Iron Absorption

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ABSTRACT

Background: Iron bioavailability can be manipulated by the nutritional composition of a meal. Ascorbic acid, and a yet-to-be-identified fraction sourced from meat commonly termed the “meat factor” both appear to enhance the bioavailability of non-haem iron, however not all meat factor sources have been identified, and the enhancing mechanism still remains elusive.

Objectives: The objective of this study was to identify whether salmon enhances non-haem iron absorption in an *in vitro* model of human iron absorption. This model is commonly used for drug and nutrient uptake assays.

Design: Caco-2 cells were cultured until tight junctions were mature. Raw salmon homogenate and reconstituted egg albumin were digested using an *in vitro* process. ^{55}Fe was used as a tracer to measure iron uptake into the cells. Caco-2 cell monolayers were incubated with the uptake solutions for 60 minutes. Ascorbic acid was used as a positive control.

Results: Ascorbic acid enhanced non-haem iron absorption to the highest degree ($28.8\% \pm 3.69\%$). Salmon significantly enhanced iron absorption ($12.31\% \pm 2.15\%$) compared to egg albumin ($4.87\% \pm 0.49\%$). Differences between means of all treatments were statistically significant ($p < 0.01$).

Conclusion: Salmon flesh digested *in vitro* enhanced non-haem iron absorption in Caco-2 cells to a similar magnitude to that reported with beef digestate. Further investigation into the mechanism of enhancement and its impact in human subjects is justified.

INTRODUCTION

Ascorbic acid, red meat, pork, poultry and fish all significantly enhance iron absorption when consumed as part of a vegetable-based meal in human subjects (Cook and Monsen 1976). Of all meat sources beef has been repeatedly reported to enhance iron absorption to the highest degree in human subjects (Cook and Monsen 1975; Cook and Monsen 1976; Armah et al. 2008), however increasing meat consumption in order to improve iron status will also increase saturated fatty acid consumption which may compromise cardiovascular health (Astrup et al. 2011), thus the identification of alternative iron absorption-enhancing foods requires further investigation. Although ascorbic acid may fulfil these criteria, it is not stable and will quickly degrade during most cooking processes or prolonged exposure to sunlight.

The effect of oily fish on iron uptake has been investigated both in humans and rats, however protocol inconsistencies have led to contradicting results (Rodriguez et al. 1996; Seiquer et al. 2002; Navas-Carretero et al. 2008) and further investigation is justified.

The aim of this study is to investigate the effects of New Zealand King salmon digestate on non-haem iron uptake in Caco-2 cell monolayers compared to ascorbic acid, a positive control and egg albumin, a negative control.

MATERIALS AND METHODS

Cell culture

Caco-2 cells were sourced from American Type Culture Collection (Manassas, USA) and cultured in T75 flasks (Nunc, Rochester, NY) in 15 mL of cell culture medium (D10) corrected to pH 7.4 containing Dulbecco's modified Eagle's minimal essential medium (Invitrogen, CA, USA), foetal bovine serum (10%) (Invitrogen, CA, USA), Penicillin-Streptomycin-Neomycin (1%) (Invitrogen, CA, USA), non-essential amino acids (1%)

(Invitrogen, CA, USA) and Glutamax (1%) (Invitrogen, CA, USA) at 37°C with 5% CO₂ and 90% humidity. Cells were used between passage 30 and 35. Cells were seeded at a density of 200,000 cells/cm² on hydrated 12-well Transwell plates (Corning, NY, USA). The medium was replaced every 2 days and monolayers were used 27 days post-seeding after monitoring the formation of cell junctions using trans-epithelial electrical resistance (TEER) measurements. Only wells with a TEER value between 250 Ω cm² and 800 Ω cm² (after subtraction of the membrane resistance) were used.

Treatments

Fresh Salmon (Countdown, New Zealand) was trimmed from the shell to a total weight of 400 g. Egg albumin (Zeagold, New Zealand) was reconstituted to the biological concentration of raw egg albumin by 1 part egg albumin to 7 parts de-ionised water to a final weight of 400 g.

In vitro digestion

Porcine pepsin (Sigma P-7000, St. Louis, Mo., U.S.A.) was diluted in 0.1M HCl (BDH chemicals, UK) to a working solution containing 7812 units/ml. Aliquots of GLM, beef and egg albumin homogenates were incubated at 37°C for 30 minutes. Each 20 g aliquot was titrated with 1M HCl (BDH chemicals, UK) to pH 2.4 and combined with 0.67 ml of pepsin solution. Samples were incubated at 37°C for 2 hours in a shaking water bath.

Porcine pancreatin 2.4 mg/ml (Sigma P-1625, activity 3 x USP specifications) and porcine bile salts 6.25 mg/ml (Sigma B-8631, glycine and taurine conjugates of hyodeoxycholic and other bile salts) were prepared immediately before use in 0.1M NaHCO₃. Samples treated by peptic digest were immediately titrated to pH 6 with 1M NaHCO₃ (Sigma St. Louis, MO). Five millilitres of pancreatin/bile solution were added to each sample. Samples were incubated for 2 hours at 37°C. Digests were immediately stored at -20°C.

Digestates were diluted to 300 mOsm with deionised water. Each treatment was diluted a further 3 fold in HBSS. The non-haem iron concentration of each digestate was analysed by spectroscopy using a ferrozine method (Ahn et al. 1993). Ascorbic acid (95.5 mmol/L) was prepared in an ascorbate:Fe molar ratio of 4:1. Salmon and egg albumin digestates and ascorbic acid were combined with a pre-prepared iron working solution containing 1:10 ⁵⁵Fe and ⁵⁶Fe respectively (37 kBq) and titrated to pH 6.5.

Experimental design

Treatment aliquots (300 μ L) were applied to the Caco-2 apical reservoir in a randomised order and incubated for 60 minutes at 37°C with 5% CO₂ and 90% humidity. The apical and basolateral reservoirs were rinsed twice with both HBSS (pH 7.4) and removal solution (0.5 mmol/L EDTA in HBSS, pH 7.4). Monolayers were solubilised in 0.2M NaOH (Sigma St. Louis, MO) for 12 hours at 4°C and 100 μ L aliquots of cell lysate were removed. Time zero, basolateral and tissue samples were analysed for ⁵⁵Fe by scintillation counting (Wallac Trilux 1450 Microbeta, PerkinElmer, Waltham, Massachusetts, USA).

Statistical analysis:

All results were calculated as percentage iron in solubilised cells compared to the apical iron concentration at time zero. Statistical analysis was performed using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA). Treatments were analysed by 2-way ANOVA using the General Linear Model procedure. Where appropriate, post-hoc analysis was carried out using least square difference (LSD) analysis.

RESULTS

All Caco-2 cell monolayers had a TEER value between 250 Ω cm² and 650 Ω cm². There was no significant decrease in TEER value after 60 minutes duration. There was no treatment effect on TEER change over the 60 minute period ($p > 0.05$).

Table 1 Iron uptake by Caco-2 cell monolayer[†]

Ascorbate (%)	Egg albumin (%)	GLM (%)
28.8 ± 3.69a	4.87 ± 0.49b	12.31 ± 2.15c

[†]Values represent percent mean iron uptake ± SE, n=8. Values marked with dissimilar superscript letters are significantly different from one another (p<0.05).

Caco-2 cell iron uptake is summarised in Table 1. Iron uptake was significantly enhanced in the ascorbic acid treatment compared to the egg albumin digestate (p<0.0001) or salmon digestate (p<0.0001). Salmon digestate significantly enhanced iron uptake compared to egg albumin digestate (p=0.005).

DISCUSSION

Ascorbic acid consistently enhances iron absorption approximately 2 to 8-fold compared to egg albumin in human subjects and cell cultures (Glahn and Van Campen 1997; Teucher et al. 2004). This effect is consistent with our results which clearly show that when compared to egg albumin digestate, ascorbic acid enhances iron absorption by 5 to 6-fold.

The present study indicates that salmon digestate enhances iron absorption by around 2.5-fold above that of egg albumin digestate, a magnitude similar to that of red meat in human subjects (Cook and Monsen 1976) and red meat in mammalian cell cultures (Glahn et al. 1996). The results also support previous observations reported by Navas-Carretero et al. (2008) who noted that salmon supplementation at a dose recommended by the American Heart Association (AHA) improves non-haem iron absorption and retention.

However, the enhancing effect of oily fish supplementation on iron status *in vivo* requires further investigation. Enterocyte iron uptake is also augmented in rats when supplemented with fish oil as their exclusive lipid source over a 16 week period (Rodríguez et al. 2003; Miret et al. 2007). This initial result suggested that oily fish improves iron status, however Rodríguez et al. (2003) reported that long term fish oil supplementation was associated with significantly reduced hepatic iron levels, a result which appears to be associated with polyunsaturated fatty acid (PUFA) enrichment of the erythrocyte membrane, erythrocyte fragility, augmented erythrocyte turnover and decreased iron retention.

Nevertheless, oily fish consumption by mildly iron deficient women at a dose twice that recommended by the AHA does not further compromise iron status (Navas-Carretero et al. 2009), while rodent studies using whole sardines as the exclusive protein source and partial lipid source reported improved iron uptake and retention compared to a lactic casein/olive oil control diet (Seiquer et al. 2002). These PUFA doses were significantly less than those reported by Rodríguez et al. (2003) or Miret et al. (2007).

In summary, we have shown that salmon digestate can enhance non-haem iron uptake in Caco-2 cells. These results further suggest that supplementation of salmon may be a healthy alternative to that of red meat in order to improve non-haem iron uptake into the absorptive enterocyte. Further investigation into the long term effects of oily fish consumption or dose on iron retention is justified.

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Assessment of nutrition knowledge and food skills in talented adolescent athletes

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ABSTRACT

Background: There is limited research on the nutrition knowledge and food skills of elite adolescent athletes. As adolescent athletes are moving through an important physiological stage of life, as well as training and competing in their chosen sports, optimal nutrition is vital for not just health and wellbeing, but also sporting performance.

Objectives: This study investigated the general and sports nutrition knowledge of this group. Influences on knowledge and food choice were also investigated.

Design: This study investigated the basic and sports nutrition knowledge of 98 talented adolescent athletes (n=59 females, n=39 males, mean age of 16.6 years) from five team sports (basketball, football, netball, rugby and underwater hockey) using a questionnaire.

Outcomes: The participants had a reasonable level of basic nutrition knowledge, but lacked knowledge of sports nutrition particularly in the areas of sports drinks, supplements and the role of protein in the body. There was a positive view of sports nutrition demonstrated by majority of the subjects, although subjects rated taste as the biggest influence on their food choice.

Conclusion: It is clear that the adolescents in this study had a positive view of sports nutrition but did not possess a high level of knowledge in this area. More research is needed to assess the overall knowledge of New Zealand adolescent athletes and the most appropriate, and effective methods of education for this group.

INTRODUCTION

Adolescents have very specific nutritional requirements due to the period of change that they are moving through. The effect of peer pressure, the role of independence and the increased need for energy requirements means that many adolescents have eating habits which are less than desirable. An athlete, whether adult or adolescent, needs a balanced diet for both health and wellbeing and optimal performance. Coupled with the fact that when athletes are adolescents they have greater energy and nutrient needs due to the large period of growth that this period entails (ADA, 1996, Story et al, 2002, Hinton et al, 2004, Croll et al, 2006); this creates a significant nutritional challenge.

In terms of performance it could be assumed that the more talented and more successful an athlete is, the more nutrition knowledge he or she will possess and the better his or her nutritional behaviour will be; however, there is a lack of research on elite athletes compared to non-elite athletes or sedentary individuals. Knowledge and food skills may play a role in positive nutrition behaviour, but more research needs to be undertaken in this area.

METHODS

Subjects

Five sporting organisations were asked to provide contact details of their athletes who met the entry criteria for the project. The entry criteria included athletes between the ages of 13 and 20 years old as at the 1st January 2007, considered talented or elite in their chosen sport as they were either carded, or part of a High Performance Academy Group, Regional Talent Identification Group or equivalent.

Ethical considerations

The study was given ethical approval by the Massey University Committee of Ethics (ref 07/52). Subjects who took part in the study were asked to sign a consent form indicating their consent, subjects who were 16 years or younger were required to provide parental consent to take part in the research.

Questionnaire

The questionnaire was developed using previous research questions modified from Parmenter and Wardle (1999), Cuspiti et al (2002) and Turconi et al (2003), and newly developed questions. A pilot trial of the questionnaire was conducted with athletes of the same age and who participated in the same sports as the targeted population. Changes were made to questions which were unclear before sending to the actual subject group. Due to time constraints, validity and reliability tests were not able to be performed. To identify those athletes who had correct knowledge, incorrect knowledge, and those who did not have any knowledge, the questionnaire used 'yes', 'no', and 'unsure' answers.

The subjects were posted a questionnaire to complete and return, along with an information sheet and consent form (parental and subject).

Analysis

The questionnaire results were coded +1 for a correct answer, 0 for an incorrect answer and 'u' for unsure. Where comments were added, these were separated into groups based on similar themes in answers. The data was analysed with SPSS v14.0. The data was examined using a Chi-Squared statistic test to assess the frequency and association between variables. Results were considered to be statistically significant if $p < 0.05$.

RESULTS

Ninety eight talented adolescent athletes ($n=59$ females, $n=39$ males, mean age of 16.6 years) from five team sports (basketball, football, netball, rugby and underwater hockey) participated in the study. Overall, the participants had a reasonable level of basic nutrition knowledge. The correct score for general nutrition knowledge ranged from 61% (basketball) to 78% (underwater hockey). There were no significant differences between any sport or gender for basic knowledge. Overall scores for sports nutrition knowledge ranged from 37% for football up to 44% for netball. A lack of knowledge was seen particularly in the areas of sports drinks, supplements and the role of protein.

Taste was rated as the biggest influence on food choice. The number of athletes who were involved in food preparation ranged from 50% (netball) to 74% (rugby). The athletes self-rated cooking skills (on a scale with 1 = non-existent, 10 = excellent) ranged from 4.85 (netball) – 7.67 (rugby).

The adolescents had a positive view of sports nutrition with 79% believing that sports nutrition was important in their sporting planning and 88% believing that specific sports nutrition strategies could enhance their performance.

DISCUSSION

The subjects had reasonable levels of basic nutrition knowledge, but there were certain areas that showed a lack of knowledge, specifically food components. This is consistent with previous research (Berning et al, 1991). Research cited in the literature is inconclusive on differences in level of knowledge between genders (Rosenbloom et al, 2002, Nichols et al 2005). Nowark and colleagues (Nowak et al, 1998) suggested that males tend to exhibit lower levels of nutrition and food knowledge compared to females, and they were not as interested in health as females. However, this study demonstrated that there was not a significant difference in basic nutrition knowledge levels between genders.

The disparity in protein knowledge seen in this study has been observed in previous research (Rosenbloom et al, 2002, Harrison et al, 1991). Subjects also showed a lack of knowledge on the topic of supplements, including vitamin and mineral supplements. Harrison et al (1991) also found that there was also confusion over the role of vitamins and minerals in adult New Zealand elite and non elite athletes.

The results of this study mirrored findings of both Pratt and Walberg (1988), and Chapman and Toma (1997) showing that many athletes believed that a sports drink was

more effective for rehydration than water. O'Dea (2003) found that, although adolescent athletes identified that sports drinks could enhance performance, they did not relate this to rehydration. Many of the athletes (87.8%) in this research identified sports drinks as being performance enhancing when they were consumed during an event but could not give reasons why.

Taste was rated the highest influence on food choice in this study, followed by cravings and parental influence which are seen in Neumark-Sztainer's (1999) model of influences on adolescent food choice. The food skills that the athletes possess may play a role in food choice, for example if they have a higher degree of cooking ability they may consume better quality and more balanced meals. There was a trend for a relationship between cooking skill rating and the athlete's role in food preparation, although not statistically significant ($p=0.131$).

CONCLUSIONS

It is clear that the adolescents in this study had a positive view of sports nutrition but did not possess a high level of knowledge in this area. More research is needed to assess the overall knowledge of New Zealand adolescent athletes and the most appropriate, and effective methods of education for this group. Because of the low levels of knowledge it is recommended that resources and educational programmes are designed specifically for adolescent athletes.

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Effect of storage conditions on bacterial content in expressed breast milk

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ABSTRACT

Background: Nursing mothers in New Zealand and many other countries use expressed breast milk (EBM) to feed infants when breastfeeding is not possible. EBM is nutritionally balanced to provide nourishment to the infant. The handling and storage of EBM can introduce the possibility of contamination.

Objective: To determine the viable Aerobic Plate Count (APC), *Escherichia coli* and *Staphylococcus aureus* counts in EBM stored at room temperature and refrigeration temperature.

Design: Breast milk samples were collected in suitable containers from eight healthy mothers based in Auckland and 1.5 mL aliquots were prepared and stored at different storage conditions. Each sample was analysed for APC, *Staphylococcus aureus* and *Escherichia coli* at baseline, 1 h, 2 h, 3 h and 4 h stored at room temperature ($23 \pm 2^\circ\text{C}$) and 4 h, 8 h, 12 h, 24 h and 48 h at refrigeration temperature ($4 \pm 1^\circ\text{C}$).

Outcomes: A significant ($p < 0.05$) increase in APCs was observed in samples stored at the two temperatures. However, the overall cell counts were lower in the refrigerated samples. At the end of 48 h of refrigerated storage, the bacterial counts had decreased or remained stable in the samples. A similar trend was observed in the *Staphylococcus aureus* counts. No *Escherichia coli* was detected in any of the samples indicating sanitary conditions during handling and storage of EBM.

Conclusion: The results of this study suggest that storage of EBM at refrigeration temperature is most appropriate to minimise microbial content and any further growth.

INTRODUCTION

The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months after which gradual introduction to solids is recommended (Kramer & Kakuma, 2001). Considering the fact that breast milk is the optimal source of nutrients and an unmatched source of indispensable anti-microbial and other protective substances, breastfeeding infants is important. Breastfeeding might not be possible at all times due to various reasons including swollen breasts due to production of milk, infant being unable to suckle or even due to the economic activities of lactating mothers (Abraham & Steven, 2006). Expressed breast milk (EBM) is a suitable alternative for feeding infant when breastfeeding is not possible. Expression of breast milk, handling, storing and subsequent provision of breast milk to infants introduces the possibility of contamination and nutrient depletion.

According to the guidelines provided by New Zealand Ministry of Health, expressed breast milk can be stored at room temperature for up to 4 hours and refrigeration temperature ($4 \pm 1^\circ\text{C}$) for up to 48 hours (Ministry of Health NZ, 2010). A study conducted by Eteng et al. (2001) reported that the bacterial content increased significantly ($p < 0.05$) when breast milk was stored at ambient temperature beyond 3 hours (Eteng et al., 2001). Another study reported a decrease in the cell counts when breast milk was stored under refrigeration for up to 96 hours (Slutzah et al., 2010). Therefore, the objective of this study was to investigate the bacterial content of expressed breast milk when stored under the recommended guidelines by Ministry of Health, NZ (Ministry of Health NZ, 2010).

MATERIALS AND METHODS

Eight healthy breastfeeding mothers based in North Shore City, Auckland, New Zealand were recruited after completion of the screening questionnaire. The participants were requested to collect the breast milk before the first feed of the day in order to standardise the collection of breast milk and they were requested to express (manual or pump method) milk from one breast only until empty leaving the other breast for the infant to feed on. Expressed breast milk collected in a sterile air-tight container (80 mL AVENT™) was transported to the laboratory in a cooler box under refrigerated conditions ($4 \pm 1^\circ\text{C}$) within 1 hour of expression. Aliquots of these samples were prepared and stored at room temperature ($23 \pm 2^\circ\text{C}$) for 4 hours and refrigeration temperature ($4 \pm 1^\circ\text{C}$) for 48 hours. Each sample was analysed for aerobic plate count (APC), *Staphylococcus aureus* and *Escherichia coli* at different time points during storage as indicated in Table 1. Standard enumeration methods were used for determining the microbial counts. Plate count agar was used for the enumeration of APCs. Baird-Parker agar was used for *Staphylococcus aureus* enumeration while Lauryl Sulfate Tryptose broth with MUG (4 methylumbelliferyl- β -D glucuronide) was used for the enumeration of *E. coli*. Data obtained in this study were analysed by the SPSS statistical software, version 18.0 (SPSS Inc., Chicago, IL, USA, 2010).

Table 1 Storage conditions for expressed breast milk samples for microbiological analysis

Storage condition	Storage time (hours)				
Room temperature ($23 \pm 2^\circ\text{C}$)	0	1	2	3	4
Refrigeration temperature ($4 \pm 1^\circ\text{C}$)	4	8	12	24	48

RESULTS

The results of the microbiological analyses showed the presence of microbial growth on the aerobic plate count agar and *Staphylococcus aureus* on the Baird Parker agar. *Escherichia coli* was not detected in any samples. The total colony forming units (cfu) ranged between 1.55 - 3.27 log cfu/mL in the samples at baseline. An increase in the APCs (range 2.02 - 3.62 log cfu/mL) was observed when the samples were stored at room temperature ($23 \pm 2^\circ\text{C}$) for 4 h (Figure 1) and a significant ($p < 0.05$) increase in the aerobic plate counts was recorded in all samples when analysed hourly. At refrigeration temperature ($4 \pm 1^\circ\text{C}$), the APCs had increased (1.96 - 3.35 log cfu/mL), but the counts remained lower than the counts in the EBM samples stored at room temperature for 4 h. After the storage period of 48 h at refrigeration temperature, the APC levels ranged between 1.87 - 3.36 log cfu/mL (Figure 2). No significant ($p > 0.05$) change in aerobic plate count was recorded in the EBM samples stored in the refrigerator for 48 h.

The *Staphylococcus aureus* counts at baseline ranged between 1.51 - 3.02 log cfu/mL, which increased significantly ($p < 0.05$) to about 2.14 - 3.76 log cfu/mL by the end of 4 h storage at room temperature (Figure 1). The counts ranged between 1.83 - 3.58 log cfu/mL in samples stored at refrigeration temperature for 4 h, indicating that the counts were higher in samples stored at room temperature. At the end of refrigeration storage (48 h) the cell counts ranged between 1.57 - 3.33 log cfu/mL with no significant ($p > 0.05$) change, indicating that the microbial growth remained stable (Figure 2).

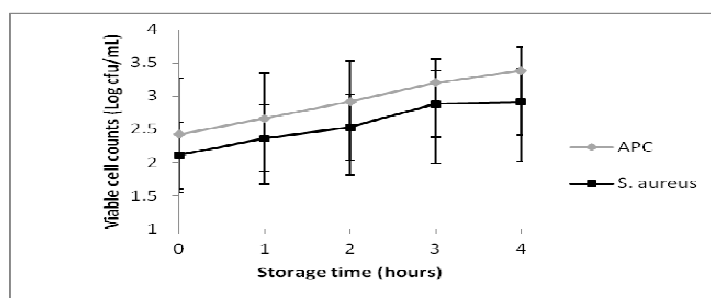


Figure 1: Growth of APCs and *Staphylococcus aureus* in expressed breast milk samples stored at room temperature ($23 \pm 2^\circ\text{C}$) for 4 h.

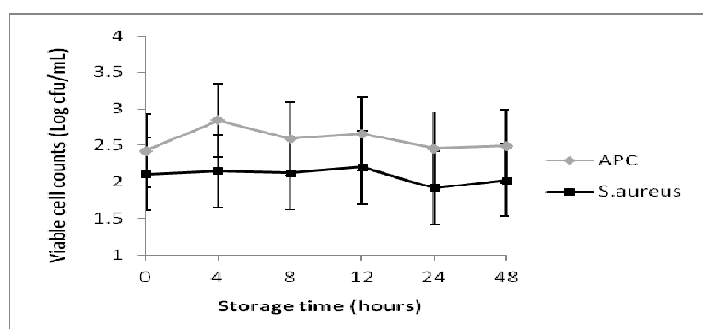


Figure 2: Growth of APCs and *Staphylococcus aureus* in expressed breast milk samples stored at refrigeration temperature ($4 \pm 1^\circ\text{C}$) for 48 h.

DISCUSSION

Various bacterial isolates found in breast milk are common skin flora including *Staphylococcus albus*, *Staphylococcus aureus*, *Streptococcus viridians* (Eteng et al., 2001). Breast milk also contains probiotic lactic acid bacteria which colonise the infant gut (Martin et al., 2003). Other bacteria isolated from human milk include *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Streptococcus australis*, *Streptococcus salivarius*, *Streptococcus mitis*, *Lactobacillus gasseri*, *Bifidobacterium breve* (Marin et al., 2009).

In a study by Igumbor et al. (2000), there was no significance in the microbial growth in samples stored at high temperature (30 - 38°C), room temperature (15 - 27°C), refrigeration temperature (4 - 10°C) and freezing temperature (0 - 4°C) for 4 h, 8 h, 24 h and 72 h respectively. Growth was observed in the samples stored beyond these storage durations and both pathogens and non-pathogens were isolated from these milk samples (Igumbor et al., 2000). In a similar study conducted by Knoop et al. (1985), the bacterial counts slightly decreased in the samples stored at higher temperature (8 - 10°C) during the storage period of 3 days. The decrease in the bacterial content in samples stored at refrigeration temperature (4°C) was more significant compared to that in the samples stored at higher temperature (8 - 10°C) (Knoop et al., 1985). Therefore, the results of our study are correlated to previous studies conducted on the bacterial content in human milk stored at different temperature conditions for different periods.

Reports have indicated that storage of breast milk at refrigeration temperature or cold storage for 48 hours does not eliminate the antimicrobial properties (Hernandez et al., 1979; Martinez-Costa et al., 2007; Ogundele, 2000), whereas storage beyond 72 hours significantly lowers the degree of bacteriolysis. This suggests that bactericidal activity remains stable in stored EBM for 48 h during refrigeration (Martinez-Costa et al., 2007). The bactericidal activity of human milk could be responsible for no change in the bacterial counts in EBM samples stored at refrigeration temperature for 48 h.

Breast milk contains diverse bacterial species, among which some organisms have antimicrobial activity against *Staphylococcus aureus* (Heikkila & Saris, 2003). A study conducted by Heikkila and Saris (2003) revealed that lactic acid bacteria (*Lactobacillus*

rhamnosus, *Lactobacillus crispatus*, *Lactobacillus lactis*) were effective against *Staphylococcus aureus*. Isolates of *Staphylococcus epidermidis*, *Streptococcus mitis* and *Streptococcus salivarius* also suppressed the growth of *Staphylococcus aureus*. Hence breast milk contains commensal bacteria which have anti-*Staphylococcus aureus* activity (Heikkila & Saris, 2003). This could be the reason for no change in *Staphylococcus aureus* counts observed in this study.

CONCLUSION

Microbiological analyses were conducted for samples stored at room temperature ($23 \pm 2^\circ\text{C}$) for 4 h and refrigeration temperature ($4 \pm 1^\circ\text{C}$) for 48 h. *Escherichia coli* was not detected in any of the EBM samples suggesting that good sanitation was maintained during the handling of EBM. However, a significant ($p < 0.05$) increase was observed in both APCs and *Staphylococcus aureus* counts in the EBM samples stored at room temperature. Meanwhile, the APCs and cell counts of *Staphylococcus aureus* in EBM samples stored in a refrigerator remained stable for 48 h. Therefore, EBM stored at room temperature for 4 h may be unsafe for infant feeding. Storing EBM under refrigerated conditions is more appropriate for providing safe and nutritive milk to the infant.

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Effect of storage conditions on the vitamin C concentration of expressed breast milk

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ABSTRACT

Background: Breast milk is the best source of nutrients for growth and development of the infant. When breastfeeding is not possible, expressed breast milk (EBM) is a suitable alternative for infant feeding. Handling and storage of EBM causes degradation of essential nutrients like vitamin C, which is important to the infant owing to its antioxidant activity.

Objective: To determine the concentration of vitamin C in EBM stored according to the guidelines recommended by the Ministry of Health, New Zealand.

Design: EBM samples were collected in air-tight containers from eight healthy mothers based in Auckland and aliquots (0.75 mL) were prepared before storage under different conditions. Samples stored at room temperature ($23 \pm 2^\circ\text{C}$) were analysed at baseline, 2 h and 4 h. Refrigerated ($4 \pm 1^\circ\text{C}$) samples were analysed after 4 h, 8 h, 12 h, 24 h and 48 h, whereas frozen (-18°C) samples were analysed after 24 h, 48 h, 7 and 14 days.

Outcomes: A significant ($p < 0.05$) decrease in the concentration of vitamin C was observed in all the samples towards the end of the storage periods. At the end of 4 hours, samples stored at room temperature contained 84.4% of the initial concentration of vitamin C while the refrigerated samples contained 92.8% of the initial vitamin C concentration. Refrigerated samples contained lower concentration of vitamin C (56.5% of baseline concentration) as compared to the frozen samples (81.1% of baseline concentration) after 48 h of storage.

Conclusion: The results of this study indicate that cold-stored samples retained higher concentrations of vitamin C as compared to the samples stored at room temperature.

INTRODUCTION

Human breast milk is a complex mixture of organic and inorganic compounds that provide a wide array of growth factors, and necessary nutrients for the growth and development of the infant. It contains valuable nutrients, with appropriate amounts of carbohydrates, proteins, vitamins, fat and minerals. Breast milk from a healthy mother provides all nutrients and supports adequate growth and development for the first 6 months of an infant's life (Kunz et al., 1999).

Breastfeeding (feeding the infant with breast milk directly from human breasts) is also known as nursing (Scott et al., 2006) and WHO recommends exclusive breastfeeding for the first six months (Kramer & Kakuma, 2001). When the mother is unable to nurse the infant directly, an alternative method is to provide expressed breast milk (EBM). EBM can be obtained either by manual expression or by use of a breast pump and fed to an infant through a bottle feeding system (Meah, 1996). According to the guidelines provided by the New Zealand Ministry of Health, EBM can be stored up to 4 hours at room temperature ($23 \pm 2^\circ\text{C}$), 48 hours at refrigeration temperature ($4 \pm 1^\circ\text{C}$) and for 14 days at freezer temperature (-18°C) (Ministry of Health NZ, 2010).

During handling and storage of milk, oxidation or degradation of heat and light sensitive nutrients such as vitamin C is possible (Fenaille et al., 2005). Loss of ascorbic acid (vitamin C) in EBM has been studied and reports suggest that storage of milk at both refrigerator and freezer temperatures have led to significant decreases in the levels of vitamin C (Ezz-El-Din et al., 2004) thereby reducing the antioxidant capacity of breast milk (Hanna et al., 2004). Therefore, the objective of this study was to determine the concentration of vitamin C in EBM when stored at room temperature, refrigeration temperature and freezer temperature in accordance with the recommendations by Ministry of Health, NZ.

MATERIALS AND METHODS

Participants for this study were recruited from Auckland, New Zealand and the selection criteria were mothers of infants aged 0 - 6 months currently and exclusively breastfeeding their infants without additional solid food. Eight eligible healthy mothers were recruited and each mother was provided with an 80 mL sterile air-tight AVENT™ container for the collection of EBM. Mothers were requested to express milk from one breast until empty before the first feed of the day. The samples were then transported to the laboratory in cooler box at refrigeration temperature ($4 \pm 1^\circ\text{C}$) within 1 h of collection and processed.

Eppendorf tubes were labelled and twelve 0.75 mL aliquots of each sample were prepared and stored at three different temperatures: room temperature ($23 \pm 2^\circ\text{C}$), refrigeration temperature ($4 \pm 1^\circ\text{C}$) and freezing temperature (-18°C). Among the twelve tubes, sample from one tube was analysed at baseline while two tubes with sample were stored at room temperature ($23 \pm 2^\circ\text{C}$) for up to 4 h. Five tubes were stored in a refrigerator (Model E38IT) ($4 \pm 1^\circ\text{C}$) for up to 48 h and the remaining four tubes were stored at freezer temperature (Model E38IT) (-18°C) for up to 14 days. The samples were stored for different periods prior to analysis (Table 1).

Table 1 Storage conditions and times of sampling for expressed breast milk samples for vitamin C analysis

Storage temperature	Storage time (hours)				
Room temperature ($23 \pm 2^\circ\text{C}$)	0	2	4	-	-
Refrigeration temperature ($4 \pm 1^\circ\text{C}$)	4	8	12	24	48
Freezer temperature (-18°C)	24	48	168	336	-

Notes (-) indicates no analysis was performed at these storage times

At the end of each storage period, each sample was mixed with a preservative (10% metaphosphoric acid and 1% oxalic acid) in the ratio 1:1 and then transferred to the deep freezer (-80°C) until required for batch analysis. Vitamin C in EBM samples was analysed by High Performance Liquid Chromatography (HPLC) method (Romeu-Nadal, Morera-Pons, Castellote, & Lopez-Sabater, 2006). Data obtained in this study were analysed by the SPSS statistical software, version 18.0 (SPSS Inc., Chicago, IL, USA, 2010). Wilcoxon Signed Rank Test and Friedman's Analysis of Variance (ANOVA) were conducted due to the non-parametric nature of the data. A p value of <0.05 was considered significant.

RESULTS

The median vitamin C concentration at baseline was 72.3 mg/L ranging from 59.7 mg/L to 82.2 mg/L in the eight samples. After four hours of storage at room temperature ($23 \pm 2^\circ\text{C}$), the median concentration measured 58.1 mg/L with 84.4% of the initial concentration of vitamin C remaining in the samples (Figure 1).

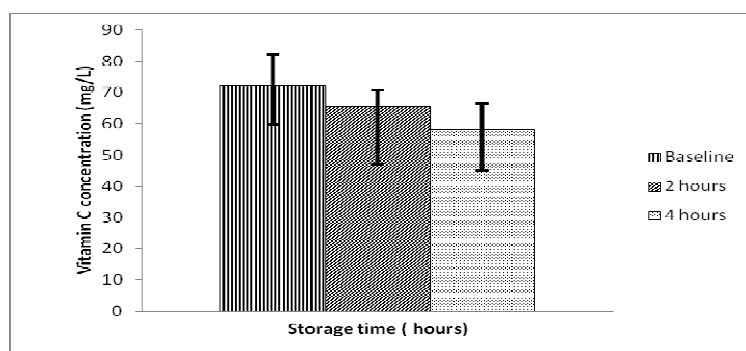


Figure 1 Median concentration of vitamin C (mg/L) in expressed breast milk samples stored at room temperature ($23 \pm 2^\circ\text{C}$)

EBM samples stored for 48 h under refrigeration ($4 \pm 1^\circ\text{C}$) contained only 56.5% of the initial concentration of vitamin C and the median concentration of vitamin C was 36.4 mg/L ranging between 27.1 - 48.3 mg/L. Samples analysed at different intervals during refrigeration storage indicated a significant decrease in vitamin C concentration compared to the baseline concentration (Figure 2). Fourteen days of storage in a freezer (-18°C) resulted in lower vitamin C concentration and 51.3 mg/L (approximately 70% of the initial concentration of vitamin C) remained in the EBM samples, with levels ranging between 52.2 - 70.8 mg/L. The median concentration of vitamin C decreased in all EBM samples stored in the freezer (Figure 3).

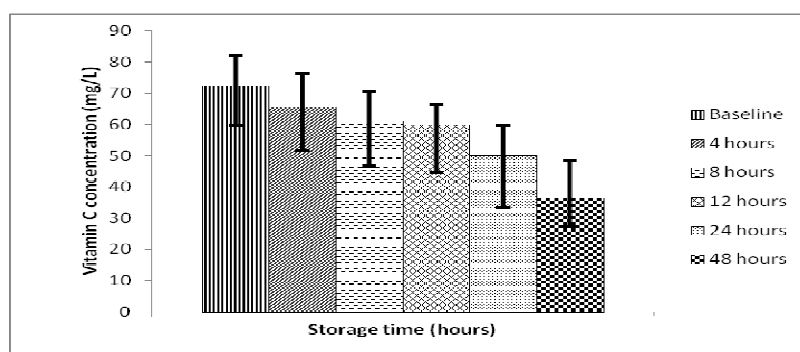


Figure 2 Median concentration of vitamin C (mg/L) in expressed breast milk samples stored at refrigeration temperature ($4 \pm 1^\circ\text{C}$).

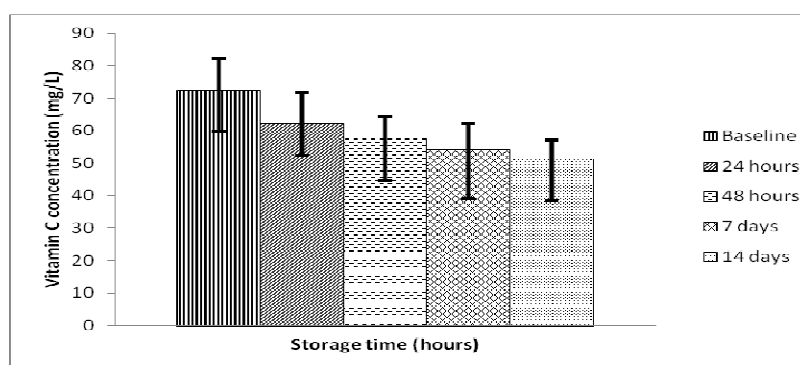


Figure 3 Median concentration of vitamin C (mg/L) in expressed breast milk samples stored in a freezer (-18°C)

The concentration of vitamin C was significantly ($p < 0.05$) lower in the samples at room temperature (84.4% of initial concentration) compared to the refrigerated samples (92.8% of initial concentration) at the end of 4 h. This study also indicated that samples stored at refrigeration temperature for 24 h contained only 71.1% of initial concentration of vitamin C

while 86.4% had remained in the frozen samples. A significant difference ($p < 0.05$) was observed between the samples stored in the refrigerator and freezer for 24 h. At the end of 48 h storage in the refrigerator, vitamin C concentration was significantly ($p < 0.05$) lower (56.5% of the baseline concentration) as compared to the frozen samples (81.1% of the baseline concentration). This indicates that significant ($p < 0.05$) reduction of vitamin C occurred at refrigeration temperature compared to freezing temperature.

The results obtained from the analysis of data using Friedman's Analysis of Variance (ANOVA) indicated a significant difference ($p < 0.05$) in the vitamin C concentration in samples stored at refrigeration temperature for 4 h, 8 h, 12 h, 24 h and 48 h. The same statistical analysis revealed a significant difference ($p < 0.05$) in the concentration of vitamin C in samples stored at freezer temperature for 24 h, 48 h, 7 days and 14 days.

DISCUSSION

In a previous study, total ascorbic acid levels in EBM were shown to decrease by an average of one-third after 24 h of storage at 4°C with wide variation between individuals (Buss et al., 2001). A preliminary study by Conlon and Cui (2009), also reported a significant ($p < 0.05$) decrease in the vitamin C concentration in EBM after 2 h of storage at ambient temperature compared to baseline concentrations. The same study also reported that vitamin C levels decreased significantly ($p < 0.05$) in samples stored at refrigeration and freezing temperature for 24 h (Conlon & Cui, 2009). Reduction in the concentration of vitamin C was also observed in other studies (Bank et al., 1985; Buss et al., 2001; Ezz-El-Din et al., 2004; Romeu-Nadal et al., 2008). Therefore the current findings from this study are in agreement with the results from previous studies.

Ascorbic acid (vitamin C) is highly sensitive and vulnerable to degradation wherein loss of vitamin C activity occurs. Degradation of the vitamin in breast milk results due to storage of milk, heat treatment, exposure to light or air (oxygen) and exposure to trace metals (Kall, 2003). Only ascorbic acid (vitamin C) and its principal oxidation product, dehydroascorbate, possess the vitamin C activity. Oxidation of these elements leads to formation α -diketogulonic acid causing the loss of vitamin activity (Kall, 2003; Naidu, 2003). It has been observed that vitamin C losses are partly due to the lactoperoxidase activity. Adding a peroxidase inhibitor, potassium cyanide to the breast milk samples provided protection against the losses of the nutrient (Buss et al., 2001). This could be a reasonable explanation for the decrease in the vitamin C concentration in stored EBM samples. According to the Ministry of Health NZ, 2005 the Reference Dietary Intake of vitamin C for infants (up to 6 months age) is 25 mg/day (Ministry of Health NZ, 2005). The recommendations on storage conditions of EBM are appropriate, as EBM stored at various storage conditions contain the required amount of vitamin C, essential for the infant under 6 months.

CONCLUSION

This study was conducted to determine the effect of selected storage conditions on vitamin C concentration of EBM samples obtained from healthy mothers. Results of the study showed that vitamin C concentration decreased significantly ($p < 0.05$) in all the EBM samples stored at the selected storage conditions. The reduction in the vitamin concentration was highest in samples stored at room temperature ($23 \pm 2^\circ\text{C}$), intermediate in those stored at refrigeration temperature ($4 \pm 1^\circ\text{C}$) and least in frozen EBM samples (-18°C). Although storage of EBM leads to degradation of vitamin C, it is still a good option to store EBM under the guidelines recommended by the Ministry of Health, NZ, as the EBM still contains the recommended levels of vitamin C. In conclusion, storing EBM under cool temperatures is best for providing safe and nutritive milk to infants.

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Fresh Fruit and Vegetables as Functional Foods

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ABSTRACT

Background: Epidemiological studies consistently show a diverse array of health and wellness benefits arising from a higher-than-average intake of fresh fruit and vegetables. Significant reported benefits include a reduced risk of developing cardiovascular disease, type II diabetes and certain forms of cancer. These benefits are rarely seen when supposedly-bioactive phytochemicals are extracted from fruit and vegetables and administered as nutraceutical supplements. There are several reasons why this may be so, including something fundamentally important about the need to provide phytochemicals in the presence of a high-fibre matrix; the need to provide a synergistic diversity of phytochemicals; and the fact that people who eat a diet rich in fruit and vegetables are generally not the same people who eat a diet rich in saturated fats and sugar. Public health efforts are therefore best served by promoting the increased consumption of fruit and vegetables, as part of a healthy lifestyle (including taking moderate exercise and avoiding smoking).

Global horticulture has tended to move to the commodity production of cheap, standardised fruit and vegetables with a long postharvest life to reduce losses through the marketing chain. Biochemical analysis of fruit and vegetables shows that when the breeding focus is on yield there can be a corresponding dilution of potentially important bioactive components. Developing a differentiated range of fruit and vegetables that is naturally enriched in health-beneficial phytochemicals is an attractive business proposition for New Zealand growers. They are faced with high production costs and long distances to deliver their products to global markets and need a point of difference if they are to command a premium in the marketplace.

Any attempt to differentiate fruit and vegetables by their composition is challenging. Plant composition is strongly affected by genotype, growing region and agronomic inputs; and composition is not fixed at the point of harvest, but continues to vary thereafter as a result of plant metabolism. Further compositional changes happen during the final steps of food preparation. Despite the complexity, the effects of these variables are quantifiable and manageable.

Conclusion: New Zealand growers need differentiated, high-value fruit and vegetable products with 'embedded intellectual property' in the form of well-validated compositional knowledge at the point of sale. These are attractive attributes for sale to affluent global consumers and are not simple to copy, so can be offered at a premium price. Combining compositional knowledge with a more detailed understanding of the 'health impact' of that composition is an urgent need; there is a great potential to tailor plant composition to deliver particular health and wellness benefits. Since it has proved very hard to encourage people to consume more fruit and vegetables, the availability of 'naturally enriched' fruit and vegetables will also offer a domestic public health benefit.

INTRODUCTION

The determination to improve health and wellbeing through diet is not a new phenomenon; Hippocrates, the father of modern medicine, wrote in about 400 BC, 'Leave your drugs in the chemist's pot if you can heal your patient with food' (quoted in Ames, 2003). What is new is the sense of urgency around demonstrating functionality in food, in terms of increased health or wellness, because of the modern epidemic of obesity that appears to accompany the adoption of a Western lifestyle. Obesity is associated with a cluster of unpleasant outcomes: increased risk of type II diabetes, cardiovascular disease and some cancers. The World Health Organisation has indicated that people who have 'Metabolic Syndrome' are at elevated risk of premature death from this cluster of causes; and they offer five 'risk factors' to determine if you are suffering from Metabolic Syndrome: central obesity,

high blood pressure, high blood sugar, high blood triglyceride content and insufficient 'good cholesterol' in the blood (Esposito *et al.* 2007). There are published 'risk thresholds' for each of these indicators, and if you exceed those thresholds in any three indicators you are considered to have Metabolic Syndrome.

Lifestyle and diet

The lifestyle changes that are needed to reduce your risk are well known: they include reducing your intake of saturated fats and sugar, getting some exercise, stopping smoking, reducing your alcohol intake and increasing your intake of fruit and vegetables. Indeed, a low intake of fruit and vegetables is associated with increased risk of ischaemic heart disease and stroke, and stomach, oesophageal, colorectal and lung cancer (Lock *et al.* 2005). In the current debate about permissible nutrition and health claims for use on foods sold in Australia and New Zealand, it is noteworthy that there is a recommendation for a pre-approved high level health claim: 'A healthy diet with a high intake of both fruit and vegetables and consisting of a variety of foods is associated with a reduced risk of coronary heart disease' (FSANZ, 2009).

What is very surprising is how poor consumer understanding is about the benefits of a diet rich in fruit and vegetables. The current understanding is that adults should eat around 600 g of fruit and vegetables a day; and, as this is well in excess of most countries' average intake, the target is often simplified to "5+ a day", meaning five or more servings a day, where a serving is around 80 g. Despite many years of public health campaigning around this figure, less than half of the New Zealand population consumes the recommended three servings of vegetables and two servings of fruit (the adult intake of fruit and vegetables in New Zealand still sits somewhere around four servings a day (Lock *et al.* 2005) and consumption actually declined between the 2002 and 2006/7 New Zealand Health Surveys (Ministry of Health, 2008). There are many reasons for our reluctance to eat more fruit and vegetables; people cite a lack of time for preparing fruit and vegetables, a dissatisfaction with the flavour of modern varieties, concerns about spray residues, lack of knowledge in terms of how to prepare or cook vegetables, and the widespread availability of pre-prepared products high in fat and sugar (Smyth *et al.*, 2009). Many of these reasons would seem trivial if only the enormity of the problem were more widely recognised. In a remarkable paper published in 2007, a group of New Zealand medical researchers calculated that, based on present-day knowledge, some 1,559 deaths in 1997 could be directly attributed to an inadequate intake of fruit and vegetables (Tobias *et al.*, 2006). Given that our newspapers are full of debate about how to reduce the road toll, it seems bizarre that a four-fold larger number of preventable deaths is simply ignored. A similar analysis on a global scale has calculated some 2.635 million people die each year from not eating enough fruit and vegetables (Lock *et al.* 2005).

The 'epidemic' of obesity that accompanies the adoption of the Western lifestyle has led to a search for 'magic bullets': short-cuts that will allow people to retain their fast pace of life and unhealthy lifestyle choices. This has helped to drive the apparently recession-proof growth of the nutraceutical supplements industry, reaching some US\$250 billion in 2008-9. Tragically, the evidence for the efficacy of these supplements is sketchy. There is an excellent website, <http://www.informationisbeautiful.net/play/snake-oil-supplements/>, that reviews the evidence for the efficacy of supplements based on the 'gold standard' of health research, the Cochrane database of randomised, placebo-controlled, double-blind clinical trials. It is clear from that website that only a few of the well-known supplements have well-attested beneficial effects; but this has not stopped the phenomenal growth of the supplements industry.

Foods for health and wellbeing

Part of the reason for the inadequacy of supplements, compared to the strong epidemiological evidence for the benefits of eating a diet rich in fruit and vegetables, may well be that there is something particularly good about eating whole fruit and vegetables: the fibre in the matrix and the combinations of diverse phytochemical compounds may work synergistically to provide a far larger benefit than individual extracts supplied in concentrated form (and specific research results are tending to support this concept). Another part of the benefit is the simple fact that few people who eat a diet rich in fruit and vegetables also eat a diet rich in saturated fats and highly refined foods; they tend to be mutually exclusive (Ministry of Health and the University of Auckland, 2003).

There is now a global trend for researchers to seek to understand the specific contributions fruit and vegetables make to human health and wellbeing. Differentiating fruit

and vegetable products around particular health benefits is a well-established route to increased sales: such as blueberries for mental acuity, cranberries for urinary tract infections, or broccoli for reducing the risk of colorectal cancer. But, as with the supplements industry, there is concern that these claims may be overblown; and unlike supplements, it is very hard to do a double-blind study when people know what fruit and vegetables they are being asked to eat.

Horticulture and Nutrition

Horticulture New Zealand (HortNZ) has developed a vision for the expansion of New Zealand's vegetable exports. The planned increase in exports will come from sustainable production and will not be accompanied by an equal growth in the area of land devoted to horticulture. Instead the vision is for increasingly high-value products exported to affluent and discerning consumers in a diverse array of markets. The horticultural sector has several recent success stories to examine as it looks for models of sustainable export growth (Figure 1). The products on this graph have all grown in exports over the last 20 years but they typify very different growth patterns. Exports of New Zealand wine have demonstrated a phenomenal record of year-on-year increases in total export value; but here there have been massive increases in productive area, and we are teetering on the brink of commoditisation with a glut of inexpensive wine threatening to devalue the NZ 'brand'. The growth of processed vegetable exports is a response to the world's growing appetite for cheap, convenient products. The growth of kiwifruit exports is the pattern New Zealand needs to keep replicating. Here, the introduction of a novel product in the last decade (the ZESPRI™ GOLD fruit) reversed a gradual downwards drift in export value for the green 'Hayward' variety in the 1990s. The market was offered something fresh and different from what was available in the rest of the world. It was a product with an attractive colour and flavour; and had the benefit of a co-ordinated and well-resourced marketing strategy.

When considering what aspects of novelty will appeal to consumers and attract higher prices, it is a given that we need to consider such drivers as taste, texture, convenience and 'freshness'. There is an additional but relatively untapped driver of consumer behaviour that our horticultural industries are all exploring. This is the fact that affluent consumers around the world are making purchasing decisions based on a belief in the functionality of their purchases, in terms of their own (and their family's) health and wellbeing.

There is enormous compositional variation between different varieties of the same species. Detailed research has shown that plant composition is strongly affected by agronomic inputs during growth (water, light, nutrients and temperature) and that composition changes markedly with plant maturity. Finally, there is a strong awareness that fresh products are not shelf-stable like processed food or nutraceutical products. The composition of fresh fruit and vegetables is not fixed at harvest but continues to change during storage and transport. Despite this variability, there are now some research programmes that aim to deliver 'fresh, functional' fruit and vegetables, based on elite germplasm, controlled growing conditions, and closely-managed protocols for postharvest handling, so as to deliver some 'guaranteed minimum' concentrations of bioactive phytochemicals at the point of sale.

Capitalising on this research and offering differentiated fresh products with validated claims for efficacy in terms of promoting health and wellbeing will be an important contributor to the growth of New Zealand's horticultural industries. To retain market leadership we will need New Zealand-unique germplasm and New Zealand-managed partnerships to deliver the products to affluent consumers, with the product composition guaranteed at the point of sale. Increasing convenience and stabilising composition through processing will provide additional product opportunities. Compositional changes during the production of chilled or frozen pre-prepared meal components, or minimally-processed fresh product mixes, or even juices and smoothies, can be quantified and managed. Providing consumers with product-specific information at the point of sale may help to emphasise the 'value proposition'.

Some nutritionists question the fact that 'elite' fruit and vegetable products with known composition and some evidence for the contribution they will make to human health should be sold at a premium. The counter-argument is that good food should be available to every New Zealander at a reasonable price. This is a question of social policy; the simple economics of needing to charge a premium to compensate for the extra costs involved in developing the products and monitoring their composition makes it impossible to deny that growers will need to receive a better price for them to choose to plant these improved varieties, particularly

since there may be a 'yield penalty' from growing high-phytochemical plants. We are aware that one political party is already recommending the removal of Goods and Services Tax on fruit and vegetables to encourage their consumption. Offering more 'nutrient-dense' fruit and vegetables may also help to deliver a higher content of beneficial phytochemicals into the diet: despite many years of promotion through campaigns such as 'Five+ a day', there has been little change in the average consumption of fruit and vegetables in New Zealand.

CONCLUSIONS

Fruit and vegetables differentiated in terms of their functionality for improved health and wellbeing should provide a global export growth opportunity for New Zealand. We have an enviable international reputation for integrity: if we claim something is spray-free or 'organic', the world understands that we will have some form of accreditation or validation process in place. New Zealand needs to trade more on our integrity, just as Switzerland is known for the reliability of its products. Trading on our integrity is a more defensible strategy than our supposed 'clean and green' image that is founded (as a former Minister of Science, Simon Upton, once famously remarked) 'on a low population density and a strong westerly wind'.

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Sustainable nutrition: an agricultural perspective

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ABSTRACT

Providing nutritious food for an ever-increasing global population in sustainable fashion within the difficulties of climate change, intensification of land use, bio-security failure, and vagaries of weather, exchange rates and customer preference... as well as the rise of what has been termed 'the cult of the amateur'... is creating enormous challenges.

Sustainability itself is now subject to many different definitions according to perspective. In food production, soil scientists made an internationally-coordinated effort (Smyth and Dumanski, 1994) to create a framework based on five objectives: "Sustainable land management combines technologies, policies and activities aimed at integrating socio-economic principles with environmental concerns so as simultaneously to: maintain and enhance productivity; decrease risks to production; protect the potential of natural resources and prevent the degradation of soil and water quality; be economically viable; be socially acceptable" Using the framework allows inclusion of concepts of peak oil, peak phosphorus, and also peak water.

New Zealand is well-placed to be able to develop, evaluate and implement sustainable production systems that ensure NZ Inc. Food Products will be in demand in premium markets, but only if validation and verification are possible. Life Cycle Assessment (LCA) is part of the validation, and research has shown that under current production systems New Zealand food can be placed in supermarkets using less energy and water than other countries can manage. The global population is expected to reach almost 9 billion by 2050, with food demand predicted by the UN to increase by 70%. Intensification of agriculture is critical, not just for increasing food production, but because it will enable avoidance of emissions from conversion of native landscapes to food and bio-fuel crops to be avoided.

The next point is education. People buy food for various reasons, not all of which are fact-based. Increased awareness of incidence of cancer has prompted concern about food's role in illness, with 'modern-day food production systems' being targeted. Considerable research, reviewed by the World Cancer Research Fund and American Institute for Cancer Research in 2007, has shown a conclusive relationship between life-style choices (over-indulgence, highly-processed foods and smoking) and incidence of different types of cancer. Research has shown no conclusive evidence that food production system pre-farm gate results in a significant difference in food quality on a dry matter basis. The problem appears to be in what we choose to put on our plates in terms of both quantity and post-farm-gate processing.

The big issue for the world lies in achieving truly sustainable production systems that are also profitable. The goal might be achieved through intensification. It might also be achieved through an organic route, as long as premiums can be commanded to offset the lower production generally achieved.

Research, extension and education are advocated so that farmers, growers and consumers are fully informed about the pros and cons of the choices they are making as the world approaches 'peak food'.

INTRODUCTION

Providing affordable, nutritious food for an ever-increasing global population in a sustainable fashion within the difficulties of climate change, intensification of land use, biosecurity failure, and the vagaries of weather, exchange rates and customer preference is creating unprecedented challenges for the food producers. Extra pressure is being caused by what has been termed 'the cult of the amateur' (Trewavas, 2008) where groups and individuals have 'persuaded an unqualified public of supposed dangers in food' leading to a

backlash against conventional agriculture. This is viewed as having potential to have serious repercussions on vital food security (Trewavas, 2008).

The Future of Food and Farming: Challenges and choices for global sustainability (Foresight, 2011), a UK Government Science report, concludes that more food must be produced sustainably through the spread and implementation of existing knowledge, technology and best practice, and by investment in new science and innovation. It also suggests that demand for the most resource-intensive types of food must be contained, waste must be minimised, and the political and economic governance of the food system must be improved to increase food system productivity and sustainability.

SUSTAINABLE PRODUCTION

The term 'sustainability' implies the use of resources at rates that do not exceed the capacity of the Earth to replace them and is now subject to many different definitions. These vary according to fashion and perspective. In food production, soil scientists made an internationally-coordinated effort (Smyth and Dumanski, 1994) to create a framework based on five objectives:

"Sustainable land management combines technologies, policies and activities aimed at integrating socio-economic principles with environmental concerns so as simultaneously to:

- maintain and enhance productivity
- decrease risks to production
- protect the potential of natural resources and prevent the degradation of soil and water quality
- be economically viable
- be socially acceptable."

Using the framework allows inclusion of concepts of peak oil, peak phosphorus, and also peak water. The 'decreasing risk' objective, is important as it has the potential to improve food security as fluctuations in supply will, at least in theory, be decreased. Similarly, individual farmer income is potentially stabilised, improving recruitment and retention in the agricultural work force. Also vital for the work force is economic viability (a fundamental component of business sustainability) – a good return from food production is paramount in attracting good people.

Sustainable production systems can be achieved in different ways, at least in part because the weighting that is put on each of the five factors above varies between people, as does what constitutes economic viability. New Zealand is well-placed to be able to develop, evaluate and implement sustainable production systems that ensure NZ Inc. food products will be in demand in premium markets, but only if validation and verification are possible. Life Cycle Assessment (LCA) is part of the validation process, and research has shown that under current production systems, New Zealand food can be placed in supermarkets using less energy (e.g. Ledgerd 2008) and water (e.g. Clothier 2009) than other countries can manage. New Zealand production systems are intensive, using fertilisers and pesticides, but are mostly pasture-based.

SUSTAINABLE INTENSIFICATION

Between 1900 and 1950, global population increased from 1.7 to 2.5 billion (47%), land area in production increased 14% but yield increased by 75%. This meant that more people were being fed at a better calorific input. In the next 50 years, population increased to 6.1 billion (244%), land area in production increased 22%, but yield increased by 276% (Smil 2008). Technology, particularly the Green Revolution involving new cultivars of cereals, plus fertiliser, pesticides and irrigation, made this possible. The fact that only 22% more land was associated with the 276% increase in yield meant that extra land wasn't required, thereby preserving native ecosystems (and all the species contained) from more rapid domestication.

There are also greenhouse gas (GHG) reduction benefits from intensification. Burney et al. (2010) from Stanford University and the Carnegie Institute of Washington compared 'real world' statistics, both the yields and the environmental impacts, with what the outcome would have been if technology had not increased production per hectare (which would have meant a far greater area of land would have had to have been brought under agriculture). The research shows that agricultural intensification between 1961 and 2005 increased GHG emissions from factors such as fertiliser production and application, but the net effect of

higher yields per hectare meant emissions of up to 161 Gigatons of carbon (GtC) (590 GtCO₂e) were avoided.

On current production levels, it has been calculated (Tilman et al., 2001) that over one billion additional hectares of land will be needed in order to feed the population predicted for 2050. Concerns are increasing that Peak Food, the moment in time when per capita availability of food in the world reaches a maximum and then begins to decline, is already here. Although he doesn't use the 'peak food' term, Lester Brown, President of the Washington-based Earth Policy Institute does point out that world reserve food stocks have fallen to dangerous levels, and increased prices have failed to push up food production (Brown, 2011). Intensification of agriculture is critical, not just for increasing food production, but because it will preserve biodiversity (Trewavas, 2000) and enable avoidance of emissions from conversion of native landscapes to food and biofuel crops.

RESEARCH

At the same time as New Zealand is positioning to become the preferred supplier of sustainably-produced, high quality food, the world is being urged to consider behaviours. The Food Climate Research Network's report (January 2010) 'How low can we go?' suggested five points for action:

1. Increase production efficiencies
2. Improve crop yields
3. Change animal feed to decrease methane
4. Use non-carbon fuel
5. Change human consumption

Points 1 and 2 require research and probably increasing intensification, as discussed above.

Point 3 is the subject of research by the New Zealand Agricultural Green House Gas Research Centre (which involves all land-based CRIs and universities); feed, rumen microorganisms and animals are being investigated. Housing animals could alleviate some of the GHG problem as waste products can be trapped and recycled, and may be the way of the future, at least for part of the year or day. Careful management would be required in terms of brand, but most northern hemisphere animals are housed for at least part of the time, so education is likely to be effective.

Point 4, using non-carbon fuel is a viable option for New Zealand as wind power 'catches on' and hydro-power management improves. Renewable energy is part of the marketing and brand story for New Zealand that can be developed under the 100% pure and clean green image.

Point 5 is the big challenge for the world, not in terms of the general understanding, or misunderstanding, of the impact of becoming vegetarian (which would mean more land being cropped) or organic (which would require even more land to compensate for the overall decreased yields currently offset by premiums (but if the whole world was organic, there would be no premiums) but in quantity and waste. Estimates are, for instance, that in developed countries over 25% of food purchases are thrown away. And, of course, obesity is of concern in many developed countries.

UNDERSTANDING FOOD CHOICES

Education is vital. People buy food for various reasons, not all of which are fact-based. Research from Nielsen (2010) on global trends in healthy eating involved 27,000 people in 55 markets. The results indicated that 76% respondents buy organic products because they believe that they are healthier (even though there is considerable research which shows that there is no consistent difference in food quality). Over half (53%) of the respondents buy organic food so that pesticides and other toxins can be avoided (even though organics production systems do apply permitted products that are pesticides). Similarly, over half (51%) purchase organic foods because they believed them to be more nutritious (even though there is no consistent evidence to support the suggestion that food production systems pre-farm gate result in a significant difference in food quality on a dry matter basis (e.g., Kristensen et al., 2008; Dangour et al., 2009; Tobin et al., 2011)).

An increased awareness of incidence of cancer has prompted concern about food's role in illness, with 'modern-day food production systems' being targeted. Genetic

predisposition appears to be part (5 - 10%) of the story, and potential trigger factors are constantly proposed in the media, with careful use of such words as 'may be linked, potentially, preliminary studies...'. Given the reliance humans have on food for nutrition, it is not surprising that food is being targeted as a possible problem.

Considerable research, reviewed by the World Cancer Research Fund and American Institute for Cancer Research in 2007, has shown a conclusive relationship between life-style choices and incidence of different types of cancer. A summary of the review is available from the European Food Information Council website (www.eufic.org). The review indicates that there is a causal relationship between body fatness and oesophageal, pancreatic, colorectal, endometrial, kidney and post-menopausal breast cancer, and probably also gall-bladder cancer. Foods containing dietary fibre probably protect against bowel cancer. Overall evidence that fruits and vegetables protect against cancer is less conclusive than previously believed. Alcohol is implicated in mouth, throat, oesophagus, breast cancer and male colorectal cancer. Consumption of large quantities of red and processed meats can increase risk of bowel cancer. Similarly, a high intake of salt and salt-preserved foods is a probable contributor to stomach cancer risk.

The report concluded that "making smart food and lifestyle choices from an early age will help reduce the risk of certain conditions. A healthy diet can be achieved through a balanced food intake, consuming certain foods in moderation and including a wide variety of different foods".

CONCLUSION

The big agricultural goal for the world lies in achieving truly sustainable production systems that are also profitable. The goal might be achieved through intensification. It might also be achieved for some growers and producers through an organic route, as long as premiums can be commanded to offset the lower production generally achieved.

Research, extension and education are required so that farmers, growers and consumers are fully informed about the pros and cons of the choices they are making as the world approaches 'peak food'.

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Fatty acid composition, cholesterol, vitamin A and E in refined hoki oil, crude hoki oil and crude tuna oil

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ABSTRACT

Analyses of fatty acid composition, cholesterol, vitamin A and E contents in refined hoki oil, crude hoki oil and crude tuna oil were carried. The oils were supplied by SeaDragon Marine Oils Ltd, New Zealand. The results obtained in this study showed that crude tuna oil contained a higher percentage of PUFA compared to hoki oils. However, refined and crude hoki oils were higher in MUFA. DHA and EPA were the main n-3 fatty acids in the three fish oils analysed. All samples showed a good ratio of n-3 to n-6 fatty acids. Higher cholesterol contents were found in the crude hoki oil and crude tuna oil compared to the refined hoki oil. A lower amount of vitamin A was observed in the crude tuna oil than the hoki oils. However, the amount of vitamin E was higher in the crude tuna oil than the crude hoki oil.

INTRODUCTION

Fish has attracted a lot of attention in the last four decades for its benefits for human health. Epidemiological studies have shown an inverse correlation between fish oil consumption and the risk of cardiovascular disease. Fish oil has high amounts of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that are important in cardiovascular care (Holub, 2002). Fish oil also contains vitamins such as A, D and E. Vitamin E (α -tocopherol) is one of the natural antioxidants that can be used to reduce lipid oxidation in the fish oils. Cholesterol can be found in the unsaponifiable fraction of the fish oil and its concentration in the crude oils range between 5000 and 8000 $\mu\text{g/g}$ (Tucker and Pigott, 2003). Hoki is one of the commercial fish species in New Zealand and hoki oil has been used in fish oil supplementation products. However, information on the physicochemical characterisation of hoki oil is limited. The objectives of this study are to determine the nutritional properties of the refined hoki oil, crude hoki oil and crude tuna oil and to assess the processing effects on the nutritional properties of the hoki oil.

METHODS

Refined hoki oil (RHO), crude hoki oil (CHO) and crude tuna oil (CTO) were supplied by SeaDragon Marine Oils Ltd, New Zealand. The oils were extracted from the frames, heads and livers of hoki and tuna fish. The RHO had gone through a refining process and supplemented with 0.15% of mixed tocopherols as antioxidants. CHO contains Barox antioxidant in approximately 500 mg/kg and no additive is added to the CTO. Fatty acid methylation was carried out according to a modified van-Wijngaarden (1967) method. Analysis of fatty acid composition was conducted by gas chromatography-flame ionisation detector (Agilent 6890N). A FAMQ005 FAME reference standard (AccuStandard, Inc., USA) was used for the fatty acids identification. Cholesterol content was determined by a spectrophotometer (Ultrospec 3300 pro; Amersham Biosciences) at 550 nm according to a modified Rudel and Morris (1973) method. The concentration of cholesterol was calculated based on a calibration curve of the cholesterol standard. Vitamin A and E (measured as α -tocopherol) analyses were conducted by high performance liquid chromatography (Agilent 1100; Agilent Technologies Inc., USA) and measured at 292 and 325 nm, respectively. Concentrations of the samples were calculated using calibration curves of the external standards. All measurements were carried out in triplicate and data were reported as mean \pm standard deviation (SD). Statistical analyses of data were performed using SPSS 17 software for Microsoft Windows. Multivariate analysis and the Tukey test were conducted to determine whether there was any significant difference between samples for each measurement at $p < 0.05$.

RESULTS

Refined and crude hoki oils contained higher percentages of monounsaturated fatty acids (MUFA; $44.84 \pm 0.92\%$ and $44.81 \pm 0.21\%$, respectively), followed by polyunsaturated fatty acids (PUFA; $28.47 \pm 1.29\%$ and $27.15 \pm 0.27\%$, respectively) and saturated fatty acids (SFA; $26.69 \pm 0.37\%$ and $28.04 \pm 0.19\%$, respectively). On the other hand, crude tuna oil contained higher percentage of PUFA ($39.92 \pm 0.05\%$), followed by SFA ($34.82 \pm 0.03\%$) and MUFA ($25.26 \pm 0.07\%$). The amounts of five nutritionally important PUFA are shown in Figure 1. The percentages of total n-3 PUFA in all fish oils analysed were higher than the n-6 PUFA. The ratio of n-3 to n-6 fatty acids in RHO, CHO and CTO were 5.52 ± 1.71 , 7.27 ± 0.26 and 8.90 ± 0.38 , respectively.

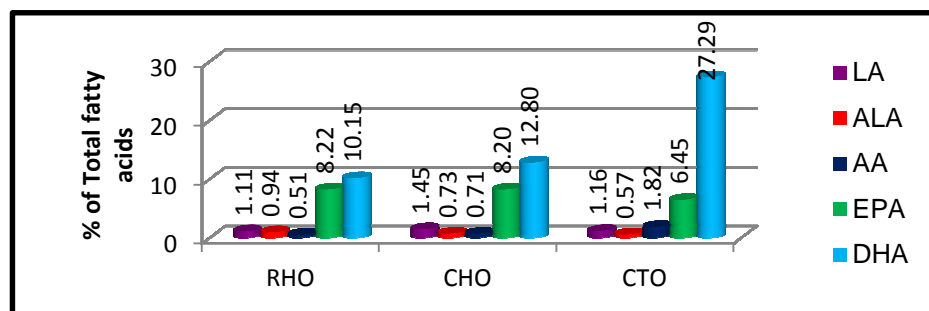


Figure 1: Percentages of five important PUFA in fish oils

RHO = refined hoki oil, CHO = crude hoki oil, CTO = crude tuna oil, LA = Linoleic acid, ALA = α -linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid and DHA = docosahexaenoic acid

The amounts of cholesterol, vitamin A and α -tocopherol in the RHO, CHO and CTO are presented in Table 1.

Table 1: Cholesterol, vitamin A and α -tocopherol found in refined hoki oil (RHO), crude hoki oil (CHO) and crude tuna oil (CTO)

Analysis	RHO	CHO	CTO
Cholesterol ($\mu\text{g/g}$)	1411.27 ± 376.48^b	5149.40 ± 770.77^a	2045.48 ± 250.82^b
Vitamin A ($\mu\text{g/g}$)	1444.62 ± 135.70^a	997.60 ± 329.06^a	110.99 ± 26.65^b
α -tocopherol ($\mu\text{g/g}$)	1107.94 ± 143.39^a	151.44 ± 57.73^c	752.49 ± 137.77^b

^{abc} Values with different superscript letters within a row are significantly different.

DISCUSSION

The fish oils in the present study have low amounts of linoleic acid (LA), α -linolenic acid (ALA) and arachidonic acid (AA) but high amounts of DHA and EPA. This finding is in accordance to the lipid characterization of marine fish as reported by Steffens (1997). Hoki and tuna oils contain high amounts of DHA and EPA which come from their natural diet. Tuna has a high amount of DHA because they use the SFA and MUFA for their energy and selectively accumulate and store DHA in their tissues (Watanabe, 1989). The ratio of n-3 to n-6 fatty acids in marine fish varies between 5 to more than 10 (Steffens, 1997) and all fish oil samples in the present study are within this range. Supplementation of fish oil with a high ratio of n-3 to n-6 fatty acids will lower corresponding dietary n-6 to n-3 ratios and may reduce the risk of atherosclerosis and coronary heart disease (Hu, 2001).

Cholesterol is the most common sterol in most marine species (Stansby, 1982) and the cholesterol contents of cod liver, herring, menhaden and salmon oils range from 4850 to 7660 $\mu\text{g/g}$ (Kinsella, 1987). The RHO in the present study contained less cholesterol than the

CHO and CTO as it has been processed. Processing can remove much free cholesterol and some cholesterol esters (Tucker and Pigott, 2003). On the other hand, the cholesterol in CTO was lower than CHO. This finding is in accordance with Kinsella (1986) where an increase in PUFA content mirrors a decrease in cholesterol content.

The livers of many fish species contain relatively large concentrations of oil-soluble vitamins that are associated with the lipid fraction (Kinsella, 1987). The vitamin A concentrations in halibut, shark and tuna liver oils are up to 210,000 µg/g (Tucker and Piggott, 2003). The vitamin A contents in hoki oils in the present study were significantly higher than the tuna oil. Tocopherol content of fish oils varied greatly among and within species and the content ranges from less than 10 to 750 µg/g. The amount of tocopherol in the noncommercial liver oil is greater than the oil that has been processed and used for commercial purposes (Kinsella, 1987). However, the α -tocopherol content in the RHO was higher than the crude oils in the present study. This is due to the amount of mixed tocopherols (1500 µg/g) added to the RHO by the manufacturer.

CONCLUSIONS

Hoki and tuna oils are a good source of n-3 fatty acids especially tuna oil which is rich in DHA. Refining can reduce the cholesterol content in the fish oil. Hoki oils contain more vitamin A than tuna oil which has a naturally higher α -tocopherol content.

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Does dietary broccoli fibre influence body composition of the healthy rat in the presence of high and low fat?

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ABSTRACT

Background: Daily intake of dietary fibre is important in preventing gut-related disorders, cardiovascular diseases, type 2 diabetes, cancer and obesity. Fermentable carbohydrates such as pectin, gums, resistant starch, and non-starch polysaccharides can enhance the metabolic absorption of minerals, including calcium, magnesium and iron from the gut.

Objective: To determine if feeding broccoli fibre in high and low fat diets alters body composition and bone density in the healthy rat.

Design: Sixty-four male Sprague Dawley rats (9 weeks of age) were fed four experimental dietary treatments (16 rats per treatment) for 17 weeks. The dietary treatments were: 1) low corn oil and cellulose, 2) low corn oil and broccoli fibre, 3) high corn oil and cellulose, and 4) high corn oil and broccoli fibre. Body composition and bone density were assessed by DEXA scan analysis. Serum levels of C-terminal telopeptides of type 1 collagen (CTX), a resorption marker, were also measured.

Outcomes: Body fat mass ($p=0.002$) and fat percentage ($p<0.001$) were significantly higher in rats fed the high fat diets. Lean mass, lumbar spine (area, bone mineral content and density), and femur measurements (bone mineral content and density) were higher in rats fed the low fat diets. Broccoli fibre supplementation increased lumbar spine area ($p=0.040$), lumbar spine bone mineral content ($p=0.077$), femur area ($p=0.079$), and femur bone mineral content ($p=0.074$).

Conclusion: Low fat diets increased lean mass and bone area, mineral content and density in the lumbar spine and femur. Broccoli fibre supplementation had only a small impact on bone health through increased lumbar spine bone area.

INTRODUCTION

Daily intake of dietary fibre is important in preventing gut-related disorders, cardiovascular diseases, type 2 diabetes, cancer and obesity. Dietary fibre, particularly fermentable undigested carbohydrates such as inulin, pectin, gums, resistant starch, and non-starch polysaccharides, can enhance the metabolic absorption of minerals, including calcium, magnesium and iron, from the gut (Cashman 2006, Alexiou and Franck 2008). Calcium absorption is thought to be improved via colonic fermentation of the dietary fibre which reduces the luminal pH thus improving the solubility and passive absorption of calcium (Cashman 2006). The active transport of calcium may also be affected by dietary prebiotics through increased calbindin expression in the colon and caecum (Roberfroid *et al.* 2010). The broccoli fibre used in the present study consisted of mixed function cell wall polysaccharides from broccoli stems, which has been shown to be fermented in the large bowel (Paturi *et al.* 2010).

We hypothesised that fibre extracted from broccoli stems may enhance calcium absorption in rats fed high or low corn oil diets. This was part of a larger study investigating the interaction of dietary fibre and polyunsaturated fatty acids and their effects on the composition of the caecum microbiota, microbial fermentation products (short chain fatty acids) and colon morphology in healthy rats (Paturi *et al.* 2010).

METHODS

Sixty-four male Sprague Dawley rats (21–23 days old, 45–50 g) were housed in family groups and fed a commercial pelleted feed for 6 weeks. At 9 weeks of age the rats were randomly assigned to experimental diets (16 rats per treatment). They were fed four experimental dietary treatments for 17 weeks. The dietary treatments were: 1) low corn oil and cellulose, 2) low corn oil and broccoli fibre, 3) high corn oil and cellulose, and 4) high corn oil and broccoli fibre. The fibre level in the diets was 7.5%, and the level of corn oil was 5% and 30% for the low and high treatments, respectively. The broccoli fibre was prepared from broccoli stems obtained from growers in the Manawatu region. The stems were harvested, and then stored at 4°C until processing in the Food Technology Pilot Plant, Massey University, Palmerston North. The fibre was prepared by a cold water aqueous extraction method that causes minimal loss of total non-starch polysaccharide including pectin, freeze-dried, finely ground (1 mm mesh), and stored at room temperature until the experimental diets were prepared. The resulting broccoli fibre contained more than 95% dietary fibre (dry weight after pepsin digestion and 80% ethanol precipitation). The compositions of the experimental diets are given in Table 1. The study was carried out with ethics approval from the AgResearch Grasslands Animal Ethics Committee (Application 11321).

Table 1: Ingredient composition (%) of experimental diets

Ingredient	Low corn oil	Low corn oil	High corn oil	High corn oil
	Cellulose	Broccoli	Cellulose	Broccoli
Lactic casein ¹	12.0	12.0	12.0	12.0
Vitamin mix ²	5.0	5.0	5.0	5.0
Mineral mix ³	5.0	5.0	5.0	5.0
Sucrose ⁴	4.0	4.0	4.0	4.0
Starch ⁵	61.5	61.5	36.5	36.5
Corn oil ⁶	5.0	5.0	30.0	30.0
Cellulose ⁷	7.5		7.5	
Broccoli fibre ⁸		7.5		7.5
Energy (kcal/kg)	3545.8	3545.8	4734.5	4734.5

¹Alacid 80 mesh, New Zealand Milk Products, Wellington, New Zealand

²A mixture prepared at Plant & Food Research that supplied (mg/kg diet): retinol acetate 5.0, dl- α -tocopheryl acetate 100.0, menadione 3.0, thiamin hydrochloride 5.0, riboflavin 7.0, pyridoxine hydrochloride 8.0, d-pantothenic acid 20.0, folic acid 2.0, nicotinic acid 20.0, d-biotin 1.0, myo-inositol 200.0, choline chloride 1500; (μ g/kg diet): ergocalciferol 25.0, cyanocobalamin 50.0

³A mixture prepared at Plant & Food Research that supplied (g/kg diet): Ca 6.29, Cl 7.79, Mg 1.06, P 4.86, K 5.24, Na 1.97; (mg/kg diet): Cr 1.97, Cu 10.7, Fe 424, Mn 78.0, Zn 48.2; (μ g/kg diet): Co 29.0, I 15, Mo 152, Se 151

⁴Caster sugar, Chelsea Sugar Company, Auckland, New Zealand

⁵Wheaten cornflour, Golden Harvest, Primary Foods Ltd, Auckland, New Zealand

⁶Davis Trading Company, Palmerston North, New Zealand

⁷Ceolus PH102, Commercial Minerals Ltd, Auckland, New Zealand

⁸Prepared at Plant & Food Research, Palmerston North, New Zealand

The rats were fed their respective diets for 17 weeks, during which time food and water were provided *ad libitum*. The animals were weighed weekly and food intake was measured during the final week of the study. After 16 weeks of feeding, dual energy x-ray absorptiometry (DEXA) was carried out on all animals under anaesthesia. The animals were weighed and anaesthetised intraperitoneally (0.06 ml/100 g body weight of Acepromazine, Ketamine, Xylazine). Body composition measurements were taken using a Hologic Discovery A bone densitometer (Bedford, MA, USA). An initial quality control scan was taken. The coefficient of variation for this data was 0.98–1.0%. Each rat underwent a total body scan for

fat and lean mass. The coefficients of variation were: whole body fat 2.12%, lean mass 0.32%, body mass 0.09%.

At the end of the 17 week feeding study, the rats were euthanized by CO₂ overdose and blood was collected via cardiac puncture and left to coagulate at 4°C. Serum was harvested following centrifugation at 2000 x g for 10 min and stored at -80°C. Serum levels of C-terminal telopeptides of type 1 collagen (CTX), a resorption marker, were measured. All data were analysed with analysis of variance including contrasts to assess the main effects of fat content, fibre type and fat content x fibre type interaction. All analyses were carried out using GenStat 12th edition release 2009 (VSN International, Hemel Hempstead, UK).

RESULTS

The rats remained healthy and gained weight during the 17 week study. There were no significant differences in the final body weights and food intake between the rats fed the experiment diets (Table 2). The rats fed the high corn oil diets had significantly ($p < 0.001$) higher mean body weight gains than those fed the low corn oil diets. The rats fed the low corn oil diets tended to eat more than those on the high corn oil diets. There was no significant difference in energy intake between the low and high corn oil fed rats. There was no significant effect of fibre type on final body weight, body weight gain, food intake or energy intake.

Table 2: Effect of experimental diets on rat body weight, food and energy intake¹

Diet	Final body weight (g)	Body weight gain (g)	Food intake Week 14 (g)	Energy intake Week 14 (kcal/kg)
Low corn oil cellulose	597 ± 12.7	171 ± 7.6	151 ± 4.0	901 ± 27.8
Low corn oil broccoli	613 ± 16.6	183 ± 6.8	151 ± 3.1	879 ± 21.1
High corn oil cellulose	626 ± 23.2	225 ± 14.5	110 ± 4.5	855 ± 31.5
High corn oil broccoli	645 ± 21.1	210 ± 14.6	111 ± 3.9	836 ± 17.8
Significance ²				
Fat content	$p = 0.117$	$p < 0.001$	$p < 0.001$	$p = 0.085$
Fibre type	$p = 0.364$	$p = 0.909$	$p = 0.984$	$p = 0.410$
Fat content x Fibre type	$p = 0.920$	$p = 0.250$	$p = 0.876$	$p = 0.942$
LSD ($p < 0.05$)	54.28	33.04	11.07	71.16

¹Values are mean ± SEM.

²Two-way analysis of variance was carried out to compare the means. The level of significance is shown as p . Degrees of freedom = 59.

Whole body fat was significantly higher ($p = 0.015$) and the lean mass significantly lower ($p = 0.002$) for the rats fed the high corn oil diets than those fed the low corn oil diets (Table 3). There was no significant effect of fat content, fibre type or fat content x fibre type on whole body bone mineral content or density. However, high fat diets significantly reduced lumbar spine area, mineral content and density (Table 4). The rats given the broccoli fibre diets had higher lumbar spine areas than the rats fed cellulose regardless of whether the diet was supplemented with high or low corn oil. Similarly, there was a reduction in femur mineral content and density for the rats fed the high corn oil diets (Table 5). There was no significant effect of fat or fibre content on the bone resorption marker CTX.

Table 3: Effect of experimental diets on whole body composition¹

Diet	Lean mass (g)	Fat (g)	Bone mineral content (g)	Bone mineral density (g/cm ²)
Low corn oil cellulose	433 ± 10.6	182 ± 10.8	16.4 ± 0.4	0.18 ± 0.002
Low corn oil broccoli	461 ± 9.9	158 ± 14.4	16.6 ± 0.4	0.18 ± 0.002
High corn oil cellulose	424 ± 11.1	210 ± 18.6	16.7 ± 0.4	0.18 ± 0.001
High corn oil broccoli	418 ± 10.4	234 ± 18.7	17.1 ± 0.5	0.18 ± 0.002
Significance ²				
Fat content	p = 0.015	p = 0.002	p = 0.334	p = 0.244
Fibre type	p = 0.288	p = 0.994	p = 0.523	p = 0.280
Fat content x Fibre type	p = 0.113	p = 0.136	p = 0.875	p = 0.978
LSD (p < 0.05)	29.86	45.43	1.24	0.004

¹Values are mean ± SEM.

²Two-way analysis of variance was carried out to compare the means. The level of significance is shown as p.

Table 4: Effect of experimental diets on lumbar spine area, bone mineral content and density¹

Diet	Lumbar spine		
	Area (cm ²)	Mineral content (g)	Mineral density (g/cm ²)
Low corn oil cellulose	2.70 ± 0.05	0.668 ± 0.026	0.246 ± 0.005
Low corn oil broccoli	2.80 ± 0.03	0.715 ± 0.018	0.255 ± 0.005
High corn oil cellulose	2.61 ± 0.03	0.623 ± 0.017	0.238 ± 0.004
High corn oil broccoli	2.69 ± 0.05	0.647 ± 0.017	0.240 ± 0.004
Significance ²			
Fat content	p = 0.023	p = 0.006	p = 0.012
Fibre type	p = 0.040	p = 0.077	p = 0.216
Fat content x Fibre type	p = 0.765	p = 0.559	p = 0.450
LSD (p < 0.05)	0.12	0.06	0.01

¹Values are mean ± SEM.

²Two-way analysis of variance was carried out to compare the means. The level of significance is shown as p.

Table 5: Effect of experimental diets on femur area, bone mineral content and density¹

Diet	Femur		
	Area (cm ²)	Mineral content (g)	Mineral density (g/cm ²)
Low corn oil cellulose	1.88 ± 0.02	0.614 ± 0.016	0.325 ± 0.004
Low corn oil broccoli	1.93 ± 0.02	0.636 ± 0.012	0.329 ± 0.003
High corn oil cellulose	1.84 ± 0.02	0.584 ± 0.012	0.316 ± 0.003
High corn oil broccoli	1.89 ± 0.02	0.612 ± 0.015	0.324 ± 0.005
Significance ²			
Fat content	p = 0.152	p = 0.058	p = 0.046
Fibre type	p = 0.079	p = 0.074	p = 0.137
Fat content x Fibre type	p = 0.917	p = 0.845	p = 0.621
LSD (p < 0.05)	0.08	0.04	0.01

¹Values are mean ± SEM.²Two-way analysis of variance was carried out to compare the means. The level of significance is shown as p.

DISCUSSION

Rats fed the diets containing broccoli fibre were shown to have greater lumbar spine bone area, and increased lumbar spine and femur mineral content and density. Studies have demonstrated that inulin-type fructans increase calcium and magnesium absorption in young growing rats (Delzenne *et al.* 1995, Pérez-Conesa *et al.* 2006), adolescent rats (Raschka and Daniel 2005), and in ovariectomised female rats, an experimental model for post-menopausal osteoporosis (Scholz-Ahrens *et al.* 2002). In humans, short and long term consumption of non-digestible oligosaccharides enhanced calcium absorption in adults and enhanced bone mineralization in pubertal growth (Cashman 2006). Weaver *et al.* (2010) compared the effect of feeding a range of dietary fibres (cellulose, resistant starch, soluble corn fibre, dextrin, pullulan, polydextrose, inulin and inulin/fructooligosaccharides) on bone calcium content and strength in young growing rats. They found that bone calcium content was increased when the animals were fed 4% resistant starch (type 2 and type 3), dextrin and polydextrose. Also whole body bone mineral content and density, bone volume, cortical thickness and area and femur breaking strength were enhanced when the rats were fed 4% soluble corn fibre and dextrin. In contrast, Galibois *et al.* (1994) found no effect of dietary pectin, oat bran or wheat bran on calcium absorption.

The broccoli fibre used in the present study has been shown to enhance the levels of *Lactobacillus* spp, increase the short chain fatty acids (acetic, butyric, formic, propionic) in the caecum, and increase colon crypt depths and the number of goblet cells per crypt in rats (Paturi *et al.* 2010). Calcium absorption is therefore likely to have been enhanced in the broccoli fibre fed rats due to this fermentation of the fibre, reducing the luminal pH and thus improving the solubility and passive absorption of calcium.

CONCLUSIONS

Low fat diets increased lean mass and bone area, mineral content and density in the lumbar spine and femur. Broccoli fibre supplementation significantly increased only the lumbar spine area. Other vegetable fibres may have greater beneficial health effects on bone density and mineral absorption, and should be investigated.

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Effect of capsaicin on satiety and diet-induced thermogenesis

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ABSTRACT

Background: One way to reduce net energy intake is by using bioactives, such as capsaicin, which has a satiating effect and can also increase energy expenditure through diet-induced thermogenesis (DIT). In terms of energy stored as body fat, and over a prolonged period, an increase in thermogenesis can have a substantial impact on obesity.

Objectives: To determine the effect of capsaicin on satiety and diet-induced thermogenesis.

Design: Rice and rice plus tabasco sauce (containing capsaicin) was fed to 10 subjects using a two-way cross over design. Height, weight, waist and hip circumference and body fat content of the subjects were measured together with blood pressure, heart rate, energy expenditure (thermogenesis), and satiety which was measured using visual analogue scale.

Outcomes: Using the area under the curve of thermogenesis versus time, DIT was greater but not significantly so when the subjects consumed rice+Tabasco sauce rather than rice only. Scores for feeling full/not hungry/having less desire to eat 2.5 h after the meal were significantly higher for rice+tabasco sauce than for rice alone. Changes in blood pressure and heart rate were not significantly different in subjects eating the diets.

Conclusion: Capsaicin increased DIT and had a significant satiating effect, but because of inter-subject variability measurement of DIT, future studies need to be more highly powered to reach statistical significance.

INTRODUCTION

Obesity, no matter how it is caused, always involves an imbalance between energy consumed and energy expenditure (EE). Weight loss can be achieved by reducing energy intake and/or increasing EE (Smeets & Westerterp-Plantenga, 2009). There are three main forms of EE: (1) basal metabolic rate (BMR) which is about 65% of daily energy intake (DEI), (2) physical activity (20-30% DEI) and (3) DIT which is about 10% of DEI (Frayn, 2008). Even though, DIT is the smallest component of EE, it could play a role in weight maintenance (Westerterp, 2004).

There is growing evidence that certain bioactive ingredients found in spicy foods or herbal drinks can lead to greater thermogenesis and in some cases to greater satiety (Westerterp-Plantenga *et al.*, 2006). Capsaicin, which gives pungency to hot red peppers (Smeets & Westerterp-Plantenga, 2009) has been reported to increase EE and fat oxidation, and reduce appetite (Kawada *et al.*, 1986; Yoshioka *et al.*, 1998; Kawabata *et al.*, 2006). Increased activity of the sympathetic nervous system caused by capsaicin seems to be associated with energy and lipid metabolism (Yoshioka *et al.*, 1998; Yoshioka *et al.*, 1999). In the experiment reported here, we present the results of a pilot study in which we replicated previous studies and experimental protocols in preparation for more detailed research.

METHODS

Subjects and Experimental Design

Ten volunteers between the age 24 and 60 were recruited. The subjects were fed rice and rice plus Tabasco sauce (source of capsaicin in this study) in a 2 way cross over design. The subjects were asked not to eat anything after 10 pm the night before the experiment and were asked to arrive by 8 am on the day. After resting for 30 min, body measurements including height, weight, waist and hip circumference, body fat content, blood pressure, and heart rate were measured. They were then fed the test meal consisting of either plain boiled white rice (66 g) or the rice plus 3 mL of Tabasco sauce estimated to deliver 3 mg of

capsaicin. DIT was measured using a Fitmate (COSMED Srl, Italy, C02874-02-91) for 30 min prior to the meal and for 2.5 h after the meal with 10 min breaks every 20 min. Satiety profile was measured with the use of anchored 100 mm visual analogue scale (VAS). On the test days the questionnaires were completed in fasting state, immediately after the consumption of the test meal and at 2.5 h when the experiment stopped. The questions were (1) "How hungry do you feel (0 not hungry at all and 10 being extremely hungry)?", (2) "How full do you feel (0 being not at all full and 10 being extremely full)?", (3) "How strong is your desire to eat (0 being not at all strong and 10 being extremely strong)?", (4) "How much food do you think you can eat (0 being a large amount and 10 being nothing at all)?". Statistical analysis (Excel) – the data are presented as mean \pm Standard error of the mean (SEM).

RESULTS

Figure 1 shows the changes in metabolic rate over 2.5 h after consuming the test meals. The curves are quite typical of this type of study but because of the large inter-individual variation there was no statistically significant difference between the treatments.

VAS rating (Table 1) of hunger, fullness, desire to eat and satiety showed no significant difference between the treatments during fasting and immediately after food consumption. However, 2.5 h later a significant decrease in feeling of hunger and desire to eat was observed in the treatment containing capsaicin, and an increased feeling of fullness and satiety.

No significant differences were observed in the haemodynamic functions.

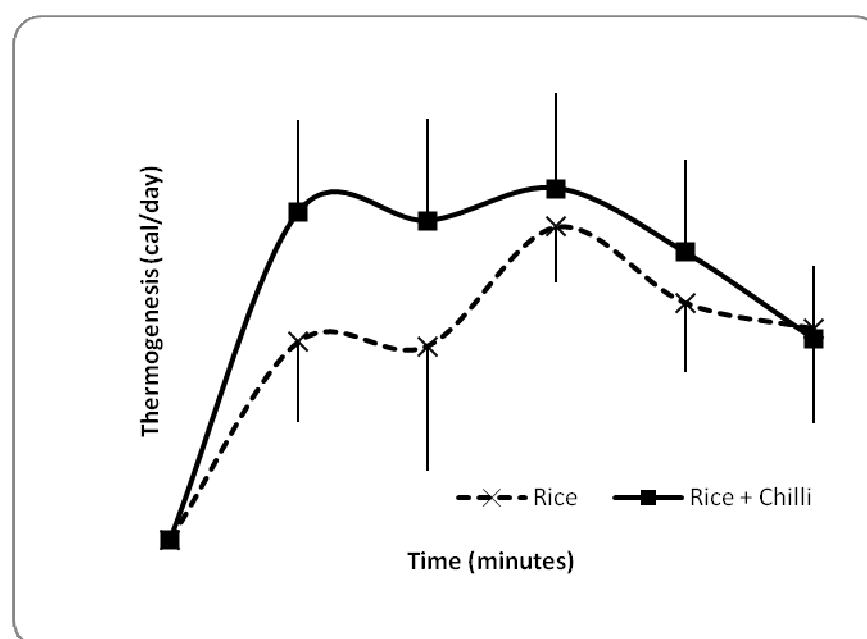


Figure 1: Rate of post prandial thermogenesis measured for 2.5 h after consuming rice or rice plus capsaicin in the form of tabasco sauce

Table 1: VAS mm rating at fasting and 2.5 h after feeding by the subjects

Satiety profile	Rice \pm SEM	Rice + Tabasco sauce \pm SEM
<u>Hunger (1):</u>		
Fasting	64.8 \pm 6	66.3 \pm 8
Immediately after eating	24.3 \pm 4	24.4 \pm 5
2.5 h after eating	65 \pm 8*	49.1 \pm 9*
<u>Fullness (2):</u>		
Fasting	16.8 \pm 4	17.6 \pm 3
Immediately after eating	68.7 \pm 3	74.7 \pm 6
2.5 h after eating	24 \pm 6*	41.8 \pm 8*
<u>Desire to eat (3):</u>		
Fasting	69.8 \pm 5	72.2 \pm 7
Immediately after eating	25.7 \pm 3	23.7 \pm 6
2.5 h after eating	69.2 \pm 9*	51.7 \pm 9*
<u>Prospective consumption of food (4):</u>		
Fasting	31.8 \pm 6	26.6 \pm 6
Immediately after eating	60.2 \pm 8	66.2 \pm 9
2.5 h after eating	26.4 \pm 8*	45.6 \pm 9*

* Significant difference $p < 0.05$

(1) "How hungry do you feel (0 not hungry at all and 10 being extremely hungry)?", (2) "How full do you feel (0 being not at all full and 10 being extremely full)?", (3) "How strong is your desire to eat (0 being not at all strong and 10 being extremely strong)?", (4) "How much food do you think you can eat (0 being a large amount and 10 being nothing at all)?".

DISCUSSION

No significant effect of capsaicin was observed on DIT probably because of the low dosage used (3 mg) combined with inter-subject variability. Other studies that used low dosage of capsaicin such as 0.03 g, 0.06 g have also observed no increase in EE (Snitker *et al.*, 2009; Galgani *et al.*, 2010). The sensitivity of subjects in this study to capsaicin limited the dose that could be given. Another reason for non significant result could be due to the small sample size. Given that intra-individual variability in DIT is 6 to 30% (Segal *et al.*, 1992; Westerterp, 1993) and within-subject variability is 43 to 48% (Ravussin *et al.*, 1986; Tataranni *et al.*, 1995), a higher powered test with more subjects is needed to confirm these results. Studies done with Japanese subjects have shown an increase in EE of up to 30% upon consumption of capsaicin (Yoshioka *et al.*, 1995; Yoshioka *et al.*, 1998). Sympathomimetic compounds like capsaicin could potentially increase thermogenesis by approximately 300-400kJ daily which could lead to substantial weight loss over time (Hursel & Westerterp-Plantenga, 2010).

From the analysis of VAS, it seems capsaicin caused suppression of hunger, lowering the desire to eat; and increased feeling of fullness and satiety which could be factors in successful weight loss. Exposure to capsaicin increased satiety in other studies as well (Westerterp-Plantenga *et al.*, 2005; Reinbach *et al.*, 2009). Because the capsaicin was able to significantly suppress appetite for 2.5 h it may be useful in reducing food intake between meals in weight management.

The fact that measurements of satiety were more significant than the DIT measurements probably reflect the larger number of factors involved in appetite regulation than in DIT.

CONCLUSIONS

Capsaicin did not increase DIT but did affect satiety and appetite. Therefore, capsaicin and compounds of similar action may be helpful in reducing energy intake and might support weight loss/maintenance by sustaining satiety and suppressing hunger. However, testing compounds for their effects on DIT requires experimental designs with enough power to overcome inter-individual variability.

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Sustainability of health & lifestyle improvements

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ABSTRACT

The poor long-term outcome of many conventional weight management treatments was the stimulus for the research described in this presentation. There is evidence from both prospective studies and randomised trials that dieting can promote increased food preoccupation, loss of control and overeating, and has been associated with subsequent weight gain. The 'non-dieting' approach aims to counteract the negative effects of dieting, takes the focus off weight loss, and advocates eating in accordance with hunger and satiety signals (termed intuitive eating) rather than deliberate dietary restriction. The objective of the first study was to compare three non-dieting interventions that focused on lifestyle change rather than weight loss, in terms of the sustainability of improvements in lifestyle behaviours, psychological well-being and medical symptoms at 2-years. In Dunedin, 225 obese/overweight women (BMI ≥ 28 ; 25-68 years) participated in a randomised, intention-to-treat trial comparing two group programs (P1, P2) and a self-guided mail-delivered program (P3). Only P1 included intensive relaxation response training. All three non-dieting interventions involved a 10-week program, followed by an eight-month support phase. Participants completed baseline, 1-year and 2-year assessments. Outcomes included behavioural, psychological and medical symptom measures and a composite success score. 118 participants completed the 2-year follow-up. Only among P1 participants were the reductions in psychological distress and medical symptoms achieved at 1-year, also maintained at 2-years. At 2-years, P1 participants had significantly greater increases in stress management behaviours than those in P2 ($p < 0.05$), and significantly greater success scores than those in P3 ($p < 0.05$). In all three programs, mean weight was unchanged at 2-years. It can be concluded from this study that inclusion of relaxation response training in a healthy lifestyle program facilitates long-term maintenance of psychological and medical symptom improvements even in the absence of weight loss. The objective of the second study was to examine the association between eating in response to hunger and satiety signals (intuitive eating) and Body Mass Index in a nationwide, representative sample of New Zealand women aged 40-50 years. In May 2009, we conducted a mail survey of 2500 women aged 40-50 years randomly selected from the New Zealand electoral rolls, including the Maori rolls (66% response rate; $n=1601$). Measures included Intuitive Eating Scale (IES) scores, current self-reported height and weight, and food patterns (frequency of binge eating, speed of eating, daily intake of fruits and vegetables, daily intake of high fat and/or high sugar foods). Total Intuitive Eating Scale (IES) scores and subscale scores were all statistically significantly inversely associated with BMI, after adjusting for potential confounding variables ($p < 0.001$). For every ten-unit increase in total IES score (potential range 21-105), there was a corresponding decrease in BMI of 6.5% (95% CI: -7.4% to -5.6%; $p < 0.001$). In conclusion, higher levels of intuitive eating are inversely associated with BMI in mid-age women. If these observations are confirmed in longitudinal studies and larger intervention trials, they may highlight a promising approach to weight management and weight gain prevention.

Integration of the Nutrition Message in Schools

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ABSTRACT

Schools have an important part to play in influencing children's food choices through formal education, school food policies and subliminal messages conveyed through teaching material and the school environment. Whilst food and nutrition is a key area of learning in the curriculum, nutrition education needs to be encompassed in every-day life within the school, and beyond.

There is a growing body of evidence to show that manipulating school food environments (such as providing free fruit to children and establishing school vegetable gardens) can have a long-term positive impact on children's eating behaviours and health but some schools are still presenting mixed messages to children.

This presentation aims to explore some of the issues surrounding the exposure of school children in New Zealand to nutrition messages and highlight areas where schools in New Zealand could improve the integration of the nutrition message through its teaching, policies and practices.

The Sport, Physical activity & Eating behaviour: Environmental Determinants in Young people (SPEEDY) study

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ABSTRACT

Previous research has shown that poor diet and a lack of physical activity are associated with overweight and obesity in adolescents. Whilst information on the determinants of diet and physical activity is available in children, the majority of studies focus on either nutrition or physical activity, and not usually both. The SPEEDY study was set up to quantify levels of physical activity (PA) and dietary habits and the association with potential correlates in 9–10 year old British school children.

Initial results indicate that whilst the majority of children meet national Physical Activity guidelines, fewer children were consuming fruit and/or vegetables daily. This talk will focus on the social and environmental factors associated with diet and activity in this cohort.

Fruits, physical activity, and wellbeing

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ABSTRACT

Background: To secure a premium food market position, one of the most popular strategies is to claim an intrinsic health-promoting ability. Fruits in general have an inherent natural 'health halo' and berries in particular are often classed as 'superfruits' because they are rich sources of different bioactive substances potentially of benefit to human health. Regular exercise has health benefits believed to be derived from adaptive responses to moderate oxidative stress. However, following exhaustive or unaccustomed exercise, excessive and prolonged oxidative stress and inflammation can be detrimental and the right balance of modulation from nutritional support via fruit phytochemicals (and vitamins) may prevent damage, and/or enhance muscular and immune function, and aid recovery.

Objectives: The New Zealand Institute for Plant and Food Research Ltd. (PFR) is a world leading plant and fruit science company with a large database of fruits and their compounds – many unique to New Zealand.

Design: PFR has developed and established a research platform to evaluate physical wellbeing and recovery. Our aim is to understand the bioactivity of fruit phytochemicals in fresh and processed functional foods with proven efficacy that offer further support to the known health benefits of regular exercise. To do this we utilise compositional analysis of fruit extracts derived from the unique New Zealand germplasm, including new varieties from breeding programs at PFR. In vitro screening of muscle cell models and tissues is used to analyse the underlying mechanism of action. Feeding and exercise trials in humans are employed to evaluate the physical health promoting effect of polyphenolic phytochemicals derived from fruit, particularly berries.

Outcomes: Data will be presented to indicate that the phytochemical composition of New Zealand cultivars has sufficient variety to provide potential strategies to improve overall body wellness. We will show that a blueberry fruit polyphenolic extract has the potential to mediate protection of muscle cells in vitro against oxidative stress and damage. We will also present human exercise trial outcomes which suggest that berryfruit consumption complements the benefits of regular exercise through the appropriate modulation of oxidative stress, inflammation and enhancement of natural immunity.

Models for Investigating Iron Nutrition

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ABSTRACT

Iron is an essential nutrient for all higher animals; it is required for oxygen transport and storage, oxidative metabolism and cellular growth. Iron is one of the most abundant elements in the Universe and the fourth most abundant element in the Earth's crust, yet iron deficiency is one of the most common nutrient deficiencies affecting Humans. Nutritionists are well placed to play a key role in preventing iron deficiency through the development and promotion of dietary strategies designed to meet the iron requirements of individuals, targeted demographic groups and whole populations. The successful development of such strategies requires a good understanding of the physico-chemical properties of iron in different foods and the physiological mechanisms that influence iron bioavailability. Numerous *in vitro* and *in vivo* techniques have been used by physiologists and nutritionists to investigate iron bioavailability; these range from simple techniques for investigating iron solubility and dialyzability, to those involving molecular biology, cell fragments, cell cultures, isolated intestinal tissues, whole animals and human subjects.

This presentation will look at the advantages and disadvantages of several *in vitro* and *in vivo* methods for investigating iron bioavailability and in so doing highlight their relevance for investigating iron nutrition in humans.

Normal BMI but Fat

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ABSTRACT

Overweight and obesity are increasing at an alarming rate not only across the globe, but also in New Zealand. Overweight and obesity are associated with various metabolic (e.g. via effects on blood pressure, cholesterol, triglycerides and insulin resistance) and cardiovascular co-morbidities. The World Health Organisation (1998) defines obesity as a condition with excess body fat (BF) to the extent that health and well-being are adversely affected. Clinicians and epidemiologists generally rely on body mass index (BMI) as a means of defining the presence of adiposity or obesity. Using BMI as a surrogate measure of BF is justified as it is a power-type index which has a relatively high correlation with estimates of body fatness and a low correlation with stature. Although BMI is measured and calculated easily, it does not reveal excess body fat. Researchers have recommended combining BMI with another measure to improve risk assessment. A possible misclassified group is those with normal BMI ($<25 \text{ kg/m}^2$) but excessive body fat. This group has been defined as having normal weight obesity (NWO). The prevalence of NWO is generally unknown in the general population. The limited evidence available indicates that the prevalence is higher in women than men and increases with age. Women with NWO present with higher levels of cardiovascular risk factors including blood pressure, lipid and glucose levels, and blunted insulin sensitivity, suggesting increased metabolic risk. Another study investigating subjects with cardiovascular disease confirmed these findings, suggesting that both BMI and BF should be assessed during screening for CVD risk. Research conducted in our laboratory confirms these findings – a normal BMI does not necessarily imply protection from the consequences of increased BF. Specific screening for NWO may be important for cardiovascular and diabetes prevention and it seems to be an important group to target for dietary and lifestyle interventions.

Going the extra Green Mile - how sustainability and nutrition are influencing future food values

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ABSTRACT

This presentation will discuss recent research on food values, both in NZ and internationally, which indicates growing social and consumer interest in lifestyles of health and sustainability, also known as LOHAS. A key concern is the issue of "food miles" - with consumers and retailers increasingly taking account of the distance and carbon costs associated with food products. The debate about "food miles" and the emergence of 'local-vores' also represents a deeper concern about the authenticity and provenance of food, including not only what we eat and but also how it is produced. This raises significant issues for industrial food production, trust in food policy and regulation, and investment in future food technologies.

A Study to Determine Whether Infant Sleep Education Influences Breastfeeding Rates

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ABSTRACT

Background: Concern has been expressed that infant sleep education initiatives which aim to promote good infant sleep habits in the first six months of life may have negative effects on breastfeeding because altering infant sleep may decrease breastfeeding opportunities.

Objectives: The aims of the study were to determine 1) whether an educational initiative to prevent the development of infant sleep problems in the first six months postpartum was associated with differences in the duration of exclusive or “any” breastfeeding, 2) the characteristics of the infant sleep educational initiative that may explain any differences in the duration of exclusive and “any” breastfeeding, 3) whether any effect on the duration of exclusive or “any” breastfeeding was modified by support and advice from a lactation consultant.

Design: The present study was designed within a larger obesity prevention initiative to follow 600 infants from birth until six months of age randomised to Control (n=200), Sleep education (n=200) or Combination (sleep education and lactation consultant) (n=200). Sleep education is given antenatally and at three weeks postpartum and is also available upon request. Lactation consultant support is given antenatally, at one week and four months postpartum and is also available on request. Telephone survey data are collected monthly to determine breastfeeding exclusivity and duration to the nearest week. A 24-hour infant sleep diary is administered at three, 19 and 26 weeks to collect infant crying, sleep and breastfeeding duration and frequency data. Exclusive breastfeeding is defined as the infant having only received breast milk from birth. “Any” breastfeeding is defined as exclusive or partial breastfeeding in the previous week. Survival analysis (Cox proportional hazard regression) will be used to determine if the infant sleep education without (Sleep group) or with (Combination group) a lactation consultant was associated with differences in the duration of exclusive and “any” breastfeeding up to six months postpartum.

Outcomes: As of September 2010, the recruitment rate is 61% and more than 500 families have consented to participate in the study. All 600 infants will have reached 6 months of age by July 2011. To date, median (25th, 75th percentile) duration of exclusive breastfeeding and “any” breast feeding across all groups is 16 (2, 21) and 26 (20, 26) weeks respectively. Interim analyses suggest no detrimental effect of sleep education on breastfeeding rates.

Conclusion: A study has been designed that will answer the question as to whether or not an infant sleep education initiative influences exclusive or “any” breastfeeding rates. The combination of the randomised design and comprehensive nature of the data collection suggests this study will furnish quality data to answer this question. Final results will be available in 2012 on completion of the infant assessment.

The Zinc Status of Non-Pregnant Women Living in Central India

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ABSTRACT

Background: The prevalence of inadequate zinc status in women of reproductive age in India is unclear. Furthermore, little data is available to compare zinc status of women from tribal and rural communities.

Objectives: The aim of the present study was to assess and compare zinc status of non-pregnant tribal and rural women aged 18-30 years and to identify dietary factors associated with serum zinc. A cross-sectional survey of 109 women was conducted in Ramtek Block, Nagpur district, Maharashtra state, India using a proportionate to population size sampling (PPS) method. Socio-demographic, anthropometric, clinical, dietary data (interactive 24-hour dietary recall) and biochemical data (serum zinc and C-reactive protein) was obtained.

Design: A cross-sectional survey of 109 women was conducted in Ramtek Block, Nagpur district, Maharashtra state, India using a proportionate to population size sampling (PPS) method. Socio-demographic, anthropometric, clinical, dietary data (interactive 24-hour dietary recall) and biochemical data (serum zinc and C-reactive protein) was obtained.

Outcomes: Serum zinc concentration was (mean \pm SD) 10.8 ± 1.6 $\mu\text{mol/L}$; 58% of tribal women had biochemical zinc deficiency (i.e. <10.7 $\mu\text{mol/L}$) compared to 39% of rural women. Dietary zinc intake (mean \pm SD) was 5.4 ± 1.8 mg/d and 78% of women had a dietary zinc intake below the Estimated Average Requirement (EAR) of 9mg/day (for women aged 14-18 years) and 7 mg/day (for women aged ≥ 19 years) for zinc. The low zinc intakes in these women reflect diets that are both low in energy and low in bioavailable zinc, as the traditional Indian diet is based on cereals and legumes and contains relatively low amounts of animal foods.

Conclusion: A low dietary zinc intake may account for the high prevalence of inadequate zinc status in non-pregnant women of reproductive age living in this area of central India.

Predicting the Glycaemic Index of Meals

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ABSTRACT

Background: The Glycaemic Index (GI) provides a measure of a person's rise in blood glucose following consumption of a test food relative to a reference food. The GI has been used to assist people with diabetes to choose foods. Although GI is tested with individual foods, a summation model has been used to predict the GI of a whole diet in which each food's GI is weighted according to its available carbohydrate contribution

$$\text{Meal GI} = \frac{\sum(\text{GI}_n \times \text{gAvailCHO}_n)}{\text{gAvailCHO}_{\text{Meal}}}$$

The validity of this model has not been robustly tested, for example the addition of fat and protein to a meal lowers the glycaemic response which is not accounted for by the model.

Objective: To assess how well the GIs of cooked meals can be predicted using the summation model.

Design: Seven test foods (potato, rice, pasta, kumara, peas, carrots, sauce) and three meals containing these foods plus 50 g pan-fried chicken were tested in 30 healthy participants. Capillary blood glucose was measured before eating and for two hours following food consumption. Blood glucose incremental areas under the curve were calculated and a mean GI obtained for all foods and meals. Total meal GI was predicted by inserting the observed GI for each food into the summation model and this was compared to the observed GI for each meal.

Outcomes: Mean GI (95% CI) values for the foods were: potato 72 (62, 85), rice 48 (41, 62), spaghetti 56 (48, 66), kumara 84 (72, 98), peas 29 (25, 34), carrots 31 (27, 36), and sauce 35 (30, 41). The predicted meal GIs were greater than the observed meal GIs in all three cases ($p < 0.05$) (Table 1).

Conclusion: The summation model overestimated GI by approximately 20% when applied to mixed meals. The glycaemic effect of major carbohydrate sources such as potato, rice and pasta was lowered when combined into a meal containing other sources of carbohydrate, protein and fat. This may be attributable to an interaction between the foods, which altered gastric emptying and the glycaemic response resulting in a lower GI.

Table 1: Predicted and observed geometric mean (95% CI) GI values

Meal	Predicted GI	Observed GI
Potato Meal	63 (56, 70)	53 (46, 62)*
Rice Meal	51 (45, 56)	38 (33, 45)*
Pasta Meal	55 (49, 61)	38 (33, 44)*

* $p < 0.05$

Consumption of ZESPRI®GOLD Kiwifruit by Children Aged 2–5 Years Reduces Symptoms and the Incidence of Upper Respiratory Tract Infection

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Background: Evidence suggests that diets rich in fruits and vegetables boost the body's natural defences against diseases caused by infection. ZESPRI®GOLD Kiwifruit is rich in vitamin C and contains several phytochemicals that may influence immune function.

Objective: We investigated the effect of consuming ZESPRI®GOLD Kiwifruit on the incidence, length and severity of symptoms of upper respiratory tract infection (URTI) in young children attending local crèches and play centres.

Design: In a randomised, crossover trial, 66 children (aged 2–5 years) were randomised into one of two groups following a 2-week washout period and consumed an equivalent of 2 servings of ZESPRI® GOLD Kiwifruit (group A) or 2 servings of banana (group B) daily for 4 weeks. This was followed by a 2-week washout period and a cross-over of the treatments, i.e. group A consumed 2 servings of banana and group B consumed 2 servings of ZESPRI® GOLD Kiwifruit for a further 4 weeks, followed by a final 2-week washout period. Parents completed a daily questionnaire of URTI symptoms using the validated Canadian Acute Respiratory Illness and Flu Scale (CARIFS), which assessed the incidence of cold-and flu-like illnesses and the severity of those symptoms.

Outcomes: The odds of not getting a cold or flu-like illness was 1.8 times greater for children while they were receiving the kiwifruit treatment than the banana treatment ($p < 0.05$). If children suffered from cold or flu-like symptoms, consuming ZESPRI®GOLD Kiwifruit reduced the severity of both the sore throat and the headache symptoms significantly ($p < 0.05$). The sum of total URTI symptoms scores over the treatment periods was significantly lower for the kiwifruit treatment than the banana treatment ($p < 0.05$).

Conclusion: Regular consumption of ZESPRI®GOLD Kiwifruit may help reduce the incidence of cold and flu-like illnesses and the severity of selected URTI symptoms in pre-school aged children.

Effect of Dietary Feedstuff on Skatole Level in Subcutaneous Fat of Female Pigs

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ABSTRACT

Background: Skatole is a compound associated with unfavourable odours and flavours in meat and, together with androstenone, is responsible for “Boar taint” in pork from entire male pigs. Recent work by our group has shown that skatole levels were higher in fat from female pigs fed garlic and/or fed diets containing the animal by-products of blood meal, tallow, and meat and bone meal.

Objectives: The objective of the study reported here was to determine the influence of different feedstuffs in pig diets on the skatole content of the resultant pork fat.

Design: Forty 35 kg female pigs were randomly allocated to 5 groups and fed diets differing in their lipid and protein sources for 38 days. Hind leg fat samples were collected after slaughter and analysed for their skatole content. The PLANT diet included soybean meal and soybean oil and the ANIMAL diet included meat and bone meal (MBM), blood meal, and tallow. Soybean meal in the plant diet was partially substituted with either MBM or blood meal at levels similar to those in the ANIMAL diet for the MDM and BLOOD groups. Soybean oil in the PLANT diet was replaced by tallow in the TALLOW group.

Outcomes: No statistically significant differences in Skatole levels in subcutaneous fat were observed between the dietary treatments (Table 1).

Table 1: Least squares means for skatole levels in subcutaneous fat of female pigs fed different diets (n=8/group).

	Treatment Group					SE	P=
	ANIMAL	BLOOD	MBM	PLANT	TALLOW		
Skatole ng/g	27.9	21.6	16.7	17.2	18.7	4.27	0.34

Conclusion: Although differences were not statistically significant, it is worth noting that Skatole levels were on average 62% higher for pigs fed the diet containing tallow, MBM, and blood (the ANIMAL group) than those fed the soybean meal and oil diet. This increase is similar to the increases of 68% and 71 % reported in previous research. However, overall the levels of skatole were below the 200 ng/g threshold levels reported for the EC or the 50 ng/g determined for the Asian market, indicating that skatole levels were not a quality issue for this pork.

Extracts from purple potato and boysenberry reduce breast cancer cell proliferation in vitro

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ABSTRACT

Background: Over 1.5 million new breast cancer cases are diagnosed annually worldwide and approximately 0.5 million women die from breast cancer yearly. The triphenylethylene drug tamoxifen, a partial oestrogen agonist as well as antagonist, is the gold standard adjuvant endocrine therapy for breast cancer. However, tamoxifen is not effective in all cases and often produces undesirable side effects; of particular concern is the potential increase in risk of endometrial cancer due its oestrogenic action at this site. Clinically, patients receiving tamoxifen can experience tumour flare at the start of the treatment, which is probably a manifestation of the transient oestrogenic action of tamoxifen. Thus there is a need for improved therapies. Epidemiological studies suggest the consumption of some vegetables and fruits contribute to a decreased risk of breast cancer. Plant-sourced phytochemicals such as anthocyanins have been shown to have anticancer activities in vitro, including inhibiting cell proliferation and inducing apoptosis.

Objectives: The current research aimed to identify plant extracts with potential therapeutic effects against breast cancer. The project is particularly relevant to New Zealand since this country has one of the highest incidences of breast cancer in the world.

Design: We compared the effects of anthocyanin-rich extracts made from purple potatoes (*Solanum tuberosum* 'Urenika') and boysenberries (*Rubus ursinus*), on the growth and survival of various breast cancer cells lines *in vitro*. Human breast cancer cell line, MCF-7, whose characteristics and growth habits have been well described, were used, with tamoxifen and 17 β -estradiol as positive control treatments. The MTT (3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was used to determine cell proliferation.

Outcomes: Tamoxifen produced a biphasic effect on cell growth, inducing a 10-20% in breast cancer cell proliferation at 10⁻⁸M while decreasing by more than 50% cell growth and survival at 10⁻⁵M. Similarly, 17 β -estradiol stimulated cell proliferation by approximately 20% at 10⁻¹⁰M while causing significant reduction on cell growth at 10⁻⁵M. The boysenberry extract also showed a biphasic effect; it stimulates cell growth at concentrations between 3 and 10 μ g/ml, while reducing cell proliferation by more than 50% at 50 μ g/ml. Interestingly, the purple potato extract similarly decreased cell growth by 40-50%, when present at 50 μ g/ml, but it did not stimulate cell growth at a lower dose as observed with tamoxifen and 17 β -estradiol treatment.

Conclusion: Our data demonstrate that both purple potato and boysenberry extracts significantly reduce breast cancer cell proliferation in culture to a degree similar to that observed with tamoxifen. It is of particular interest that the purple potato extract, unlike tamoxifen and 17 β -estradiol, did not have a biphasic effect and did not induce breast cancer cell proliferation. This extract may prove effective, alone or in combination with tamoxifen, in reducing breast cancer metastases. Future studies will assess the extracts in an animal model of breast cancer.

Assessment of the Relationship between Bone Health and Life Exposures in Black South African Women in Transition

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ABSTRACT

Background: Globally, and especially in developing countries, populations are migrating from rural to urban areas due to availability of work and better opportunities. The accompanying changes in dietary patterns and lifestyle in populations have major health consequences. These demographic and lifestyle changes may also affect bone health outcomes.

Objectives: As part of the PURE-SA study, we aimed to assess the risk for developing osteoporosis in a group of 1261 Black women from rural and urban areas in the North West Province of South Africa. Selected markers of bone health were measured in a subgroup of 658 women older than 45 years.

Design: The participants were interviewed to complete several questionnaires on socioeconomic status, self-reported diseases and bone fractures, dietary intakes, and were blood sampled.

Outcomes: Serum 25(OH) Vit D levels were significantly higher ($p < 0.05$) in the rural women (50-60 years = 30.1 ng/mL; 60-70 years = 28 ng/mL; >70 years = 26.01 ng/mL) than the urban women (50-60 years = 26.91 ng/mL; 60-70 years = 25.10 ng/mL; >70 years = 25.82 ng/mL). Bone resorption (serum CTX) was significantly higher ($p < 0.05$) in the rural women (<50 years = 0.50 ng/mL; 50-60 years = 0.58 ng/mL; 60-70 years = 0.54 ng/mL; >70 years = 0.58 ng/mL) than the urban women (<50 years = 0.38 ng/mL; 50-60 years = 0.45 ng/mL; 60-70 years = 0.38 ng/mL; >70 years = 0.48 ng/mL). Parathyroid hormone levels (PTH) increased with age in both rural and urban groups. The specific lifestyle factors that were identified in this cross-sectional study that could affect bone health, comprised reduced physical activity, use of diuretics which could increase urinary calcium excretion, smoking, a history of the use of depot medroxyprogesterone acetate which with long term use may reduce bone density, high alcohol consumption, and an increased state of inflammation which may increase bone resorption. Dietary factors identified were very low calcium intakes (especially in the rural areas) and high animal protein, phosphorous and sodium intakes.

Conclusions: The combination of low dietary calcium (less than 230 mg/day), sub-optimal vitamin D status, and raised PTH levels may result in increased bone resorption as observed in all the rural groups. This has long-term implications for their bone status. Further data is being collected currently which includes bone mineral density as well as peripheral quantitative computed tomography on this group of volunteers and will shed some light on the relationship between lifestyle, nutrition and actual bone density and turnover.

A Survey of New Zealand Health-Care Professionals: Their Knowledge about Vitamin D and the Advice they Give to Mothers

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ABSTRACT

Background: Vitamin D is an essential nutrient which is acquired mainly from exposure to sunlight via ultraviolet-beta (UVB) radiation. The relationship between lack of exposure to sunshine and the development of rickets has been known for a century or more, but now we are learning that poor vitamin D status in pregnancy, infancy and early childhood can have detrimental effects on health, in both the short and long term.

Objectives: To investigate New Zealand health-care professionals' knowledge, and the advice they give to mothers, about vitamin D.

Design: A questionnaire on vitamin D and sun exposure was designed with reference to current literature and Ministry of Health guidelines for pregnant women, infants and toddlers. The questionnaire was distributed to groups of health-care professionals around the North Island of New Zealand.

Outcomes: The questionnaire was completed by 147 health-care professionals; 41% were community or practice nurses, 22% plunket nurses, 13% dietitians, 7% midwives, 7% community health workers, 7% clinical nurses and general practitioners. The respondents were predominantly female (96.5%) and Caucasian (73%). The majority (94%) identified endogenous synthesis as the single most important source of vitamin D for New Zealanders. However, when asked for the best single source for pregnant women, respondents were less certain about sunlight (76%), with supplements (12%), and oily fish (10%) popular options. Similarly, for infants and toddlers fewer chose sunlight (68%) with infant formula, breast milk and fortified milk the other main choices. Cow's milk (fortified and unfortified) was named as the best source of vitamin D for pregnant women by only 10%, but 38% named exclusion of dairy products as a risk factor for deficiency in that group. Despite 69% of respondents being concerned about vitamin D deficiency in their clients, only 19% made recommendations to pregnant women, and 42% said that they made recommendations to mothers of infants, toddlers or small children about vitamin D. This could be because over 80% felt that there is insufficient information available to health-care professionals about vitamin D, and 81% did not feel confident that they would recognise signs of vitamin D deficiency in infants and toddlers. Forty percent said that they had encountered actual or suspected cases over the past 5 years.

Conclusion: The results of this survey show that although health-care professionals have some knowledge about vitamin D, they are aware of significant gaps in their knowledge. The respondents largely lacked confidence in their ability to give advice and clearly expressed a desire for more information. Given the importance of adequate vitamin D levels throughout the entire lifecycle, and especially during infancy, it is important that these front-line health-care professionals are better equipped and more confident to provide effective advice.

The development and administration of a retrospective questionnaire to assess dairy consumption and physical activity during childhood and adolescence

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ABSTRACT

Background: The consumption of calcium-containing food, especially dairy products, and participation in regular physical activity during childhood and adolescence have been shown to be important for adult bone health, and correlate with the continuation of these behaviours during adulthood. Previous studies have attempted to recall childhood consumption in postmenopausal women, and the most widely used questionnaire asks only about milk consumed with meals, as this is seen to be the most reliable recalled data so long after the fact.

Objectives: To develop a questionnaire to retrospectively assess the consumption of a comprehensive range of dairy products, during both childhood and adolescence, for administration to a study population of 19-29 year old New Zealand women.

Design: The dairy categories included in the web-based questionnaire were selected after reviewing the New Zealand Children's Nutrition Survey 2002 and the available literature. Dairy products included in the food frequency section were: milk as a cold drink, milk as a hot drink, milk on cereal, yoghurt and other dairy desserts, and ice-cream. There were also questions about perceived changes in consumption of dairy products. Childhood was defined as 5-10 years, and adolescence as 11-16 years. The questionnaire was completed by 119 of the participants (n=137) in a study investigating bone health and factors affecting it in 19-29 year-old women living in Auckland. The participants also completed a 4-day food diary and a food frequency questionnaire to assess current dietary intake including calcium-containing foods. Retrospective physical activity (PA) was classified according to levels of incidental PA, participation in school physical education and extracurricular sport. Current PA was assessed using the validated EPIC-Norfolk RPAQ.

Outcomes: Consumption of dairy during adolescence was highly correlated with that in adulthood ($r=0.409$, $p<0.001$) and in childhood ($r=0.674$, $p<0.001$). Calcium intake (4-day food record) was correlated with reported adult frequency of dairy consumption ($r=0.490$, $p<0.001$). The significant change in dairy consumption occurred between adolescence and adulthood (mean reported age 15.9 years), with 74% reporting a decrease. The most highly cited reasons were: prefer water or soft drinks to milk (67%), change in living circumstances (31%), do not like milk (30%), stopped eating breakfast (20%) and milk is fattening (17%). Consumption patterns changed, with a reduction in consumption of milk as a drink with increasing age, but adult consumption of milk on cereal and milk in hot drinks significantly increased compared to that reported retrospectively. There was a significant correlation between childhood PA and current total recreational activity ($r=0.293$, $p=0.002$).

Conclusion: Although previous studies have established that milk consumption does decline with age, this questionnaire provided insights into the actual patterns of change, the timing and the reasons why. These findings support the importance of establishing good dairy consumption and PA behaviours in childhood which continue to adulthood and are likely to influence later bone health.

Increasing Fruit and Vegetables in the Diet of Midlife Women: A Feasibility study

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ABSTRACT

Background: Optimum bone health in an ageing population is the result of many factors of which diet is one. The link observed between better bone health and higher consumption of fruits and vegetables has been attributed to several facets of the diet. Primarily, research has focussed on the lower renal acid load created when a higher proportion of the diet consists of alkaline forming vegetables and fruit. Other important dietary determinants may be the specific micronutrients and bioactive found in vegetables and fruit.

Objectives: The purpose of this feasibility study was to investigate if a group of midlife women (n=21) not currently consuming five serves of fruit and vegetables every day could increase their intake to nine serves/day (three fruit and six vegetable) and whether specific bone friendly vegetables can be incorporated in the diet on a daily basis. In addition, the effect of the dietary change on estimated Net Endogenous Acid Production (NEAP), food group serving numbers and urine pH was assessed.

Design: The eight week study involved the women increasing their daily intake of fruit and vegetables to nine or more serves. There was a two week ramp up period to allow physiological adjustment. The women could self adjust their intake from other food groups if/as they needed. Three day diet diaries were completed at baseline and end of study and assessed for NEAP (estimated) and the number of serves from the various food groups. Urine pH dipsticks were provided for the participants to assess urine pH and record daily.

Outcomes: Most women reached or exceeded the study requirements for the increase to nine serves of fruit and vegetables. There was a corresponding reduction in the number of serves from the bread/cereal food group. NEAP (estimated) was reduced significantly over the 8 weeks alongside an increase in the mean urine pH of 0.8 pH units (95% CI 0.46-1.14).

Conclusion: This study demonstrated a group of midlife women can change their diet over eight weeks by significantly increasing fruit and vegetable serves (including specific vegetables) resulting in a concomitant reduction in the number of serves from the bread/cereal food group. The dietary changes were seen alongside a significant decrease in estimated total NEAP and an increase in alkalinity of urine pH.

The Effects of Fish Oil Supplementation on School Achievement in 8-13 Year Old Children from a Mainstream School Population – Selected Preliminary Results

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ABSTRACT

Background: Basic research has established the fundamental role of long chain (LC) omega-3 fatty acids, found in fatty fish and fish oil, on brain structure and function. The effects of these fatty acids on healthy mainstream school children are unknown.

Objectives: To investigate whether supplementation with fish oil for a period of 15 weeks improves selected aspects of school achievement in 8-13 year old children from a mainstream school population compared to placebo treatment. A secondary objective was to assess the intake of fish by this population group.

Design: A randomised placebo controlled double-blind study design was used. Children were recruited from a low socio-economic school in Auckland. Of the 256 children aged 8-13 years, 174 children met the inclusion criteria (no medication or fish oil supplements, no allergies to fish, parental consent) and completed the intervention. Of the 174 children (99 boys and 75 girls), 42 were NZ European, 46 Maori, 66 Pacific Island and 20 from other ethnic groups. Children were stratified by age and gender and then randomly assigned to two groups receiving either 4 capsules/day of fish oil (540 mg EPA and 360 mg DHA/day) or placebo (vegetable oil) 5 days of the week for 15 weeks. Supplements were consumed daily at school under controlled conditions. Standardised national tests assessing school achievement were administered at baseline and end of the study. Only selected aspects of word fluency, spelling, basic facts and reading are reported. Parents completed a food frequency questionnaire regarding their children's usual intake of fish and seafood. The children's previous day's fish intake was recorded daily at time of supplement distribution.

Outcomes: Although the fish oil group's score in 8-9 year old children for division basic facts tests improved significantly compared to placebo (mean [95% CI] change of 3.81 [2.76, 4.86] vs. 2.16 [1.08, 3.23], $p=0.03$), supplementation did not significantly affect addition, subtraction and multiplication. Using the adapted Thurstone word fluency test the number of spelling errors for four letter words starting with "C" were significantly reduced with fish oil supplementation compared to placebo and the effect was greatest in 8-9 years old boys (change of -0.22 [-0.80, 0.36] vs. 0.82 [0.23, 1.36], $p=0.01$). Fish intake was low; over the 15 weeks less than 9% of the children consumed fish and seafood >1x/week. Parents reported their children's fish and seafood intake as 0.5 [0.5, 2] servings/week (median [25, 75 percentile]). Fish types most frequently eaten were low-fat fish and battered fish.

Conclusion: Fish oil supplementation particularly of 8-9 year old children from a mainstream school population with low habitual intakes of fish and seafood over a period of 15 weeks resulted in some improvements in school achievement compared to placebo treatment. Further analysis of the data to gain more in-depth understanding of the effects of fish oil supplementation on school achievement and children's behaviour is continuing.

Project Energize: Survey of the Food Habits of Primary School Children's in the Waikato

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ABSTRACT

Background: Project Energize is a through-school programme provided to all Waikato District Health Board primary schools. The aim is to improve childhood health through a range of school-based interventions that encourage daily moderate and vigorous physical activity and healthier eating patterns.

Objective: To investigate patterns of breakfast and lunch consumption and food choices of primary school children.

Design: In terms 2 and 3, 2009, a one page, 13 question survey was delivered to all families (20,238) in 116 Energize schools and 6,050 (30%) were returned. Responses were analysed in terms of the, socioeconomic status (school decile), ethnicity and the food and beverage classification system. Each school received a summary of the findings for their school.

Outcomes: Six out of seven children have breakfast every day and four out of five children brought lunch from home to school every school day. In general low decile and Maori and Pacific children were less likely to have breakfast at home and to bring lunch from home every day than high decile and NZ European children. Everyday foods sandwiches (90%) and fruit and vegetables (86%) were most frequently brought for lunch but occasional foods; muesli bars and cake (78%) and chippies (50%) were also reported. Snacks defined as chippies, cake, biscuits, chocolate and lollies were on average consumed 5.8 times a week with one in six consuming these items eight or more times a week.

Conclusion: These findings have provided school-specific information that allows interventions to be targeted. The survey will be repeated in 2011 as one of the measures of the effectiveness and sustainability of the programme.

How can we help improve the food environment around schools?

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ABSTRACT

Background: The obesogenic environment that promotes the consumption of nutritionally poor and energy-dense food and beverages is a major contributing factor to childhood obesity in New Zealand. Many interventions have been delivered through schools to address the foods sold in school canteens/tuck shops. However, food stores outside school generally offer mostly cheap and high fat/sugar foods to students on their way to and from school. A closed gate policy alone cannot stop students going across the road as some students often skip lunch and save their money for a better-value fast-food snack or meal on the way home. Regardless of what goes on inside the school, the wider school environment must be a target for intervention.

Objectives: To look at ways to improve the external food environment around schools.

Design: A pilot to work collaboratively with local food operators, schools, and other health providers was carried out in Auckland. A multifaceted approach has been utilised:

1) Food outlets in close proximity to schools were approached and encouraged to introduce new healthy options. Some high volume high fat foods, such as hot chips and pies are addressed through delivering Best Practice Guidelines to improve their nutritional profile. In order to help food operators sustain the provision of healthy foods, strategies to improve business efficiency and to increase business profit were used, such as support to introduce healthier combos, placement of healthier options at eye-level and the pair-up of supplier and market.

2) In order to increase students' awareness of the healthier alternatives and their motivation to select them, Student Health Councils were involved in the introduction and promotion of the healthy foods. Students were surveyed on what food they would like to see become available at the food outlets near school and about their views on the price and accessibility of healthy foods around school. Tastings of new healthy food were held in schools. Students' suggestions and comments were fed back to food operators.

3) This initiative was also linked with other existing school health promotion programs, such as School Food and Beverage Classification System, Healthy Heart Award from the Heart Foundation, Get Wise 2 from Diabetes Project Trust, Health Promoting School and the Healthy Kai program, to ensure greater reach and sustainability.

Outcomes: 8 out of 10 approached food outlets participated actively in this one-year pilot project. New healthy items, such as pastry-less pies, sushi and wraps have been successfully introduced and have attracted more and more students. For example, the sales of sushi in one shop reached to 100 packs per week just three weeks after its introduction. A combo consisting of a chicken wrap and yoghurt has become the most popular item ordered by parents for their kids on their way to school. The provision of healthy foods has allowed one food outlet to become a lunch supplier for five local schools. The food outlets have enhanced their reputation as a supplier of healthy food within the local community. A beneficial relationship between school and local food operators has empowered involved schools to engage with the food operators and to address the food environment outside the school gate.

Conclusion: The Introduction of healthy food has to be paired up with a market. A win-win approach, students' involvement and collaborative working partnerships are the key successful elements of this pilot.

Implementing the Clinical Guidelines for Weight Management in New Zealand

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ABSTRACT

Background: The Clinical Guidelines for Weight Management in New Zealand Adults and the Clinical Guidelines for Weight Management in New Zealand Children and Young People (the Guidelines) are a new tool to help address the growing health issue obesity presents in New Zealand, particularly in Māori, Pacific and South Asian populations. The Guidelines are evidence based and structured around the 'four-step approach' of best practice whereby frontline health care workers:

- (1) raise awareness
- (2) identify need and the context for action
- (3) determine options for action (the FAB trio: Food/diet, physical Activity and Behaviour strategies)
- (4) maintain contact and support

Objectives: The Guidelines and their implementation aim to provide guidance for individual and group weight management, to be principally used in primary care and community-based settings. Implementation will provide a consistent approach across the many weight management initiatives, programmes, and tools in the private and public sector.

Design: Activities will support frontline health care workers who provide healthy weight management advice and treatment to patients/consumers. They will focus on communicating the Guidelines to frontline health care workers and raising awareness of the Guidelines to patients/consumers, particularly Māori, Pacific and South Asian populations. This will be done using a number of approaches, including development of:

- digital communication tools and resources
- training tools and resources

Outcomes: The success of implementing the four-step approach will be measured by an increase in weight loss initiation and improved weight loss maintenance.

Conclusion: By integrating healthy weight management and lifestyle advice into the day-to-day activity of the health sector, the number of New Zealanders with a healthy weight will increase.

Tailored nutrition education: is it really effective?

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ABSTRACT

Background: There has been a growing interest in tailored nutrition education over the previous decade, particularly as developments in technology have enabled efficient personalisation and delivery of information to large numbers of individuals. Since 2006, four systematic literature reviews have suggested significant potential of tailored nutrition education for improving dietary habits. However, the majority of included trials have used subjective self-report dietary outcome measures such as food frequency questionnaires and dietary recalls. Because participants in pragmatic nutrition education trials cannot be blinded to whether or not they receive the intervention, it is possible that the positive findings of these reviews are the result of information and reporting bias, rather than a true effect of tailored nutrition education.

Objective: To assess the likely true effect of tailored nutrition education for improving dietary intakes and food purchasing habits.

Design: Randomised controlled trials assessing the effectiveness of tailored nutrition education and employing objective measures of diet and food purchasing (i.e. biomarkers, till receipts, or electronic sales data) were identified through systematic literature reviews and a literature search to September 2010. The findings of trials employing objective measures of diet were compared with the findings of trials using subjective self-report dietary outcome measures, the latter from previous systematic literature reviews.

Outcomes: Four randomised controlled trials undertaken in supermarket settings used objective till receipts or electronic sales data (n=1,778 participants in total). Two were undertaken in the United States (1997 and 2001, respectively), one in Australia (2006), and one in New Zealand (2010). Two trials delivered education via a kiosk housed in a supermarket, one used an Internet supermarket shopping website, and the remaining trial mailed participants monthly packages of tailored print materials. Three trials delivered the education over six-months, and one over a five-month period. All trials followed-up at the end of intervention, and one followed-up six months after the intervention had finished. In contrast to the findings of previous systematic reviews, three of the four trials reported largely negative findings, with no effect of tailored nutrition education on any food groups or nutrients assessed, at any time point. The exception was one of several outcomes (total fat) for one trial, at six-months. The remaining positive (Internet-based) trial found a significant effect of tailored nutrition education on the primary outcome (saturated fat) at five-months.

Conclusion: Although commonly understood to be potentially effective, the findings of trials employing objective outcome measures suggest that tailored nutrition education is unlikely to be useful as a stand-alone intervention for improving dietary intakes and food purchasing habits. Consequently, other levers for behaviour change such as environmental or structural strategies should be the focus of future nutrition research. Nevertheless, education may still play a role in generating social understanding and acceptance of policy changes to improve nutrition.

Iodine knowledge and behaviour of pregnant and lactating women within New Zealand

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ABSTRACT

Background: Recent studies have indicated iodine deficiency is re-emerging in New Zealand, especially for women during pregnancy and lactation. Inadequate iodine status during pregnancy and lactation can impair infant mental and physical development.

Objective: To explore the current knowledge and behaviour regarding iodine deficiency during pregnancy and lactation in a self-selecting population within New Zealand.

Design: Pregnant and breastfeeding women were recruited from the Palmerston North area via advertisements in the local media and posters at medical and health centres and throughout New Zealand via national media. Questionnaires were administered through telephone interviews. In total, 66 pregnant women over 26 weeks of gestation, and 89 lactating women at least three weeks post partum, completed the questionnaires.

Outcomes: Between 62% (pregnant) and 72% (lactating) women were personally aware of iodine deficiency in New Zealand. The majority did not recognise dairy products as a good dietary source of iodine, and more than half did not recognise eggs as a good dietary source of iodine, although most participants were able to indicate others, such as fish (68% pregnancy; 65% lactation), and seaweed (85% pregnancy; 89% lactation). One third of women incorrectly believed sea salt is a good source of iodine. In terms of salt intake, 27% pregnant and 33% lactating women used only iodised salt; 14% (pregnancy) and 11% (lactation) used only non-iodised salt; 59% (pregnancy) and 56% (lactation) used iodised salt together with other types of salt. Interestingly, of 85% pregnant and 71% lactating women who took supplements, only 20% and 33% respectively took supplements containing iodine. The most common source of dietary information used by participants were the internet (45-53%) and pamphlets (65-66%), whereas only approximately 25% received information from health professionals.

Conclusions: This study shows pregnant and lactating women have some knowledge that iodine deficiency exists in New Zealand but limited knowledge of dietary sources of iodine. Use of iodine containing supplements is low and women do not always choose iodised salt when use salt. It is important that pregnant and breastfeeding women are made aware of how to improve their iodine intake. It is essential that women are made aware of the recently launched government subsidised iodine supplement for pregnancy and lactation.

Selenium intake during pregnancy and lactation in Palmerston North, New Zealand

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ABSTRACT

Background: New Zealand has low levels of selenium in the soil and hence in the food supply consequently selenium deficiency has been shown to exist in the population. Selenium requirements increase during pregnancy and lactation, and selenium deficiency can compromise the antioxidant status of a newborn infant. Selenium deficiency can also exacerbate iodine deficiency which has been shown to exist in New Zealand, including Palmerston North. Inadequate iodine status during pregnancy and lactation can affect foetal mental development and during infancy can impair both mental and physical development.

Objective: To explore the current selenium intake during pregnancy and lactation in a self-selecting population within the Palmerston North area of New Zealand.

Design: Pregnant and breastfeeding women were recruited from the Palmerston North area via advertisements in the local media and posters at medical and health centres. Twenty-four hour urine samples were obtained from pregnant women (n=25) greater than 26 weeks of gestation and breastfeeding women (n=32) at least three weeks post partum. Breast milk samples were also collected (n=31) from lactating women. Three 24-hour recalls were collected for each participant, the dietary data was analysed using Food Works Professional 2009. Selenium concentration was determined in urine and milk samples using inductively-coupled plasma mass spectrometry.

Outcomes: Over the three days median selenium dietary intake was 46.4 (IQR 37-63) µg/d during pregnancy; 64% (16/25) of intakes were <EAR (55 µg/d) and a further 16% (4/25) <RDI (65 µg/d). Assuming 55% of selenium intake is excreted in urine median intake was estimated to be 48.7 (IQR 39-59) µg/day, with 60% (15/25) of intakes <EAR and a further 20% (5/25) <RDI. During lactation median 3 day dietary selenium intake was 47.4 (IQR 40-71) µg/d; 69% (22/32) of intakes were <EAR (65 µg/d) and a further 13% (4/32) <RDI (75 µg/d). Median selenium concentrations in breast milk were 11.3 (IQR 10-13) µg/l. Using a mean breast milk intake of 750 ml/d only 35% (11/31) would provide the recommended 10 µg/d.

Conclusions: This study shows low selenium intakes during pregnancy and lactation amongst this population. Of greatest concern is the low levels of selenium found in breast milk, which puts infants at risk of deficiency. This finding has potential adverse consequences for both mothers and their infants.

Effects of banana and ZESPRI® GOLD Kiwifruit dietary supplementation on T- and B-lymphocyte frequencies in healthy elderly humans.

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ABSTRACT

Background: Immune function is known to decline with age, with a decrease in B lymphocyte numbers being a prominent feature of immune senescence. Some immune cell numbers and function may also be related to sex, environment/climate, health status, and diet. ZESPRI® GOLD Kiwifruit is a good source of vitamin C and other micronutrients and may improve immune function when included as part of a healthy diet.

Objectives: To compare the effect of banana versus ZESPRI® GOLD Kiwifruit dietary supplementation on peripheral blood leukocyte numbers and function.

Design: In a crossover trial, 32 healthy elderly human subjects consumed the equivalent of 2 bananas or 4 ZESPRI® GOLD Kiwifruit each day for 4 weeks, with a 4 week washout period between each treatment. Blood samples were drawn at the end of each 4 week period and assessed for leukocyte numbers, subpopulation frequencies, and immune cell function using standard complete blood counts (CBC) and flow cytometry.

Outcomes: Total white blood cell counts and total lymphocyte, monocyte, and neutrophil counts were not significantly affected by dietary treatment. Monocyte and neutrophil phagocytosis of bacteria was higher in summer than in winter. Conversely, NK cell-induced tumour cell cytotoxicity was lower in summer and significantly reduced in participants who experienced cold or flu symptoms as self-reported in a WURSS™ daily questionnaire. Neither phagocytosis nor NK cell function was significantly affected by dietary supplementation. Treatment with both fruits resulted in a significant increase in the ratio of memory:naive T lymphocytes without changing total T lymphocyte counts; the memory:naive ratio returned to basal levels following the washout periods. In females but not males, the consumption of kiwifruit significantly increased B lymphocyte frequencies and counts in the blood by >15%, while banana consumption had no effect. This kiwifruit effect resulted in females having a significantly increased B:T lymphocyte ratio compared to their baseline ratios, which extended into the washout period.

Conclusion: ZESPRI® GOLD Kiwifruit consumption results in a significant increase in B lymphocyte frequency and total cell numbers in elderly females, which may correlate with an increase in humoral immune cell function. Both kiwifruit and banana consumption transiently alter the memory:naive T lymphocyte ratios in elderly males and females, possibly via vitamin E-driven induction in T lymphocyte proliferation. NK and phagocytic cell functions in the elderly are affected by climate and health status but not dietary supplementation.

Could impaired gastric digestion be a primary cause of protein malnutrition in the elderly?

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ABSTRACT

Background: With ageing, the pH of gastric juice may increase, gastric pepsin diminish and digestive discomfort become more pronounced, than for younger people.

Objectives: To measure the effect of pH on protein hydrolysis in the presence of pepsin, Zyactinase (a protease derived from kiwifruit) and pepsin with Zyactinase.

Design: *In vitro* study of the effect of pH (range <1.5 to 6.4), on the proteolytic activities of pepsin, Zyactinase and pepsin with Zyactinase, for 60 minutes (gastric pH <1.5 to 5.0) and 120 minutes (duodenal pH 6.4) at 37°C using a standardised meat protein. Nitrogen in the supernatant was measured by the Kjeldahl method.

Outcomes: Protein hydrolysis by pepsin was maximal at a pH less than 1.5, Zyactinase at a pH of 3.2 and the effects of the proteases were additive from pH 2 to 5. At pH 4, in the presence of both proteases, protein digestion was twice that of pepsin by itself. At a duodenal pH, equivalent to 6.4, both proteases were inactive.

Conclusion: Reports of digestive discomfort being relieved by the consumption of kiwifruit may be a result of enhanced protein hydrolysis in the stomach reducing reliance on primary protein hydrolysis by pancreatic proteases and minimising the incidence of the large intestine loading with undigested protein.

High dose selenium reduces ventilator associated pneumonia and illness severity in patients with systemic inflammation

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ABSTRACT

Background: The systemic inflammatory response syndrome (SIRS) involves a complex chain of events culminating in the adhesion and migration of neutrophils from the circulation into inflamed tissue where toxic free radicals are released. In the upper respiratory tract SIRS is characterised by capillary congestion, leukocyte and macrophage infiltration into the alveolar spaces. If this inflammatory response occurs in the intensive care unit (ICU) more than 48hr after intubation, it is called ventilator-associated pneumonia (VAP); usually defined as bacterial nosocomial pneumonia that develops with acute respiratory failure. It is a common and major complication that affects up to 30% of the most vulnerable ICU patients by increasing morbidity, mortality, length of stay and hospital costs. The ability of Selenium (Se) to behave both as an antioxidant and anti-inflammatory agent through selenoproteins such as Glutathione Peroxidase (GPx-3) confers a potentially important role for supplementation in Se-depleted ICU patients

Objectives: To evaluate the safety and efficacy of high dose Se, administered by continuous infusion, following an initial loading bolus, of Selenite (SeO₃), on pharmacodynamics and clinical outcome in Se-depleted critically ill patients with SIRS.

Design: Prospective, placebo-controlled, randomised, single-blinded phase II study of 35 patients with SIRS, age >18, and APACHE II > 15 in a multidisciplinary University Hospital ICU. Two groups of patients received either placebo or intravenous SeO₃ as a bolus-loading dose of 2000 µg Se over 2 hours followed by continuous infusion (CIV) of 1600 µg Se per day for 10 days. The primary clinical outcome of illness severity was determined by Sepsis-related Organ Failure Assessment (SOFA) score. VAP, adverse events (AE) and other parameters were monitored as secondary safety endpoints. To assess GPx-3 and other variables, blood samples were obtained: before randomization (day 0), then days 3, 7 and 10.

Outcome: SOFA score reduced significantly only in SeO₃ group at day 10 compared to day 0 (7.8 ± 3.8 vs. 4.7 ± 2.0 , $p=0.02$). During the study period, early VAP was lower in SeO₃ group (6.7% vs. 37.5%, $p=0.04$). Pharmacodynamic profiles showed that GPx-3 activity increased more in the SeO₃ group from day 3 (0.52 ± 0.28 vs. 0.26 ± 0.08 U/mL ($p=0.001$) and reached a maximum at day 7 (0.62 ± 0.24 vs. 0.28 ± 0.14 U/mL, $p=0.001$) in SeO₃ and placebo group, respectively but then declined by day 10 (0.39 ± 0.09 vs. 0.27 ± 0.09 ($p=0.09$). ICU length of stay, days of mechanical ventilation, AE and other safety parameters were similar between both groups.

Conclusion: High dose parenteral SeO₃ significantly increases Se status, improves illness severity (SOFA score) and lowers incidence of early VAP in critically ill SIRS patients. Daily infusion of 1600 µg Se (as SeO₃), following an initial bolus of 2000 µg, is novel and without obvious adverse effects in ICU.

The associations between magnesium and cardiovascular risk in overweight and obese women randomised to an energy-restricted high protein or high fibre diet

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ABSTRACT

Background: Dietary magnesium intake has reduced in developed countries over the last century, and consequently average magnesium intakes in New Zealand are well below the recommended dietary intake. Plausible biological mechanisms and a large body of cross sectional research have indicated a link between both dietary magnesium intake and magnesium status and cardiovascular risk factors. However, dietary interventions to modify magnesium intake and status and examine the effect on cardiovascular risk have not been previously carried out.

Objectives: To examine the effect of a hypocaloric high carbohydrate, high fibre diet compared with a hypocaloric high protein diet on dietary magnesium intake and status, and to examine corresponding modifications in cardiovascular risk.

Design: A randomised controlled weight-loss trial was carried out with eighty-three overweight and obese women, who were free from medicated diabetes and dyslipidaemia. Fasting plasma magnesium was measured, and dietary magnesium intake was assessed using a three-day diet record at baseline and at the end of the study.

Outcomes: Over eight weeks of follow-up, plasma magnesium and dietary magnesium intake did not change significantly in either diet group. In the high fibre diet group, an increase in 100 mg of dietary magnesium daily was associated with a 1.3 kg reduction in weight ($p=0.005$), an 8% reduction in fasting insulin concentration ($p=0.043$) and an 8% reduction in HOMA-IR ($p=0.015$). An increase in plasma magnesium concentration by 1 mg/L in the high protein diet group was associated with a 600 g weight loss ($p=0.013$), 350 g reduction in trunkal fat ($p=0.016$) and an increase in the McAuley insulin sensitivity index ($p=0.020$).

Conclusion: Increases in both dietary magnesium and plasma magnesium were associated with an improvement in cardiovascular risk factors. This study suggests that weight loss in conjunction with an increase in magnesium intake and improvements in magnesium status, may work synergistically to reduce cardiovascular risk in an at risk population.

Caffeine consumed with and without sugar: acute effects on fat and carbohydrate oxidation and heart and respiratory rate variability.

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ABSTRACT

Background: Over the last two decades caffeinated beverage sales including energy drinks and shots have increased exponentially. In New Zealand young people consume large quantities of “energy” formulations which often contain large amounts of sugar. Preliminary work suggests that consuming caffeine and sugar together may cause the formation of new fat: de novo lipogenesis, in sedentary young women. Furthermore the pattern of respiratory rate and depth appears to change.

Objective: To investigate the effects of caffeine, sugar and caffeine with sugar on substrate metabolism and variability in heart and respiratory rates.

Design: A cross-over experimental design with randomisation of subjects to the order of the three treatments. Breath-by breath oxygen consumption and carbon dioxide production were measured for 30 minutes before and 30 minutes after consuming each drink.

Outcomes: Carbon dioxide output increased at a faster rate than oxygen consumption at in all participants after consuming all three types of drinks indicating that fat oxidation was suppressed. In 7 out of 10 participants the consumption of caffeine with sugar increased the ratio of carbon dioxide to oxygen to more than 1.0 throughout the measurement period which may mean that de novo lipogenesis was occurring. Hyperventilation would not be physiologically possible for this period of time.

Conclusion: Given the variable levels of response of participants further work will measure biomarkers of caffeine and substrate metabolism in urine and blood samples. Potential differences between active and sedentary people will be explored.

Enhancers or inhibitors of iron in dietary patterns of women with low iron stores compared to iron replete women

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ABSTRACT

Background: The most recently available data indicate that 23% of New Zealand (NZ) women have low iron stores (Serum Ferritin (SF) $<20 \mu\text{g/L}$, Haemoglobin (Hb) $\geq 120 \text{ g/L}$), and that 39-45% of NZ women aged 15-44 have inadequate intakes of iron. Iron absorption from dietary sources may be affected by dietary enhancers (e.g. ascorbic acid (fruit, vegetables), meat, fish and poultry, alcohol, citric acid) or inhibitors (e.g. phytates (legumes, cereals), phenolic compounds (tea, coffee), oxalates (spinach), and calcium (milk) which inhibits both haem and non-haem iron consumed within an hour of the meal. Differences in the dietary patterns of iron replete women compared to women with low iron stores may explain why some women do not achieve iron sufficiency.

Objective: This study aimed to investigate the enhancers or inhibitors of iron within the dietary patterns of women with low iron stores compared to women who are iron replete.

Design: Women aged 18-44 years, with low iron stores (LIS) (serum Ferritin (SF) $<20 \mu\text{g/L}$, Haemoglobin (Hb) $\geq 120 \text{ g/L}$) ($n=84$) and normal iron stores (NIS) (SF $\geq 20 \mu\text{g/L}$, Hb $\geq 120 \text{ g/L}$) ($n=64$) were compared. Venous blood samples (SF; Hb) were taken and dietary data collected using a computerised iron habits assessment tool consisting of a non-quantitative food frequency questionnaire and a dietary practices questionnaire.

Outcomes: Women were grouped based on their iron stores. The women with LIS had significantly lower median (IQR) SF than those with NIS (19 [9.8] vs. 48.5 [27] $\mu\text{g/L}$). Daily serves of fruit and vegetables showed weak positive correlations with SF ($r=0.193$, $p=0.02$). If meat was eaten at lunch, LIS women were 3.48 times more likely to consume coffee than NIS women ($p=0.028$). LIS women were 2.84 times more likely to consume milk products ($p=0.016$) and 7.36 times more likely to consume milk drinks ($p=0.032$) with meat containing evening meals compared to women with NIS who were 2.48 times more likely to consume milk products between meals ($p=0.010$). The LIS women were also 7.56 times more likely ($p=0.043$) to consume milk as a drink in the evening and 10.2 times more likely ($p=0.012$) to consume a chocolate-based drink at breakfast than NIS women.

Conclusion: Low iron status is linked to milk consumption patterns. It appears that the consumption of coffee and milk at meal times may contribute to women's low iron stores. Vegetable, fruit and milk intakes between meals were associated with iron repletion. Further research is needed to investigate if calcium and iron rich foods displace one another in the diets of LIS women. Recommendations regarding milk consumption should be included in dietary advice given to women with low iron stores.

Selenium-containing black and green teas: Total phenolic content, total antioxidant and free-radical scavenging activities

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ABSTRACT

Background: Tea is second only to water as the most common plant-based beverage and has been consumed worldwide for centuries due to its beneficial health properties. Tea is a major source of flavonoids, a subclass of polyphenols which have been shown to have several biological activities. The most widely recognised biological properties of tea polyphenols are the antioxidant activities, due to their free radical scavenging and metal chelating abilities. Excessive free radicals produced in cells have been shown to be involved in the pathogenesis of numerous chronic degenerative diseases. Selenium is an important trace mineral that acts as an antioxidant against free radicals that damage DNA and enhances the immune response. Selenium may delay or prevent the onset of cancer and also have anti-ageing effects.

Objectives: To determine the total phenolic contents (TPC) and the antioxidant capacity of selenium-containing green (Se-GTE) and black (Se-BTE) teas in comparison with normal green (N-GTE) and black (N-BTE) teas.

Design: TPC was measured by the Folin-Ciocalteu method while antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and by ferric reducing antioxidant power (FRAP) assays. 1% aqueous tea extracts were prepared at different extraction temperatures (50°C, 70°C, 90°C and 100°C) and different extraction times (2, 5 and 10 min).

Outcomes: The results demonstrated that Se-GTE contains the highest TPC, followed by N-BTE, N-GTE and Se-BTE ($p < 0.05$). Similarly, Se-GTE exhibited the highest antioxidant and DPPH-radical scavenging activities compared to other teas ($p < 0.05$). However, N-BTE showed significantly higher TPC, antioxidant and DPPH-radical scavenging activities than Se-BTE, which may be explained by different environmental conditions and agricultural practices. The TPC, total antioxidant and DPPH-radical scavenging activities significantly increased with increasing extraction time from 2 min to 10 min. Similarly, the TPC, total antioxidant and DPPH-radical scavenging activities significantly increased with increasing extraction temperature from 50°C to 100°C. However, the optimal temperature was found to be 90°C as the extracts prepared at this temperature showed significantly higher TPC, antioxidant and DPPH-radical scavenging activities than their counterparts extracted at other temperatures. In general, total antioxidant activity and DPPH-radical scavenging activities positively correlate with TPC ($p < 0.05$), which may indicate that the phenolic compounds are the major contributors to the antioxidant activity. Our findings clearly reflected that tea extraction conditions, such as extraction temperature and extraction time are important factors for determining the TPC and antioxidant activities.

Conclusion: The results of this study further our understanding of how the potential health giving benefits of tea are maximised by the extraction temperature and extraction time during its preparation, therefore potentially increasing the health benefits of consuming tea.

Antioxidant capacity, phenolic contents and anthocyanin concentrations of various cultivars of New Zealand highbush blueberries

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ABSTRACT

Background: Blueberry fruits are recognised as one of effective antioxidants due to their abundant anthocyanin content, which may protect against several chronic diseases. Although the anthocyanin content and the antioxidant capacity of blueberries have been studied widely, these can vary greatly within the plant variety and agronomic conditions.

Objectives: The objectives of this study were: (1) to determine the anthocyanin level, total phenolic content (TPC) and the antioxidant capacity in the extracts of berries from different varieties of highbush blueberries grown in New Zealand and (2) to evaluate the effect of different solvents (water/ water plus 5% formic acid/ mixed solvents) used to prepare extracts for TPC and antioxidant activity assays.

Methods: Eight cultivars of highbush blueberries were used: Bluecrop, Brigitta, Burlington, Dixi, Duke, Elliott, Jersey and Reka (obtained from Mamaku Blue, Rotorua). All of the cultivars assayed were grown under the same agronomic conditions to avoid environmental and agronomic effects on phenolic composition. The anthocyanin composition of water plus 5% formic acid blueberry extracts were analysed by High Performance Liquid Chromatography (HPLC). For the total phenolic content and antioxidant capacities, all cultivars were extracted using either water, water plus 5% formic acid, or mixed solvents (acetone: methanol: water: formic acid 40:40:20:0.1% v/v). The TPC was determined by the Folin-Ciocalteu procedure, and antioxidant activity was measured by Ferric Reducing Antioxidant Power (FRAP) and diphenyl-picrylhydrazyl (DPPH) radical assays.

Outcomes: The concentrations of anthocyanins, TPC and antioxidant capacities varied substantially between the cultivars. Elliott had the highest total anthocyanins, TPC and antioxidant capacity as measured by FRAP and the scavenging activity toward DPPH-radical. In contrast, Jersey cultivar showed the lowest values for most of the parameters mentioned above. The order of total antioxidant capacity among different blueberry cultivars was as follows: Elliott > Burlington > Bluecrop > Duke > Brigitta > Reka > Dixi > Jersey. Using different solvents resulted in significant differences in the outcomes of all assays used in this study. Overall, mixed solvents (acetone: methanol: water: formic acid 40:40:20:0.1% v/v) showed the highest TPC and antioxidant activity followed by water extracts plus 5% formic acid, whereas just water extracts showed the lowest values. TPC of all blueberry cultivars showed a positive correlation with the antioxidant activity with a positive correlation coefficient ($R^2 = 0.65-0.98$) which may indicate that polyphenolic compounds are the major contributors to the antioxidant capacity.

Conclusion: Extracts from blueberries may supply substantial antioxidants, which may provide health promoting advantages to consumers. These activities are influenced significantly by the amount of phenolic compounds present in each cultivar.

Microbiological contamination in non-commercial enteral feeding solutions for patients in a Mexican Hospital

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ABSTRACT

Background: Enteral Feeding Solutions made from ingredients such as milk, fruits, vegetables, etc., are common in Latin American hospitals and bacterial contamination occurs easily

Objectives: To determine the presence of microbiological contamination of enteral feeding solutions made in the hospital, identify and quantify the microbial agents and ascertain the prevalence of gastrointestinal complications in patients.

Design: Observational and prospective study in a Mexican hospital. Twenty two samples were analysed, eleven were taken immediately after their preparation (0 h) and eleven at the end of their administration (20 h). Gastrointestinal complications shown by the patients were recorded. Hazard Analysis and Critical Control Points were used.

Outcomes: Of the eleven patients who were administrated the formula eight had a nasogastric tube, two had feeding gastrostomies and one was fed orally. Gastrointestinal complications presented were diarrhea 73% (8/11), vomiting 27% (3/11), bloating 36% (4/11) and nausea 45% (5/11). 100% of the samples were found with contamination with significant increase in bacterial colony counts (ufc/mL) at 0 h and at 20 h of their administration. Prevalence of microorganisms: *Escherichia coli* 91% (20/22), *Enterococcus sp* 73% (16/22), *Acinetobacter sp* 64% (14/22). For Hazard Analysis and Critical Control Points, each of the ingredients used in the enteral formulas were qualitatively analysed, not finding an evident source of contamination. Nevertheless, the employees who prepared the formulas did not meet the necessary guidelines for hand hygiene and utensils. Afterward, another three formulas were analysed, and once again were found with high levels of contamination.

Conclusion: All hospitals should make protocols and procedures to control contamination in enteral formulas, due to the ease of contamination and the possible consequences associated with increased complications and increased health care costs. As a result of our investigation it was decided to use commercial formulas in our hospital.

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- Smith AB (1980) Fat digestion in Teal. **In:** *Birds of New Zealand*. (Ed EF Jones). P 21-29. Palmerston North, ADB Press.
- Smith AB (1981) Analysis of faecal chromium. *British Journal of Nutrition* 81, 22-28.
- Smith AB, Brown CD (1982) A multienzyme technique for estimating protein digestibility. *New Zealand Journal of Science* 5, 123-130.
- Smith AB, Jones EF, Brown CD (1979) Problems in the determination of soluble fibre. *Journal of Animal Science* 47, 81-85.

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