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INSTRUCTIONS TO AUTHORS

#### MURIEL BELL MEMORIAL LECTURE 2008

#### Nutrition research – to specialise or not to specialise

#### C.M. SKEAFF

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It was a privilege to be invited to deliver the 2008 Muriel Bell Lecture, an annual lecture of the New Zealand Nutrition Society to honour the pioneering contribution of Dr Muriel Bell to medical and nutritional sciences. It was not difficult to find historical records of Dr Bell's research activities but equally accessible and impressive were the accounts of her efforts to improve the quality of the New Zealand food supply, and educate New Zealanders about good nutrition (Mein Smith, 2007). Her drive to improve the health of the nation through good nutrition was accomplished through official roles in the Department of Health, as well as involvement in a number of health professional bodies and scholarly association. Interestingly, she was also a regular contributor in the Listener magazine in the 1940s and 50s. Dr Bell's lifelong commitment to learning how good nutrition affects health and her dedication to translating that knowledge into action are, in the light of history, ample testimony that she was worthy of the distinction of being the first woman awarded an MD by the University of Otago.

The title of this year's Muriel Bell lecture was "Nutrition Research – to specialise or not to specialise" and was a play on the wording of the conference theme "To eat or not to eat". All who choose a career in nutrition research will face at least once, if not more frequently, the decision to focus their lifelong research efforts on a single topic, or choose a diverse range of topics to which a common set of investigative skills can be applied. The former choice often enables individuals to generate and disseminate discoveries about a topic that, experience shows, evolve in their uniqueness and insightfulness only through decades of singular and tireless research. Such individuals tend to become recognised experts in that topic. The latter choice – a diversity of topics – has a captivating allure because the researcher is forever engaged in learning about and responding to new areas of knowledge and in acquiring a diverse set of approaches to research. The diverse approach can foster a broader understanding of nutritional sciences, however, diversity can lead to superficiality because there is less time to learn about and research each of the many topics.

Collaboration with other researchers fosters discovery whether one chooses a single or diverse research focus, but, one fact is certain, the diverse approach is all but impossible without collaboration.

The research career I have pursued involves a range of research topics, all of which are the outcome of collaboration with other scientists. The remainder of the Muriel Bell Lecture will touch on a few of these research topics and the knowledge that has been generated from them.

1. Can a meat-rich diet improve iron status in women with non-anaemic iron deficiency? A randomised controlled trial (Heath *et al.*, 2001).

2. A diet high in fruit and vegetables improves plasma concentrations of antioxidants but has not effect on plasma cholesterol concentrations (Zino *et al.*, 1997).

3. A diet high in vitamin E-rich foods has little effect on plasma vitamin E concentrations (McGavin *et al.*, 2001).

4. Tuatara in captivity are hyperlipidaemic and have lower proportions of plasma n-3 fatty acid in comparison with their counterparts in the wild (Cartland-Shaw *et al.*, 1998; Blair *et al.*, 2000).

5. Margarine consumption was a major predictor of trans fatty acids status in New Zealand before the advent of 'trans-free' margarines (Skeaff and Gowans, 2006).

6. Total and HDL cholesterol concentrations are in decline in New Zealanders (Skeaff *et al.*, 2001).

7. Red blood cell n-3 fatty acids are inversely associated with prostate cancer risk (Norrish *et al.*, 1999).

8. A once-a-week supplement of folic acid can increase blood folate concentrations to levels that are associated with the lowest risk of neural tube defects (Norsworthy *et al.*, 2004).

9. Homocysteine-lowering with B-vitamins does not improve cognition in older people. A randomised controlled trial (McMahon *et al.*, 2006).

10. Homocysteine-lowering with B-vitamins does not affect blood pressure, markers of bone turnover, of serum n-3 long chain polyunsaturated fatty acids (*Green et al.*, 2007; McMahon *et al.*, 2007; Crowe *et al.*, 2008).

11. The vitamin D status of New Zealand children and adults is lower than previously anticipated. The major predictors of serum 25 hydroxyvitamin D concentrations in children and adults were season, ethnicity, sex, and BMI (*Rockell et al.*, 2005; Rockell *et al.*, 2006).

12. The seasonal variation in serum 25 hydroxyvitamin D concentration in residents of Invercargill is accompanied by seasonal changes in serum parathyroid hormone concentrations (Rockell *et al.*, 2008).

13. Serum 25 hydroxyvitamin D concentrations are markedly higher in Fijian women living in Fiji than in Pacific People living in New Zealand (Heere in press).

So what is it that leads to good collaboration? The foundation of good collaboration is that the research is the outcome of "we" not "I". When two or more minds combine to enquire into the reality of things, it is an anathema to claim that an idea is owned by "me" or that the research is "mine".

Postscript: Prof Christine Thomson in thanking Prof Skeaff on behalf of the Nutrition Society reminded the audience that Dr Bell's own research interests were diverse.

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# Food Security: Current research initiatives, globally and in New Zealand

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#### **INTRODUCTION**

During the mid 1990s the condition of 'food security/food insecurity' was studied and described in developed countries, where previously it had been considered only in third world countries. The accepted internationally recognised definition, pertinent to developed countries, is that:

Food Security is achieved when there is access to nutritionally adequate and safe foods, and the assured ability to acquire personally acceptable foods in a socially acceptable way (LRSO, 1990).

Since then, a variety of studies have been undertaken in order to 'measure' food insecurity at a population level, to describe its effects on the lives and health of the food insecure and to find solutions to food insecurity in populations and among specific groups.

This paper will first present and discuss research on the prevalence of food insecurity, the health outcomes of food insecurity and research efforts to find solutions, i.e. to determine intervention strategies which might enhance food security. Secondly, some new data on the family food environment in New Zealand, relevant to food security, will be presented.

#### A. Food insecurity in New Zealand: Prevalence

The prevalence of household food insecurity in New Zealand was presented to the Nutrition Society in 2005 (Parnell *et al.*, 2005) using data derived from the 1997 National Adult Nutrition Survey, NNS97 (Russell *et al.*, 1999) and the 2002 National Children's Nutrition Survey, CNS02 (Parnell *et al.*, 2003). The indicator(s) used to define and assess food insecurity were developed specifically for New Zealand, using the same processes as those used in the US to derive the 'US Core Food Security Model'. Both the US and NZ models are based on the use of a series of food security indicator statements, each addressing a specific aspect of food insecurity, which are then 'ranked' in order of severity; 18 statements in the US, and 8 in NZ (Parnell, 2005). By having a household member respond to each statement on a 'list' of indicator statements it is then possible to assign them to a food security 'category'. The categories which have been developed for use in NZ are: fully/almost fully secure, moderately food secure, and low food security.

		Fully food secure	Moderate food security	Low food security
National Survey 1997	Nutrition	72%	24%	4%
Children's Survey 2002 <sup>*</sup>	Nutrition	50%	38%	12%

*Table 1: Household food security prevalence in New Zealand, by food security category* 

\*Households in 2002 all included children

#### Effects of food insecurity

Current food security research in the US has burgeoned in the epidemiological arena, but there has been little progress in exploring specific associations of self reported household food security status with actual food or nutrient intakes of a household member. This is probably because of the effort and complexity of collecting reliable data on foods consumed and deriving nutrient intakes. Nevertheless the US Core Food Security Model has now been employed in numerous population based studies to explore relationships between aspects of health and food security status (severity).

#### Food security status and adverse health outcomes

In Canada household 'food insufficiency' (one component of food security) has been associated with poorer adult health status across multiple dimensions of health including: self-reported heart disease, diabetes, high blood pressure and food allergies. Individuals in 'food insufficient' households appear to be more likely to rate their health as poor or fair, to have restricted activity, poor functional health, and to have major depression than those in food sufficient households (Vozoris and Tarasuk, 2003). Similar findings have emerged in a study within a disadvantaged region of the US (Stuff *et al.*, 2004).

One large US study, the Children's Sentinel Nutrition Assessment Project (C-SNAP) has explored the relationship between children's health status and their household food security status. Children in moderately food insecure households were 75 percent more likely to report their health as 'fair/poor' compared to children within food secure households (AOR 1.75, 95% CI, 1.48-2.02). Those in households designated as 'food insecure with hunger' were almost two and one-third times more likely to report their health as fair/poor (AOR 2.31, 95% CI 1.89-2.82) (Cook *et al.*, 2004). Several other studies have described associations between 'food insufficiency' (but not food insecurity) and child health. Associations with cognitive, academic and psychosocial development in school aged children, and suicide symptoms in adolescents, have been described (Alaimo *et al.*, 2001; Alaimo *et al.*, 2002). The authors of the above studies have been cited as 'evidence' that food insecurity will lead to obesity, diabetes and micronutrient deficiencies.

#### Food security status and its effect on body weight

There is continuing research and debate on the association/influence of food security status and body weight status with the major focus being on the prevalence or

absence of obesity. In adulthood food insecurity of a severe or prolonged nature is probably associated with underweight or at the very least, not associated with overweight (Olsen, 1999; Gulliford *et al.*, 2003). However a number of studies support the paradoxical association which has been found between overweight/obesity and moderate food insecurity in women (Olsen, 1999; Gulliford *et al.*, 2003; Tarasuk and Beaton, 1999; Kaiser *et al.*, 2004; Townsend *et al.*, 2001; Parnell, 2005). These cross sectional studies collectively do not support a clear conclusion of causality between food security status and overweight status. However there are several conceptually plausible explanations for the association, such as that food insecurity will result in a lower intake of healthy foods, and a greater intake of obesogenic foods. Households where restricted income contributes to food insecurity may have members who ration foods or omit particular foods from their diet. Another explanation is that food insecurity is a 'stressor' and stress has in itself been specifically linked to sub-optimal eating behaviours and hence obesity (Laitinen *et al.*, 2002).

Few studies have found any association between household food insecurity and overweight in children. New Zealand cross sectional data failed to find any association in the CNS02 (Parnell *et al.*, 2003). Casey *et al.* (2006) examined the National Health and Examination Survey (NHANES) 1999-2002 data set for food security status and body weight status among 3-17 year olds. They did find an association between level of household food insecurity and 'risk of overweight status' (BMI  $\geq 85^{\text{th}}$  percentile) but not for overweight (BMI  $\geq 95^{\text{th}}$  percentile) when they controlled for ethnicity, gender, age and family poverty index level.

Research teams working in the arena of the measurement of food security status and exploring the associated health outcomes including obesity, have concluded without fail that: food insecurity is an ongoing issue, and food insecurity needs to be alleviated. Their work is necessary to keep the 'condition' of food insecurity on health and economic agendas and also to track changes in rates of food insecurity over time.

#### Enhancing food security

Another body of public health research in New Zealand, 'solution-oriented research', is attempting to address the problem of food insecurity. The Health Research Council (HRC) together with the Ministry of Health, funded research to this end. A first step has been to examine the existing research literature nationally and internationally. Frameworks such as the ANGELO framework (Analysis Grid of Environments Leading to Obesity) have been applied to identify potential environmental influences on food security, both at the macro- and micro-levels (Clinical Trials Research Unit, 2008). Further to this, qualitative research (focus groups) was undertaken to identify factors to enhance food security, particularly among low income New Zealanders (Lanumata *et al.*, 2008).

The brief given by the funding bodies was to examine food insecurity together with physical inactivity and to determine if there are interactions between the factors affecting these two 'conditions' or states. It is not explicitly stated why or what assumptions lie behind the co-examination of these two conditions. One possible explanation is that both might be expected to contribute to overweight or obesity. The Report 'Enhancing Food Security and Physical Activity' (Clinical Trials Research Unit, 2008) summarised from the international literature, the physical, economic, sociocultural and political factors which can be considered to be associated with food insecurity. While this review is thorough, the predominance of studies included are from countries other than NZ. Careful interpretation is therefore required before concluding that any factors found to be associated with food insecurity in a particular economic and cultural context (e.g. US) can be transferred to another (e.g. NZ). Selected issues arising from this report are considered and discussed below.

#### Home or community gardens

Presently, no overseas or NZ studies have demonstrated a direct relationship between gardening (fruits and vegetables) and food security status. As one NZ study reports, environmental conditions on the West Coast of NZ are not always suitable for year round food production (Barry, 1997). Climatic conditions vary throughout NZ from sub-tropical to temperate, and few consumers choose to source all of their food (fruits and vegetables in particular) locally. Supermarkets are provisioned by transporting fresh foods from the regions where they grow best to the national market. Gardening requires suitable land, skills, tools and time investment, whether it is carried out by individuals or in community. There is a clearly enunciated point of view by NZ Maori concerning food security. This is that Maori have had strong spiritual and cultural connections to the land, waterways and coastal areas of NZ. Loss of access to land, and pollution of waterways have impinged on their ability to grow food and to generate income which in itself affects ability to afford healthy food (Te Hotu Manawa Maori, 2007).

#### Location of food outlets

Ready access to healthy foods in NZ depends primarily upon supermarket location(s) in relation to residential areas, and transport to reach them. It has been hypothesised that the location of 'fast food' outlets, both multi-national chains and local outlets, will have an influence on access to, and consumption of, less healthy foods. Studies using Geographic Information System (GIS) software have addressed this issue. Pearce *et al.* (2008a) examined the association between travel time to supermarkets and convenience stores and ability to meet the recommended number of daily serves of fruits and vegetables. Proximity to supermarkets and convenience stores did not relate to achieving recommended intakes of fruit, and proximity to supermarkets did not influence vegetable serves.

The research team also examined neighbourhood access to fast food outlets in relation to diet and body weight of local residents (Pearce *et al.*, 2008b). Residents furthest from multi-national outlets were more likely to eat the recommended number of serves of vegetables but also to be overweight. Access to local fast food outlets was not associated with achieving recommended serves of vegetables or fruit or with being overweight.

There are areas of NZ where location of supermarkets, and associated transport costs to reach them are considered to be less than ideal. This has been considered to affect the food security of residents on the sparsely populated West Coast of the South Island and in Northland (Barry, 1997; Garry, 2000). It therefore appears that access to healthy food is appropriate to achieve food security for most, but not all New Zealanders, particularly those living in geographically isolated semi-rural or rural areas.

#### Money available to spend on food

While there is a relationship between food security and household income, it is not direct. Some people living in 'poverty' are food secure while others well above the poverty line are food insecure. New Zealand is the only country where prevalence of food insecurity at the household level has been explored in national studies across the whole population regardless of income or poverty level (Russell *et al.*, 1999). Change in income (income volatility) has been demonstrated to impact on food security in the US (Gunderson and Gruber, 2001) but in NZ we have only anecdotal descriptions. If income from paid work ceases, e.g. through illness or redundancy, and this situation is sustained, the effects on food security are potentially greater than a sudden loss or reduction of income followed by re-entry into the paid work force. Income volatility is most likely to escalate in an economic recession, so it is a potentially important but unexamined cause of food insecurity in this country.

Household income normally covers expenses and debts. When housing costs (mortgage or rent) escalate, money available for food can be encroached upon. Money for food can be regarded as the only modifiable portion of income when other costs are fixed, and prioritising 'other' expenses above food has been noted particularly among Pacific households in New Zealand. Pacific households have also been described as a group where re-paying high interest debts may be more common than in other population sub-groups as they lack access to credit at reasonable interest rates (Cheer *et al.*, 2002).

It has been noted repeatedly that those dependent on government welfare benefit(s) are at risk of food insecurity and more likely to have to access charitable food banks (Uttley, 1997). Long-term welfare dependency is associated with poorer food choices (Parnell, 2005) and it must also be said that accessing food through food banks is a 'stop gap' measure and not one which is socially acceptable. Thus charitable food banks in NZ do not alleviate food insecurity although they provide food to households. They are not intended to be a long term solution to hunger, poverty or food insecurity.

#### Cultural considerations – including family composition

Both from the international and NZ literature it is clear that sole parent families and households with the most people experience the highest rates of food insecurity (Parnell *et al.*, 2003; Parnell, 2005). Sole parents are more likely to utilise food banks, indicating a lack of wider support systems available to them in the community. Both household size and number of children are associated with food insecurity (Russell *et al.*, 1999; Parnell *et al.*, 2003). Pacific households in NZ are more likely to include extended family members (Cheer *et al.*, 2002) and both the absolute number, and fluctuations in household size, might be expected to affect money available to be spent on food, and hence food security status.

While qualitative studies in NZ have linked 'eating alone' with food insecurity among the elderly (Quandt and Rao, 1999), quantitative evidence (Russell *et al.*, 1999) clearly demonstrates that older New Zealanders have significantly lower rates of food insecurity than those in their middle years. Elderly people living alone, compared to those living with others, may be more at risk of food insecurity but this hypothesis has not been adequately tested.

#### **Political/Policy issues**

There is indirect evidence that changes in government policies impinge on food security. For example, food banks in NZ monitor the numbers and characteristics of clients requiring their services (McPherson, 2006). They noted a marked increase in use alongside the government benefit cuts of the early 1990s and have commented on the increase in clients with mental health problems who have been de-institutionalised. Any labour market reforms, changes in GST, taxation reforms, housing market reforms have the potential to impinge on the prevalence of food insecurity. The mechanism is

simple. If these structural changes reduce household income and/or increase food prices, such economic constraint will affect a household's ability to purchase healthy food. A frequent response to this argument is that there is no guarantee that making more money available to a household will increase their food purchases and in particular increase the purchase and consumption of healthy foods. However one Canadian study (Ricciuto *et al.*, 2006) has demonstrated that an increase in income was associated with purchasing more food and that fruit and vegetables were most responsive to increases in income.

#### B. The family food environment and barriers to food security: Selected findings

A study of 136 NZ families, with children, in Dunedin and Wellington has recently been undertaken with the aim of describing environmental and behavioural factors with respect to access to food, food purchasing practices, meal planning and patterns (Smith *et al.*, 2009). It was designed to determine how these factors differ by socio-economic status and how they relate to food security. A lack of information about what New Zealanders actually 'do' in these areas prompted the research, recognising that many educative or public health initiatives had to be undertaken without consideration of baseline data.

#### Prevalence of food security and income

Overall 40% of the households experienced some food insecurity and 10% fell into the category 'low food security'. Food security occurred across all of the income categories (high, medium, low) but was most prevalent in the low income group.

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	То	otal	Hi	gh <sup>a</sup>	Med	ium <sup>a</sup>	Lo	w <sup>a</sup>
Food security status	n	% <sup>b</sup>	n	% <sup>a</sup>	n	% <sup>b</sup>	n	% <sup>b</sup>
Fully/almost food secure	82	60	54	84	23	68	5	13 <sup>d</sup>
Moderate food security	41	30	8	13	10	29	23	61 <sup>c</sup>
Low food security	13	10	2	3	1	3	10	26 <sup>c</sup>

Table 2: Food security status by household income group

<sup>a</sup> Annual household income: High >\$70,000, Medium \$30,000-69,999, Low <\$30,000

<sup>b</sup> Percentage within income group

<sup>c</sup> Low > Medium and High (p < 0.05, Fishers exact test)

<sup>d</sup> Low < Medium and High (p<0.05, Fishers exact test)

#### Use of 'ready-to-eat food'

The use of ready-to-eat foods is often viewed as the prerogative of either the poor or food insecure. This study examined the actual use of a range of ready-to-eat foods by income group and by household food security status. On average the families in the sample used these foods 13 times in a month and the High Income group slightly more at 14.4 times per month.

	Total	High <sup>a</sup>	Medium <sup>a</sup>	Low <sup>a</sup>
Chain restaurants	1.4	1.3	1.0	1.8
Fish and chip shops	1.6	1.6	1.3	2.0
Local Asian takeaways	0.9	1.0 <sup>b</sup>	0.5	1.0
Cafés	2.4	3.5 <sup>e</sup>	1.4	1.6
Restaurants	0.9	1.2 <sup>c</sup>	0.9	0.3
School canteens	2.6	2.3	1.0	4.4 <sup>d</sup>
Workplace café	1.4	1.3	2.6	0.5
Bakery	1.5	1.8	1.3	1.2
Total Ready-to-Eat	13.1	14.4 <sup>b</sup>	10.4	13.3

*Table 3: Mean number of occasions ready-to-eat food used in a month, by household income group* 

<sup>a</sup> Annual household income: High > \$70,000, Medium \$30,000-69,999, Low < \$30,000

<sup>b</sup> High > Medium (Wilcoxon rank sum test p<0.05)

<sup>c</sup> High > Low (Wilcoxon rank sum test p < 0.05)

<sup>d</sup> Low > Medium (Wilcoxon rank sum test p<0.05)

<sup>e</sup> High > Medium and Low (Wilcoxon rank sum test p<0.05)

Cafés, restaurants and local Asian takeaways were accessed more often by the High Income group, school canteens by the Low Income group. Similarly the less food secure households were more likely to use school canteens, but food security status was not otherwise related to frequency of use of ready-to-eat foods. The higher use of the school canteens among food insecure and low income groups can be explained by the low decile schools offering cheap and healthy lunch deals (e.g. smart lunches) in the areas sampled.

	Total	Fully/almost food secure	Low/moderately food secure
Chain restaurants	1.4	1.4	1.4
Fish and chip shops	1.6	1.5	1.9
Local Asian takeaways	0.9	0.9	0.9
Cafés	2.4	2.9	1.8
Restaurants	0.9	1.1	0.5
School canteens	2.6	1.8	3.8 <sup>a</sup>
Workplace café	1.4	1.8	0.8
Bakery	1.5	1.6	1.5

*Table 4: Mean number of occasions ready-to-eat food used in a month, by household food security status* 

<sup>a</sup>Low/moderately food secure > Food secure (Wilcoxon rank sum test p<0.05)

It appears that most NZ households use pre-prepared foods and that they will access them from the sources which are most affordable to them.

#### CONCLUSIONS

From the studies reviewed, including those qualitative, quantitative and descriptive, it is clear that many NZ households experience a degree of food insecurity. There is much to learn about the effects of the duration of this experience and whether or not circumstances can be mitigated to alleviate it. There can be no disagreement that economic factors have the most significant influence on the experience of food insecurity. Interventions which do not address the economic underpinnings are likely to be unsuccessful.

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#### Obesity: More complex than just a case of too much junk food

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#### ABSTRACT

This paper reviews the current research on the genetic and physiological mechanisms that regulate energy balance in the context of a case scenario, based on Mrs X, a middle-aged woman who has a long-standing obesity problem. The implications of our increasing understanding of the biology of energy homeostasis with respect to nutritional and public health promotion strategies for obesity and its related health issues will be discussed.

#### **INTRODUCTION**

The link between our current affluent lifestyle with increasing obesity, cardiovascular disease (CVD), type 2 diabetes (DM2) and the associated morbidity and mortality, has generated a large amount of research and public health interventions (Simopoulos, 2005; Baghurst, 2003). Overall, public health interventions have been in the form of simple dietary guidelines, 'anti-obesity' messages particularly in the form of TV advertisements and more recently encouragement of increased physical activity, but all, frustratingly, with limited success (Parsons *et al.*, 2005). Therefore the important challenge is to explore some of the reasons for this limited success.

Mrs X at 59 years of age has a BMI of 49 kg/m<sup>2</sup> which classifies her as clinically obese. She is a health professional and well aware of the health risks associated with her excess weight. She is also a woman living in a society where a slim body is perceived as an essential requirement for feminine beauty and fashion. Over the years she has tried most non-fad diets as well as various drugs in her attempts to lose weight. Instead there has been a relentless increase in weight. A simplistic understanding is that excess weight is merely a case of too many kilojoules consumed compared to those expended and hence an energy imbalance where weight loss can be readily achieved by addressing that imbalance. But is it as simple as that?

#### METHODS

Two interviews and a 16 day dietary dairy which was analysed using the New Zealand food database (Foodworks version 7, New Zealand).

#### RESULTS

#### Weight changes

Mrs X demonstrates a progressive weight increase over the years from an overweight child and young woman to a seriously obese adult (Table 1).

Birthweight	4.5kg
Age 12	69 kg, plump child who loved to read
Age 21	82 kg, BMI 28.5
	After birth of two children, avid walker,
	tramper, reader, lots of fasting as part of a
	spiritual journey
Age 35	98kg, BMI 33
Age 48	Injuries which significantly affected her
	mobility.
Age 59	136 kg, BMI 49

Table 1: Weight gain history for Mrs X from birth to 59 years of age.

#### Energy intake

According to the Nutrient Reference Values for Australia and New Zealand (Ministry of Health, 2005) the estimated energy requirements for women aged 51-70 years and very sedentary, as Mrs X, is 6.9 MJ. Though there are marked daily fluctuations (range 3.1 to 10.8 MJ/day), Mrs X's average daily energy intake is not excessive at 6.11 MJ/day (Figure 1). She consumed her usual diet over this period, which was consistent with no changes in her body weight during this time.

Figure 1: Average energy intake MJ/day.



#### DISCUSSION

Mrs X is not atypical in her life-long battle with obesity. This is apparent from the rising tide of obesity despite many public health messages (Simopoulos, 2005; Parsons *et al.*, 2005) as well as societal pressures for a slim body, especially for women. Clearly, many find it more difficult to achieve what is promoted as the ideal slim body especially in an obesogenic society hallmarked by abundant food and an increasing sedentary life style. Too often for some, attempts to lose weight leads to frustration, guilt and ultimately failure and a pre-occupation with food and dieting that may lead on to eating disorders (Carryer, 2001; Carryer and Penny, 2008).

Cheap, energy dense food in generous portions that is too easily accessible and a sedentary life style, in conjunction with socioeconomic factors such as food insecurity,

educational status and lack of nutritional understanding have been the subject of much research and certainly are important contributing factors to becoming obese. But the question remains, why, in any community there are some who find it easier to remain slim, despite sharing the same obesogenic environment, while others such as Mrs X, a health conscious, well informed professional, becomes increasingly obese. Insight into this is emerging from the newer experimental techniques in neuroscience and molecular biosciences. This new research provides a plausible biological basis for the concept of a body fat 'set point', a hypothesis formulated by Kennedy in the 1950s. This hypothesis challenged the widely held concept that losing weight was just a matter of the right diet and will-power. He proposed that the body had physiological mechanisms that regulated its fat stores and would defend these as a physiological response to calorie restriction. Much research, particularly in the last decade, has confirmed this early hypothesis (Broberger, 2005; Levin, 2007; Lopez et al., 2007; Farooqi et al., 2009). The initial breakthrough came with the classic experiments of Coleman (1973) on genetically obese mice that led on to identification of leptin and its role as an 'adiposity and appetite regulator'. These experiments established a clear physiological link between genetic predisposition, obesity and energy homeostasis. Energy homeostasis consists of a complex neural circuitry particularly in the hypothalamus as well as other parts of the brain and that interacts with peripheral tissues particularly the digestive system and adipose tissue and that, in addition to leptin, includes a large number of different signalling molecules. This system regulates eating behaviour to ensure adequate energy stores for survival as well as to meet different physiological and environmental energy demands such as for growth, pregnancy and lactation.

There is human data in support of similar energy homeostasis mechanisms operating at a subconscious level. Cases of human obesity linked with leptin or leptin receptor deficiency have been identified and confirm the existence of similar regulatory pathways in humans, but these are rare causes of human obesity (Farooqi *et al.*, 2009). Instead the common feature of human obesity is leptin resistance resulting in a blunted response to the anorexic action of leptin occurring alongside elevated blood leptin levels. Directly relevant to weight control is that fasting or calorie restriction causes a marked drop in blood leptin levels. The resulting decrease in its central anorexic action in conjunction with a reduction in the basal metabolic rate combine to defend body fat stores. This is also observed in many post-obese humans who, unless they markedly increase their energy expenditure by exercising, have a persistent reduction in their resting metabolic expenditure and an apparently irresistible drive to regain lost weight (Levin, 2007).

That some are more at risk of obesity than others even though both share the same obesogenic environment is also supported by research into the human genome. A significant number of large scale genetic linkage studies have confirmed earlier twin and population based studies and an estimated 30-80% of weight variation may be determined by genetic factors including ethnicity (Neel, 1962; Loos and Bouchard, 2003; Goulding *et al*, 2007).

Both in utero deprivation, especially in combination with very rapid 'catch-up growth' after birth as well as exposure to high levels of glucose in utero as a result of gestational diabetes have been linked with predisposition to obesity due to a permanent 'resetting' of the energy homeostasis mechanisms (Barker and Osmond, 1986; Vickers *et al.*, 2000; Gluckman and Hanson, 2004; Cottrell and Ozanne, 2007).

Existing alongside the energy homeostasis regulatory mechanism there is the hedonic system, that signals the pleasure derived from an enjoyable meal and includes

the endocannabinoid system, the opioid system and the dopamine reward system which is linked with craving and addiction and thus can exert a powerful drive (Lutter and Nestler, 2009).

Therefore obesity in people like Mrs X may arise from disturbances or resetting of the energy homeostasis pathways as a result of genetic or early environmental factors or because of the hedonic signalling pathway. Weight gain itself, occurring gradually over a period of time may cause a resetting of these mechanisms and an upwardly mobile body weight set point. However at any given point along this upwardly sloping weight gain curve, the current body weight is strongly defended, becoming self-perpetuating and effectively irreversible in the vast majority of individuals (Levin, 2007).

As health professionals the vital challenge is to incorporate this current understanding of the bioscience alongside our nutritional expertise to develop more effective strategies for dealing with obesity by putting it in the wider context of health promotion. Early intervention is important but what about people like Mrs X? After CVD and DM2, cancer is the next major cause of morbidity and mortality in many societies. An obesogenic diet is also likely to be low in vegetables, fruit and wholegrains all foods that have been established as protective dietary factors with regards to cancer risk. Therefore is the appropriate message for Mrs X dieting or healthy eating? The limited long-term success of many weight-reducing regimes is well documented (Levin, 2007). Appropriate targets for Mrs X, and other like her, should be health promoting in its widest context, realistic and do-able, affirming and empowering in an environment of understanding and support (Carryer and Penny, 2008). A focus on health eating, healthy action possible for all, what ever body size, rather than a narrow focus on BMI as the only relevant indicator of human health and wellbeing?

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#### Food, Families and Whānau: Understanding the Family Food Environment in New Zealand

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#### ABSTRACT

The family environment is an important influence on children's nutrition. The research described here provides rich insights into eating practices in New Zealand homes and the factors that influence these practices. The research shows that, while parents and caregivers were aware of key nutrition behaviours, healthy eating was often a low priority in comparison with other more pressing family concerns. Factors internal to families, such as having a supportive partner and avoiding conflict, and environmental factors, such as food advertising, availability of foods, time, money, and social pressures all had central roles in shaping families' eating behaviours.

#### **INTRODUCTION**

New Zealand has experienced a rapid rise in the rate of obesity in both adult and child populations. Findings from the 2006/07 New Zealand Healthy Survey (Ministry of Health, 2008) showed that one in five children were overweight and a further one in twelve were obese. As a part of the Healthy Eating, Healthy Action (HEHA): Oranga Kia – Oranga Pumau Strategy, the Health Sponsorship Council (HSC) was funded by the Ministry of Health to deliver a programme to contribute to preventing obesity and maintaining healthy weight by helping New Zealanders adopt and maintain healthy nutrition practices. The name given to the programme was *Feeding our Futures* and its focus was on supporting parents and caregivers to provide healthy diets for their children.

This programme employed a social ecological framework, which positions the child relative to multiple spheres of influence, all of which affect the child's consumption behaviours (see Figure 1). The Feeding our Futures programme focuses on the family environment as a key sphere of influence, while acknowledging the importance of the wider environment.

In 2007 the HSC commissioned two research projects to explore how health, and in particular, healthy eating, is viewed and dealt with in the family and whānau context in order to inform the development of the *Feeding our Futures* programme. In exploring the family/whānau context, individual factors influencing parents and caregivers were assessed. These included parents' awareness of the obesity issue, their knowledge and understanding of healthy eating, their concern around their child's diet and their motivation to prioritise healthy eating. These factors were identified from Social Cognitive Theory (Bandura, 1989) as determinants of behaviour change.

In exploring the family/whānau context, it also was critical to look at the barriers and enablers that surround healthy eating for families. These factors paint a picture of the day-to-day realities of families and set the scene in which eating practices occur. The findings from these two research projects are summarised in this paper.

#### Figure 1: Social ecological framework



#### **METHOD**

HSC commissioned two audience research projects in 2007: the *Social Marketing Audience Research* project (TNS, 2007 a and b) and the *New Zealand Children's Food and Drinks Survey* (National Research Bureau (NRB), 2008).

The Social Marketing Audience Research project was large-scale, qualitative research carried out by TNS New Zealand to explore health and healthy eating in the family/whānau context. The research included 12 focus groups with parents and caregivers, 18 family/whānau focus groups, 10 in-depth interviews with children, and 48 in-depth interviews with parents and caregivers.

The *New Zealand Children's Food and Drinks Survey* was a nation-wide, inhome survey of 1,133 parents and caregivers carried out by NRB to provide baseline information on children and families' consumption behaviours and information about the home environment.

Both research projects included Māori, Pacific and Asian peoples, and people of European and Other ethnicities (people of European ethnicity also are referred to as Pakeha) from a range of socio-economic backgrounds and locations in New Zealand. Māori and Pacific parents and caregivers, and parents and caregivers from low socio-economic backgrounds were over-represented in the samples selected for both projects because these groups are affected disproportionately by overweight and obesity, and morbidity and mortality associated with overweight and obesity, as well as nutrition-related risk factors. All three research reports, including full details of the methods, can be found at www.feedingourfutures.org.nz/research.

#### **RESULTS AND DISCUSSION**

#### Awareness, knowledge, and understanding

Parents and caregivers generally showed a high level of awareness of the obesity issue. Pacific families, in particular, were aware of the obesity issue and were often aware that they were being targeted as a population group in many obesity prevention initiatives. Most parents and caregivers also had a basic understanding of behaviours central to healthy eating for children. Sixty-six percent (66%) of parents and caregivers mentioned 'eating plenty of fruit and vegetables' as central to children eating and drinking healthily. Forty percent (40%), mentioned 'drinking plenty of water', 24% mentioned 'eating balanced meals/a variety of foods from all groups', 21% mentioned 'eating meat/red meat', and 15% mentioned 'eating healthy food not junk food'. Similar understandings of healthy eating emerged from the qualitative research. However, the qualitative findings highlighted the limitations of parents and caregivers' knowledge. Eating fruit and vegetables was seen as the pinnacle of healthy eating by many parents and caregivers, however, most were at a loss as to understand why this was necessary.

"I have no idea, no idea at all. All I know is that it's [eating vegetables] good for you." Pakeha Woman – Auckland

Although eating a balanced diet was associated with healthy eating in both research projects, the qualitative research revealed that 'balance' was poorly understood. Balance was commonly understood to mean eating healthy foods for part of the week to 'balance' out the consumption of unhealthier foods during the rest of the week. It is likely that the use of the term 'balance' in food industry marketing, along with weight and dieting information, has contributed to the erosion of the traditional meaning of a balanced diet, which refers to eating a variety of nutritious foods in appropriate proportions.

"I might be under a misconception, but I think that because we're eating veges I don't worry too much about what else we eat...its not like we're having meat pies seven days a week. I think as long as you balance it all out".

Pakeha Woman - Wairarapa

Limiting junk foods also was associated with healthy eating, however, understanding of what 'junk foods' were was limited to foods that were obviously high in sugar and fat, such as, biscuits and fried takeaway foods.

Nearly all parents and caregivers (92%) believed that children could get problems from not eating and drinking in healthy ways. However, parents tended to focus on the immediate and more obvious risks of unhealthy eating. Thirty-eight percent (38%) mentioned '*overweight/obesity*', 31% mentioned '*getting sick*' and 31% mentioned '*not having energy*' as likely outcomes for children of an unhealthy diet. 'Rules of thumb' for assessing children's health, found from the qualitative research, also demonstrated parents' focus on the immediate outcomes of diet rather than the long-term effects. Many parents believed that, if their child was not obviously overweight or was physically active and had enough energy, then they need not be concerned about the quality of their diet.

"Like there's a whole lot of it about childhood obesity and everything – well my kid's not fat, he's [a] skinny little runt." Pakeha Woman – Auckland

#### Prioritisation and concern

Ensuring that children had a nutritious diet was a low priority in many families. Furthermore, 63% of parents and caregivers worried 'hardly ever/never' or 'less often/once in a while' that their child was eating too many of the unhealthy kinds of foods and drinks. Health overall was not a major concern and tended to be superseded by more pressing issues such as lack of money and time, parenting concerns and struggles, and unexpected events like redundancy or pregnancy. For some families the emphasis was on providing affordable, filling food on a tight budget.

"Bills. If something is overdue or if I have missed a payment I think, 'how am I going to catch up that payment and what am I not going to pay this week in order for me to catch up for last week?" I think a lot about that." Pacific woman – Auckland

#### Barriers to healthy eating

In the qualitative research a number of factors, both internal and external to families, were identified as influencing families' eating behaviours. A lack of time was considered a major barrier to having children eating healthily by most families. Many parents found providing healthy meals to be a time- and energy-consuming task, and when these were lacking parents would turn to takeaways and convenience meals that were less nutritious. A lack of money was another major barrier for parents, as many perceived nutritious food to be more expensive and less filling than unhealthy food and drinks.

"It is more expensive to make your kids healthier [i.e. have them eat more healthily] and you get smaller quantities as well. Junk food – you get big bags of chips, and you get healthier food and its smaller quantities and it's more expensive." Pakeha Woman – Auckland

Some parents described food industry marketing which targeted children as undermining their efforts to provide a healthy diet for their family. Children were easily influenced by food advertising and would pester their parents, who often gave in to them in order to preserve household harmony. Children also would pester their parents to buy unhealthier items that were popular among their peers at school. Parents would often give in to these demands, as they did not want their children to feel left out at school.

> "...they want those particular breakfast things like 'Fruit Loop' or whatever they are called. They want those and they're all sugar-coated, and that's what we're fighting – the TV adverts". Pacific Man – Wellington

Confusion caused by multiple and often conflicting health messages also posed a barrier for some parents. Inconsistency in healthy eating messages built a perception among some parents that there was no definitive evidence that a healthy diet was beneficial. Conversely, messages coming from schools around children's diets were valued by some parents, as they were consistent with their attempts to enforce healthy eating behaviours in the home.

Easy access to unhealthy food from takeaway shops, dairies and service stations also was noted as obstructing parents' attempts to provide healthy diets for their children. A few participants also noted that unhealthy foods were more accessible in low-income areas. Cultural pressure also was noted as a barrier to eating healthily by some Pacific families as they felt unable to reject food in social situations.

Other factors in the home that influenced children's diets were having an unsupportive partner and prioritising household harmony over trying to get children to eat healthy foods. In particular, parents who worked long hours did not want conflict to impinge on the scarce amount of time they had with their family. Having an unsupportive partner also undermined some parents' good intentions or efforts to get children eating healthily.

> "I will dish it up... and say to him [my husband] 'how am I meant to educate these kids when they say 'dad doesn't have to'. So [my son] will leave his vegetables, which I find really bad." Pakeha Woman – Auckland

#### CONCLUSIONS

This research has reflected the realities for many families, for whom ensuring a healthy diet for their children is a challenging and time-intensive task. Although there was a high degree of awareness of the obesity issue and a basic understanding of healthy eating behaviours, many parents focused on the immediate, rather than longterm outcomes, of a healthy diet. Furthermore, for many parents, concern about diet was overridden by more pressing concerns around time, money and good parenting. The barriers to healthy eating highlighted by this research emphasise the importance of taking the wider social context into account in order to ensure that children have a healthy diet.

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# Industry making changes for the better, but don't tell the consumer

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#### ABSTRACT

**Background:** Consumers can be broadly classified into two types when making food choices. Active consumers are engaged in making positive lifestyle choices and are receptive to dietary messages. Passive consumers may have an awareness of diet and health, but they have greater priorities influencing their food purchase decisions. We have many passive consumers, and some of these can actually be unmotivated by nutrition messages.

**Objectives:** To develop and implement a sodium reduction programme that will enable both active and passive consumers to benefit from improved sodium levels in a broad range of foods.

**Design:** Our sodium reduction programme was established in 2005 and incremental improvements will be made for many years ahead. Criteria are specific to the many different food categories within the company, based on levels recommended by an external organisation. Existing products are considered as part of ongoing recipe review process. New products are made with careful regard to the sodium criteria. Sodium reduction is done whilst continuing to meet our food safety requirements and consumers' expectations for taste and value.

**Outcomes:** The programme is proving to be successful. A significant number of foods now have less sodium by 5% up to 35%. Although some flavour profiles prove to be more challenging.

**Conclusions:** Industry is well placed to deliver effective sodium reduction as they can maximise results working within their other constraints. Communicating such changes to consumers has not been a focus of our programme thus far as we know that consumers' perceptions of foods lowered in salt can be negative.

#### **INTRODUCTION**

Food industry (Industry) is one of many key stakeholder groups involved in the implementation of *Healthy Eating - Healthy Action (HEHA)*; the New Zealand Government's strategy on improving nutrition, increasing physical activity and reducing obesity. Industry is a crucial step from 'policy to plate' as it can use marketing and food development to help implement HEHA and impact on consumers' health.

Industry has intimate knowledge of key consumer drivers and needs, largely reflected through purchasing behaviour, but also through ongoing qualitative and quantitative research. Consumers have a right to access food that is safe, and have a strong drive for food that meets their expectations for taste. Other priorities usually include food that provides value for money, variety, good nutrition and is convenient.

Whilst qualitative research will usually highlight nutrition as an important priority for many consumers, this is often not reflected in actual purchasing behaviour. This is because the consumer, at the point of purchase makes their decision based on a greater priority, such as taste or price.

Consumers can be considered broadly as two types: passive and active. The active consumer is highly driven by health and places nutrition as a key priority when making food choices. These people will seek out healthier versions of foods they enjoy. A passive consumer may or may not be aware of the impact of diet on their health. If they are aware, they may not be motivated to make any dietary changes or may have greater priorities when they make food choices.

#### **METHODS**

To effectively implement responsibilities under HEHA, Heinz Wattie's NZ Ltd (Wattie's) has developed and implemented a sodium reduction programme that should enable both active and passive consumers to benefit from improved sodium levels in a broad range of foods. The programme is a long term strategy, with small improvements being made over many years.

Initially a pilot trial was done on several popular foods in the Wattie's range including; Wattie's Creamy Tomato Soup and Wattie's Creamy Chicken Soup. Samples were prepared using three different levels of reduced sodium: 3%, 5% and 10%. The results showed that consumer acceptability varied considerably and was dependant on the nature of the recipe. Consumers could accept reductions of around 5% in tomato soup, but could detect the reduction in chicken soup at 3% and it was considered unacceptable at 5%.

The programme was reviewed to allow different targets to be set for each category of foods. These sodium targets (guidelines) were set based on external criteria and all savoury foods marketed by Wattie's in New Zealand were individually assessed and classified into one of three groups: 1. Acceptable (at or below guideline), 2. Borderline (within 20% above guideline), or 3. High (greater than 20% above guideline).

The programme was launched in 2005 and the Marketing and Product Development teams were both educated on the importance of reducing sodium in diets of New Zealanders and the role that food industry can play in assisting this. All teams were given the assessed data sheets for their categories and also the new guidelines to consider when developing new recipes. Teams were encouraged to '*do the best they can*' within their constraints. The key constraint in this programme is meeting consumers' expectations for taste. Any new or revised recipe must be favourable in order to meet the needs of the passive consumer. They must also ensure foods always meet food safety requirements. A company decision was made that reductions will be achieved without the use of salt substitutes.

Every year a 'Sodium reduction review' is completed, charting progress and presenting the findings back to both Marketing and Product Development. This includes awards to encourage ongoing interest in the programme.

#### **RESULTS AND DISCUSSION**

Both the Marketing and the Product Development teams have been very supportive of the sodium programme since launch in 2005. Over the following years, they have worked hard to develop new recipes in line with the guidelines. When existing foods are being reviewed, the opportunity to assess sodium is included.

The initial reviews demonstrated the success in setting targets that are relevant to each different category, rather than fixed on a percentage across all categories. This flexibility prevents the risk of under-achieving in many foods. If a set percentage had been used, this would have been conservative (approx 3-5%) to manage the acceptability of recipes very sensitive to sodium, such as chicken soup. Instead, encouraging teams to 'do the best they can' allowed a review of the Dressings category to achieve up to 27% reduction in one single product. Since then some soups have achieved up to 35% reductions in some recipes. In other more sensitive recipes, reductions have been made at 3-5% and the intention is to continue to make these reductions regularly over many years, allowing the consumer to gradually adjust their taste preference for salty foods.

Having guidelines specific to different categories of foods has enhanced acceptability and willingness to implement the programme. For example; the guideline for soups (a comparatively large serving size) is 300mg/100g where as a dressing (comparatively small serving size) has a guideline of 750mg/100g.

Using these guidelines as part of new product development is also proving successful. It has shown to be easier to keep sodium levels lower in new recipes than adjust recipes for which consumers have already developed a taste expectation. In the most recent annual review, 86% of all new foods made in the year prior were in group one or two, (73% of these being at or below guideline).

In a range of new flavours in baked beans (the "Bean There" range), using the new guidelines encouraged lower sodium levels (between 290mg and 375mg/100g) compared to the original baked bean recipe (495mg/100g).

Due to the high total number of foods marketed by Wattie's in New Zealand significant changes in overall sodium levels in the company's range will be many years in the making. There are constraints in reaching the balance between reducing sodium and providing acceptable taste that consumers are willing to pay for.

Any change to a recipe, and development of a new recipe, must be trialled through the pilot plant before going to factory production. The pilot plant facilities are a constraint in themselves as running at full capacity, reviewing all foods in the higher sodium group would take several years.

The development time in reviewing recipes is a direct cost to Industry and one that cannot easily be passed on. Few consumers are willing to pay more for healthier products, particularly in the current economic climate. Salt itself is a low cost ingredient, and it has several functions in food including preservation and texture, as well as taste.

#### CONCLUSIONS

Industry is well placed to deliver effective sodium reduction as they can maximise results working within their other constraints, but require sufficient time in order to make gradual change. The most difficult constraint is ensuring the reduction of sodium does not compromise the taste of the food.

For genuine outcomes from government policy, the consumer must actually support the initiatives through purchase and consumption of 'healthier' foods. If these foods remain on supermarket shelves or end up in the rubbish bin, it is poorly implemented policy with no real benefit for consumer health.

#### Vegetarianism, vitamin B<sub>12</sub> status and insulin resistance in South Asian women

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#### ABSTRACT

**Background**: Asian Indians have been identified as an at risk group for vitamin  $B_{12}$  deficiency, due to their limited dietary intake. Additionally they have a high incidence of type 2 diabetes.

**Objectives**: To describe the vitamin  $B_{12}$  and folate status of women of South Asian origin living in Auckland, as part of the larger Surya study looking at health and lifestyle. To assess the relationship between vitamin  $B_{12}$  and vegetarian status with insulin resistance (IR) in this group.

**Design**: Cross-sectional study of 179 women, aged  $\geq 20$  years. Initial data collection included serum vitamin B<sub>12</sub>, serum folate, measures of IR, anthropometry, and dietary information.

**Outcomes**: A total of 135 women met inclusion criteria. Non-vegetarians (NV; 73%) had higher mean serum vitamin  $B_{12}$  levels at 257 pmol/L (95% CI 235, 281) than vegetarians (V; 27%) at 181 pmol/L (159, 206), p<0.001. Vitamin  $B_{12}$  deficiency (vitamin  $B_{12}$ <br/>states of NV. Serum folate did not differ between V and NV. No correlation was found between serum vitamin  $B_{12}$  and measures of IR. A significant correlation between being NV or V and HOMA2-IR disappeared after controlling for BMI.

**Conclusion**: This population has low vitamin  $B_{12}$  status, especially if vegetarian, but being vegetarian as an adult may have a protective effect against IR, perhaps due to lower BMI and waist circumference.

#### **INTRODUCTION**

Vitamin  $B_{12}$  deficiency worldwide may have significant public health consequences, including anaemia, neurological disease and birth defects (de Benoist, 2008), and additionally, evidence suggests that sub-clinical deficiency (asymptomatic patients) or inadequate long-term status could have detrimental health consequences (Carmel, 2008; Selhub *et al.*, 2008).

Asian Indians (persons originating from the sub-continent of India including India, Bangladesh, Pakistan and Sri Lanka), have long been identified as an at risk ethnic group for vitamin  $B_{12}$  deficiency, as their lifelong and multigenerational lacto-vegetarian dietary practices result in prolonged inadequate dietary intake of vitamin  $B_{12}$  (Carmel *et al.*, 2002; Yajnik *et al.*, 2006). In populations with low vitamin  $B_{12}$  intake, low vitamin  $B_{12}$  status contributes more to hyperhomocysteinaemia than folate status, by way of creating a functional folate deficiency (Yajnik *et al.*, 2006).

Asian Indians also have a high prevalence of insulin resistance (IR) compared to other ethnic groups, which is also associated with increased prevalence of type 2 diabetes and cardiovascular disease (CVD) (Chambers and Kooner, 2002; Chandalia *et al.*, 2003). Insulin resistance has been found to be associated with hyperhomocysteinaemia (Meigs *et al.*, 2001), and increasing evidence suggests

oxidative stress to be the link between elevated homocysteine and IR (Papatheodorou and Weiss, 2007; Park *et al.*, 2009).

This study aims to describe the vitamin  $B_{12}$  status of Asian Indian women in Auckland, and to assess the relationships between vitamin  $B_{12}$  and vegetarian status with IR in this group.

#### **METHODS**

#### Subjects and design

This was a cross-sectional study using a subset of 179 participants, recruited from the Surya study that investigated the health and lifestyle of women of South Asian origin (von Hurst *et al.*, 2008). The participants were a self-selected sample, aged  $\geq 20$  years, recruited throughout Auckland. Exclusion criteria included diabetes or any other major systemic disease.

Additional exclusion criteria for this investigation included the use of vitamin B supplements, certain medications (proton pump inhibitors), and serum vitamin  $B_{12}$  levels >800 pmol/L. Only 135 participants met the inclusion criteria.

Initial assessment included demographic and medical history, anthropometric measurements, 4-day food diaries with follow-up interviews (von Hurst *et al.*, 2008), and measures of IR using the homeostasis model assessment (HOMA) 2 computer model (Wallace *et al.*, 2004). Blood analysis for serum insulin, glucose, vitamin  $B_{12}$  and folate was carried out by LabPlus, Auckland.

Vegetarian or non-vegetarian status was defined by whether participants were meat-eaters or not, in-line with the only other New Zealand (NZ) study investigating vitamin  $B_{12}$  status in this population (Rush *et al.*, 2007).

#### Statistical analysis

Statistical analyses was performed using SPSS software (version 15; SPSS Inc, Chicago, IL). Variables were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed continuous variables are expressed as mean values  $\pm$  standard deviation, while non-normally distributed data was log-transformed, again tested for normality and if normally distributed they are expressed as geometric means and 95% confidence intervals. Comparisons between groups were performed using independent t-tests for parametric data. Correlations were performed using Pearson and Partial correlations for parametric data variables.

#### Ethics and funding

Ethical approval was granted by the Massey University Human Ethics Committee (Southern A), Reference No. 06/67, and written informed consent was obtained from all subjects. Funding for the Surya study was provided by a New Zealand Lottery Board Grant.

#### RESULTS

The main country of origin of most participants was India, and the majority (84%) were migrants to NZ in the last 10 years. Using the specific cut-offs for BMI and waist circumference for Asian Indians (International Diabetes Federation, 2005), 72.6% were overweight or obese, and 50.4% had central obesity. For those whose vegetarian status was available (n=124), 27% were vegetarian.

Table 1 reports the characteristics of participating subjects by vegetarian status. Significant differences were observed between the two groups for vitamin  $B_{12}$  levels t(122)=4.14, p<0.001; BMI t(122)=2.32, p=0.02; waist circumference t(122)=2.44, p=0.02 and HOMA2-IR t(122)=2.38, p=0.02. The vegetarian group had lower serum vitamin  $B_{12}$  levels, and higher prevalence of vitamin  $B_{12}$  deficiency and marginal deficiency, compared to the non-vegetarians. Serum folate levels did not differ significantly.

*Table 1: Comparison of anthropometric and metabolic variables in participants by vegetarian status*<sup>1</sup>

	Vegetarians (n=34)	Non-vegetarians (n=90)	p value <sup>2</sup>
Age (years)	$36.6 \pm 10.1$	$40.3\pm9.08$	0.056
BMI $(kg/m^2)$	23.9 (22.6, 25.3)	25.9 (25.0, 26.9)	0.022
Overweight/obese, $\geq 23 \text{ kg/m}^2 (\%)^3$	61.8	76.7	
Waist circumference (cm)	$75.8\pm9.88$	$81.0\pm10.6$	0.016
Central obesity, $\geq 80$ cm (%) <sup>3</sup>	35.3	53.3	
Vitamin $B_{12}$ (pmol/L)	181 (159, 207)	257 (235, 281)	< 0.001
Vitamin $B_{12}$ deficiency <150 pmol/L (%) <sup>4</sup>	23.5	8.9	
Marginal status 150-221 pmol/L (%) <sup>5</sup>	35.3	28.9	
Folate (nmol/L)	20.0 (17.5, 22.9)	18.5 (17.3, 19.9)	0.271
Folate deficiency, $<10 \text{ nmol/L} (\%)^4$	2.91	1.1	
HOMA2-IR	1.00 (0.83, 1.22)	1.30 (1.17, 1.46)	0.019

BMI; body mass index, HOMA2-IR; homeostasis model assessment model for insulin resistance

 $^{1}$  Values are mean  $\pm$  SD or geometric means (95% CI) and percentages

<sup>2</sup> Independent *t*- test, log transformed data used

<sup>3</sup> Specific for South Asian population (International Diabetes Federation, 2005)
 <sup>4</sup> WHO cut-offs for deficiency as determined by 2005 technical consultation (de Benoist, 2008)

<sup>5</sup> Proposed marginal status (Allen, 2009)

No correlation was found between  $B_{12}$  status and HOMA2-IR. Low vitamin  $B_{12}$  levels were significantly correlated with being vegetarian; r=-0.351, n=124, p<0.001, while higher HOMA2-IR was significantly correlated with being non-vegetarian, r=-0.211, n=124, p=0.019. This HOMA2-IR correlation disappeared after controlling for BMI.

#### DISCUSSION

This study confirmed widespread low serum vitamin  $B_{12}$  levels confirm Asian Indians as an at-risk group for low vitamin  $B_{12}$  status, particularly amongst vegetarians. Nearly 60% of the vegetarians had levels below the recommended cut-offs for deficiency (<150 pmol/L) and marginal status (150-221 pmol/L). The participants also showed high levels of central obesity (increased waist circumference),

overweight/obesity (increased BMI), and elevated HOMA2-IR. Folate deficiency was rare.

These findings of high levels of vitamin  $B_{12}$  deficiency are in line with those of other migrant studies (Carmel *et al.*, 2002; Chambers *et al.*, 2000; Chandalia *et al.*, 2003). The vitamin  $B_{12}$  levels in this current study are lower than in the only other study in Asian Indians in New Zealand, in 12 pre-adolescent girls (9 to 11 yrs) that reported mean levels of 543 and 232pmol/L in meat-eaters and non-meat-eaters, respectively (Rush *et al.*, 2007). This difference is not unexpected as serum vitamin  $B_{12}$  appears to decrease with increasing age (Selhub *et al.*, 2008).

There was a correlation between vitamin  $B_{12}$  levels and vegetarian status, reflecting animal-based products as the primary dietary source of this vitamin. However, almost 40% of non-vegetarians still had levels below both cut-off values. Previous studies have noted that, even amongst non-vegetarians, intake of animal products is frequently low (Refsum *et al.*, 2001; Yajnik *et al.*, 2006).

In the present study, no relationship was found between vitamin  $B_{12}$  levels and IR. There was a relationship between IR and vegetarianism. However, weight status overshadowed this relationship, with non-vegetarians showing higher levels of central obesity and incidence of overweight/obesity than vegetarians. Increasing adiposity is linearly and inversely related to IR (Abate *et al.*, 1995; Karter *et al.*, 1996).

Although, vitamin  $B_{12}$  deficiency was not associated with insulin resistance in this group, low vitamin  $B_{12}$  and an imbalance with folate levels should be monitored in this population group, as they could potentially be associated with other adverse effects. A recent commentary, reviewing 10 years of fortification in the US and Canada, noted issues such as epigenetic effects and increased cognitive decline in the elderly with low vitamin  $B_{12}$  and high folate levels (Smith *et al.*, 2008).

In conclusion, as has been seen in other countries, the vitamin  $B_{12}$  status of this migrant group in New Zealand is low, especially if they are vegetarian. While no relationship between vitamin  $B_{12}$  and IR was seen, the high levels of adiposity in this group are likely to overshadow any other contributing factor to IR. In fact, being vegetarian as an adult, while potentially leading to low vitamin  $B_{12}$ , status may have a protective effect against IR, due to lower BMI and waist circumference.

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# Bone density, calcium intake and vitamin D status in South Asian women living in Auckland, New Zealand

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## ABSTRACT

The aim of this study was to investigate the bone health and associated risk factors of a group of South Asian women living in New Zealand. Studies on the Indian sub-continent suggest a high incidence of low bone mineral density (BMD) in women with poor vitamin D status and low dietary calcium. Subjects were women of South Asian origin (n=91) living in Auckland, New Zealand. They completed a 4-day food diary, provided a blood sample and BMD was measured using dual X-ray densitometry. Mean age of premenopausal (n=71) and postmenopausal (n=20) women was  $39.8 \pm 7.8$ and 55.3  $\pm$  5.4 years respectively. Osteoporosis (T-score  $\leq$  -2.5) was present in 32% of postmenopausal and 3% of premenopausal subjects, but only in the lumbar spine. Adequate 25(OH)D levels (>50 nmol/L) were found in only 22% of premenopausal, and 26% of postmenopausal women. Women <30 years appeared at increased risk of osteoporosis, with 30% incidence of osteopenia and median serum 25(OH)D3 of 20(18, 42) nmol/L. The high incidence of osteoporosis in the postmenopausal group could be associated with the early age of oophorectomy or menopause together with low vitamin D status. There is an urgent need for further research to establish the level of osteoporosis risk in young South Asian women.

## **INTRODUCTION**

Although many aspects of diet and lifestyle impact on bone, the three key environmental factors which determine the fate of the skeleton are dietary calcium, vitamin D and physical activity (Layne *et al.*, 1999; Nieves, 2005; Vieth, 2005; Hind *et al.*, 2007; Sakuma, *et al.*, 2007).

We have previously reported low vitamin D status in South Asian women living in New Zealand. Of 235 women tested for serum 25(OH)D during 2007, 84% had concentrations less than the currently accepted adequate level of 50 nmol/L (Working Group of the Australian and New Zealand Bone and Mineral Society *et al.*, 2005), and 43% had concentrations less than 25 nmol/L (Von Hurst *et al.*, 2007). The implications of this low vitamin D status for osteoporosis in this population group are unknown. However, serum concentrations of 25(OH)D $\leq$ 25 nmol/L are associated with reduced bone mineral density and increased risk of fracture (Working Group of the Australian and New Zealand Bone and Mineral Society *et al.*, 2005), and calcium absorption is reduced when serum 25(OH)D is less than 80 nmol/L (Heaney *et al.*, 2003). We therefore hypothesised that South Asian women living in New Zealand are at risk of osteoporosis and osteoporotic fracture as they age.

In New Zealand, the South Asian population is expanding rapidly due, mainly, to immigration from India (Statistics New Zealand, 2006). As in most other developed countries, the population in general is ageing and osteoporosis, a disease of older age, will increase in incidence as will the associated health costs.

#### METHODS

This bone mineral density study was a sub-study of the Surya Study (Von Hurst *et al.*, 2008). The Surya Study aimed to investigate the effect of vitamin D supplementation on insulin resistance in women 20 years and over, who were vitamin D deficient and insulin resistant. To find participants for the trial, 249 women of South Asian origin were screened. Invitations to participate in the bone mineral density study were extended to all subjects who participated in the screening phase of the Surya Study; 91 women volunteered.

Volunteers for the Surya Study were excluded if suffering from significant renal dysfunction, major systemic illness, or diabetes requiring medication. Use of vitamin D supplements exceeding 1000 IU/day (i.e. prescription dose), or any form of calcitriol  $(1, 25(OH)_2D_3)$  were also exclusion criteria.

The women were weighed, waist and hip circumference and height were measured and fasting blood samples taken for the analysis of serum 25(OH)D and parathyroid hormone (PTH). Ethnicity was confirmed with a questionnaire which established country of birth for subject, her parents and all grandparents. Demographic information, medical history, nutritional supplement and medication use was obtained by interviewer-based questionnaires. Methods, including biochemical assays, are described in greater detail elsewhere (Von Hurst *et al.*, 2008). Subjects were recruited and tested over a period of nine months, from February to November, and some seasonal variation in vitamin D status was expected. Participants were also requested to complete a 4 day food diary which was then followed up with an interview conducted by a dietitian to probe and confirm specific aspects of the diet including calcium-containing foods. Women were defined as postmenopausal if 12 months or more had elapsed since they last menstruated.

Bone densitometry was measured at 2 sites, lumbar spine (L1–L4) and right total hip (Hologic QDR 4000, Hologic, Waltham, Mass.); daily quality control scans were performed on the scanner and the co-efficient of variation ranged from 0.37–0.45% during the study. Individual results were compared with the manufacturer's normative data base for a Caucasian population, age and gender matched. World Health Organization standards were used for reporting osteopenia and osteoporosis. A T-score of  $\leq$ -2.5 is diagnosed as osteoporotic, between -1.0 and -2.4 diagnosed as osteopenia, and >-1.0 regarded as normal (World Health Organization, 1994).

## RESULTS

Physical characteristics, calcium intake, biochemical measurements and bone mineral density of the 91 women who elected to participate in the bone mineral density study are shown in Tables 1 and 2. BMD at both sites was significantly lower in the post-menopausal women; lumbar spine (p<0.001) and hip (p=0.005). There was no significant difference between the groups in height, BMI, serum vitamin D, parathyroid hormone (PTH) or dietary calcium intake.

Osteoporosis was present in 3% of the premenopausal, and 32% of the postmenopausal women, but only in the lumbar spine. A further 42% of the postmenopausal women had osteopenic T-scores in both lumbar spine and total hip. In the premenopausal group incidence of osteopenia was 40% in the lumbar spine and 32% in the total hip.

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	Pre-menopausal	Post-menopausal
	n=71	n=20
Age (years)	$39.8 \pm 7.8$	$55.3 \pm 5.4*$
Height (cm)	$158 \pm 6.5$	$155 \pm 5.8$
$BMI (kg/m^2)$	$25.6\pm4.6$	$26.1\pm3.9$
Years in New Zealand	6 (4, 9)	17 (6, 37)**
Calcium intake (mg/day) (n=51 & 17)	832 (638, 1029)	721 (528, 992)
25(OH)D (nmol/L)	32 (21, 49)	39 (29, 57)
Adequacy >50 nmol/L	22%	26%
PTH (pm/L) <i>Ref. range</i> 1.7 – 7.3	$4.7 \pm 1.6$	$4.9 \pm 2.0$
Number of subjects reporting:		
HRT or oestrogen-based birth control use	1	1
Family history of osteoporosis	8	1
Calcium supplement use	7	6
Vitamin D supplement use (<1000 IU/day)	8	8

Results are given as mean  $\pm$  standard deviation or median (25<sup>th</sup>, 75<sup>th</sup> percentiles). \*p<0.001, \*\*p=0.001. There was no significant difference between the groups in any variable except age and number of years in New Zealand. Abbreviations: BMI – body mass index, PTH – parathyroid hormone, HRT – hormone replacement therapy

Ages in the postmenopausal group ranged from 40 to 63 years. The median number of years since the last period was 5 (4, 18), and ranged from 1 to 28 years. The mean age at which menopause or hysterectomy occurred was  $44.2 \pm 6.0$  years.

Twelve women were taking calcium supplements ranging from 500 mg to 1000 mg per day, and 16 women reported use of some form of vitamin D supplementation including cod liver oil and multivitamins. The dose available in the supplements reported ranged from 1.2  $\mu$ g (48 IU) in cod-liver oil capsules, to 10  $\mu$ g (400 IU) in multi-vitamins.

Table 2: B	sone scan	results	stratified	by	meno	pausal	status
			./	~ ~			

	Premenopausal	Postmenopausal	Significance of
			difference between
			groups
Total hip BMD (g/cr	$n^2$ ) 0.89 ± 0.11	$0.81\pm0.09$	t(89)=2.873,
			p=0.005
T-sco	ore $-0.43 \pm 0.95$	$-1.06 \pm -0.74$	t(89)=2.734,
			p=0.008
Z-sco	ore $-0.200 \pm 0.97$	$-0.33 \pm 0.64$	
Lumbar 1- 4 Bl	MD $0.99 \pm 0.15$	$0.85 \pm 0.13$	t(89)=4.013,
$(g/cm^2)$			p<0.001
T-sco	ore $-0.49 \pm 1.28$	$-1.78 \pm 1.22$	t(89)=4.171,
			p<0.001
Z-sco	ore $-0.18 \pm 1.28$	$-0.66 \pm 1.13$	•

Results are given as mean  $\pm$  standard deviation. Abbreviations: BMD – bone mineral density. Z-scores are age and gender based, so difference between groups is not meaningful.

Vitamin D levels increased slightly with age (r=0.238, p=0.02), and a moderate, inverse relationship was found between serum 25(OH)D and PTH (r=-0.257, p=0.02). There was also a correlation between BMI and total hip BMD (r=0.301, p=0.004) and T-score (r=0.312, p=0.003), but no relationship could be found between BMI and lumbar BMD or T-score.

In the premenopausal group there was no difference between total hip and lumbar T-score (p=0.53), however, in the postmenopausal women the lumbar T-score was significantly lower than the total hip T-score (p=0.005).

The premenopausal group included 10 women between the ages of 20 and 29 years. When compared to the other premenopausal women, their mean BMD was not significantly different but prevalence of osteopenia was 30% in both sites, BMI was significantly lower t(69)=-2.06, p=0.04, and there was a trend towards lower serum 25(OH)D and dietary calcium intake (Table 3), but differences were not statistically significant.

Age (years)		$24.9 \pm 3.5$
Height (cm)		$161.6\pm5.3$
BMI (kg/m <sup>2</sup> )		$22.9 \pm 4.0$
Years in New Zea	aland	5 (3, 10)
Serum 25(OH)D	(nmol/L)	20.0 (18, 42)
Calcium (mg/day	r)	550 (512, 715)
PTH (pmol/L)		$4.4 \pm 1.7$
Total hip	BMD $(g/cm^2)$	$0.864 \pm 0.009$
Total hip	T-score	$-0.75 \pm 0.91$
	T-score Range	0.30 to -2.40
	Z-score	$-0.71 \pm 0.87$
Lumbar 1-4	BMD $(g/cm^2)$	$0.965 \pm 0.014$
Lumbar 1-4	T-score	$-0.60 \pm 0.81$
	T-score Range	0.70 to -2.10
	Z-score	$-0.56 \pm 0.80$
Osteopenia preva	lence	30%

Table 3: Characteristics of a subgroup of young women (n=10) 20 to 29 years of age

Results are given as mean  $\pm$  standard deviation or median (25<sup>th</sup>, 75<sup>th</sup> percentiles) Abbreviations: BMI – body mass index, PTH – parathyroid hormone.

## DISCUSSION

The 32% prevalence of osteoporosis in the postmenopausal women is cause for concern given that the mean age of this group is 55.3 years. A recently published study of bone health in Indian males (n=683) and females (n=858) aged 5–70 years from Pune, India, reported osteoporotic lumbar spine T-scores (<-2.5) in 16.9% of males and 22.4% of females in the 50–59 age group. These proportions increased in the next decade (60 to 69 years) to 23.9% (males) and 31.7% (females) (Kadam *et al.*, 2009). The post-menopausal group in the Surya Study had a mean age of 55.3  $\pm$  5.4 years, and

32% were osteoporotic in the lumbar spine, suggesting that they are at higher risk than their contemporaries in India. The Pune study di not report vitamin D status.

Socio-economic status appears to be an important determinant of bone health in India, and is closely associated with nutritional status (Shatrugna *et al.*, 2005). The women in the present study were mostly well-educated and were relatively new migrants.

Dietary calcium intake was below the current Australia/New Zealand recommended daily intake (RDI) (*Commonwealth Department of Health and Ageing et al.*, 2006), but higher than has been reported in Indian women in India (Shatrugna *et al.*, 2005), and similar to the average intake of the New Zealand adult female population (Russell *et al.*, 1999). Calcium supplementation, reported by 14% of participants, was often taken only sporadically and was not included in the 4-day food diaries. Food sources of dietary calcium were identified in a subset (n=102) of the Surya Study participants: milk contributed 29%, curry dishes (containing cream and yoghurt) 15%, and yoghurt 8% of calcium in the diets of these women (Tsai, 2008). The high contribution of dairy products to calcium intake reflects that of the general New Zealand population (Russell *et al.*, 1999), and is not dissimilar to that seen in upper socio-economic groups in India (Ganpule *et al.*, 2006; Puri *et al.*, 2008).

Dietary calcium absorption appears to increase proportionally with increasing vitamin D concentration up to a threshold around 80-90 nmol/L serum 25(OH)D (Heaney *et al.*, 2003). Similarly, an inverse relationship occurs between serum vitamin D and PTH, but the threshold for decreasing PTH may be as high as 100–110 nmol/L serum 25(OH)D (Dawson-Hughes *et al.*, 1997). None of the women in this study achieved serum 25(OH)D concentrations of 80 nmol/L, in fact, the median levels were well below the currently recommended minimum of 50 nmol/L (Working Group of the Australian and New Zealand Bone and Mineral Society *et al.*, 2005). Therefore it is likely that bone health in these women is compromised more by their poor vitamin D status, than by dietary calcium intake.

The results for the small group of women (n=10) aged 20-29 years, highlight the need for further investigation of bone health in young South Asian women in New Zealand. This is the age that peak bone mass should be achieved, but T-scores were low, and 30% were osteopenic at both sites. One 26-year old had a total hip T-score of -2.40. Z-scores, which are age and gender matched, indicate that they are already falling behind the mean for the Caucasian reference group.

Median serum 25(OH)D was very low in this group at 20 (18, 42) nmol/L, although not significantly different to the rest of the women, possibly due to the small numbers. The little information available about the bone health of other South Asian women in this age group suggests that BMD is strongly linked to vitamin D status (Alekel *et al.*, 1999; Tandon *et al.*, 2003).

## CONCLUSIONS

The findings of this study are constrained by the small numbers, and the wide range of ages of participants. They do, however, indicate that the bone health of South Asian women living in New Zealand is more likely to be adversely affected by low vitamin D status than inadequate dietary calcium intake, especially once the protective effect of oestrogen is lost after menopause. There was a high proportion of osteoporosis in the postmenopausal group given their relatively young age, possibly related to oestrogen loss at an early age. Thus, the combination of early menopausal age or oophorectomy, and very low vitamin D status is of major concern in this population group.

From the very small sample of younger women in this study, it appears that young South Asian women living in New Zealand could be at high risk of poor bone health and subsequent osteoporosis. Further investigation is needed in this sub-group to establish a clearer picture of their skeletal health, together with their behaviours and attitudes with regard to calcium consumption, sun exposure and physical activity. Such findings could be of value to other countries with temperate climates and burgeoning numbers of South Asian immigrants.

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# Effect of low dose iron supplementation during pregnancy on maternal iron status

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## ABSTRACT

**Background:** Iron deficiency during pregnancy is associated with poor birth outcome for mother and infant. Treatment of women with iron deficiency is usually with high dose iron supplements which are often poorly tolerated by pregnant women.

**Objective:** To investigate the effect of low dose iron supplementation on maternal iron status in a low income, ethnically diverse population in East London, UK.

**Design:** Women were recruited at their first antenatal (booking) appointment. Venous blood was obtained at booking (n=390) and 34 weeks of gestation (n=311). Participants took either a multiple-micronutrient supplement (containing 20 mg iron) or placebo daily. Haemoglobin (Hb) and haematocrit (PCV) were measured in EDTA-stabilised whole blood using a Coulter STKS analyser. Serum ferritin (SF) was determined separately using immunometric assay (n=305, 97).

**Outcomes:** At booking 13% of participants had anaemia (Hb<11 g/dL), 16% low haematocrit (PCV<0.330 L/L) and 11% low iron stores (SF<15  $\mu$ g/L). By 34 weeks of gestation participants receiving treatment had higher mean concentrations of Hb (11.3 *vs.* 10.9 g/dL; p=0.002), PCV (0.338 *vs.* 0.330 L/L; p=0.007) and SF (15.8 *vs.* 10.4  $\mu$ g/L; p=0.002); also the increase in the proportion of women with low iron status was reduced (36 *vs.* 55% low Hb, 42 *vs.* 53% low PCV and 44 *vs.* 71% low iron stores) compared to controls.

**Conclusions:** This study adds weight to the argument that treatment with low dose iron supplements may be preferable to using high doses in improving iron status during pregnancy.

## **INTRODUCTION**

Iron requirements are increased in pregnancy for both maternal and fetal tissues; this is partly offset by cessation of menstruation, increased absorption and mobilisation of iron stores. Maternal blood volume expands during pregnancy due to an increase in both plasma and red cell mass; however, these do not increase equally resulting in a fall in haemoglobin concentration. This physiological anaemia of pregnancy, known as haemodilution, complicates the assessment of iron status.

The WHO defines anaemia in pregnancy as haemoglobin <11 g/dL (WHO/UNICEF/UNU, 2001), although this takes haemodilution into account there is debate as to whether this level should be lower (Steer *et al.*, 1995). During pregnancy haemodilution has the same effect on haematocrit as haemoglobin; a haematocrit value below 0.33 L/L is considered anaemic (Centers for Disease Control and Prevention, 1989). Serum ferritin reflects the amount of iron stored in the body; concentrations below 15 µg/L indicate low iron stores. However, serum ferritin is an acute phase reactant therefore levels are increased by infection or inflammation, thus high levels

must be interpreted with caution (Eskeland *et al.*, 2002). Moderate anaemia is associated with adverse pregnancy outcomes such as Low Birth Weight (LBW) (Rasmussen, 2001) and preterm birth (Scanlon *et al.*, 2000). Conversely, high maternal haemoglobin is also associated with poor obstetric outcomes, although this association is due to conditions such as hypertension and smoking which have adverse effects on pregnancy and also elevate haemoglobin (Yip, 2000). Iron needs in pregnancy can only be met if a woman has good iron stores before conception, if not supplementation will be required to prevent anaemia (Milman, 2006).

Prophylactic iron supplementation in pregnancy is common practice in some affluent countries. Although iron supplementation cannot increase haemoglobin higher than the optimal concentration needed for oxygen delivery, there are concerns for women with iron loading diseases such as haemochromatosis receiving excess iron (Yip, 2000). Also patients often show poor compliance with high dose iron supplements; thought to be a result of gastric side effects such as heartburn, nausea, vomiting, constipation and diarrhoea (Beard, 2000). The aim of this study was to investigate the effect of a daily multiple micronutrient supplement containing a low dose of iron (20 mg) on iron status during pregnancy in a low income, ethnically diverse population.

## **METHODS**

Between June 2002 and May 2004 women were recruited at their booking (first) appointment from hospital and community antenatal clinics in East London, UK. Volunteers were aged 16 years or older with a singleton pregnancy in the first trimester of pregnancy (<13 weeks of gestation). Women with chronic disease or taking vitamin or mineral supplements (excluding folic acid or iron) were excluded. Participants were recruited into a placebo controlled, double blind, randomised controlled trial and given either a multiple-micronutrient supplement (containing 20 mg iron) or placebo. Ethical approval was obtained from the East London and the City Health Authority Research Ethics Committee. Informed, written consent was obtained from all participants; non English speaking women were recruited if a suitable advocate was available. Gestational age at recruitment was determined by a qualified technician using ultrasound scan and after delivery birth details were obtained from the obstetric notes.

Blood was collected from the antecubital vein into a lithium-heparin bottle. Full blood counts were determined on samples of EDTA-stabilised whole blood using a Coulter STKS analyser and included haemoglobin and haematocrit (PCV) measurements. Serum ferritin was measured using a DPC Immulite 2000 analyser, a fully automated immunoassay analyser. All analysis was carried out at the Homerton Hospital. Anaemia was defined as haemoglobin <11g/dL, low PCV defined as <0.330L/L and low serum ferritin defined as <15 $\mu$ g/L.

All data was entered into SPSS (Statistics Package for the Social Sciences) for windows version 15 (Chicago, USA) and this was used for data analysis. Normality of data was assessed using the Kolmogorov-Smirnoff test and visual inspection of Q-Q plots. Data that was normally distributed was analysed using parametric tests, i.e. independent t test (2-tailed) and one way Analysis of Variance (ANOVA). If one-way ANOVA detected a significant difference, this was further explored using *post hoc* analysis using the conservative Scheffe test. If data was not normally distributed a transformation was attempted (e.g. by log<sub>10</sub>) to achieve normality, if this was possible then the transformed data was analysed using parametric tests.

#### **RESULTS**

Four hundred and two women agreed to participate in the study, however only 161 completed the study and blood samples were not available for all participants at all visits. Maternal age ranged from 16-42 years with a mean of 28 years. The median length of gestation at recruitment was  $11^{+5}$ , with a range from  $5^{+0}$  to  $17^{+6}$  weeks (subsequent to recruitment 13 women were found to have a gestation greater than 13 weeks).

Participants reported over 50 different ethnicities, which were reduced to five ethnic groups: African, Asian, Caucasian, West Indian and Other. African was defined as women either born in or who provided a Sub-Saharan African country as their ethnic origin. Asian comprised women predominantly from the Indian subcontinent, but also a small number of women from China and South East Asia. Caucasian women were predominantly of European descent but also included a small number of women from North Africa and the Middle East. West Indian women were of originally of Sub-Saharan African descent who had lived in the West Indies or were descendents of such. Others were predominantly women of mixed race, or who did not fit into the categories above, this included a small number of women who described themselves as "Black British".

At recruitment 13% of participants were defined as anaemic (haemoglobin <11g/dL), 16% low haematocrit (PCV<0.330 L/L) and 11% had low iron stores (serum ferritin <15  $\mu$ g/L). There were no significant differences for any of the biochemical measures at booking by treatment group allocation. However, by 34 weeks of gestation participants receiving the treatment had significantly higher mean haemoglobin (11.3 *vs.* 10.9 g/dl; p=0.002), PCV (0.338 *vs.* 0.330; p=0.007) and serum ferritin (15.8 *vs.* 10.4  $\mu$ g/L; p=0.002) concentrations compared to those receiving placebo (all independent t-tests). The proportion of women with low iron status by 34 weeks of gestation had increased for both groups since booking, however, the proportion was less for those receiving the treatment compared to controls; 36 *vs.* 55% with low haemoglobin, 42 *vs.* 53% with low PCV and 44 *vs.* 71% with low iron stores.

There were no significant ethnic differences at recruitment regarding mean serum ferritin (ANOVA), however there were significant ethnic differences regarding haemoglobin and PCV concentrations (p<0.001 for both; ANOVA). *Post hoc* analysis (Scheffe) showed African women had lower mean haemoglobin and PCV concentrations than both Caucasians (p=0.001, p=0.006 respectively) and Asians (p=0.003, p=0.006; respectively). Conversely, by 34 weeks of gestation there were no differences in mean haemoglobin or PCV concentrations by ethnicity (ANOVA). However, there were disparities in serum ferritin levels by ethnicity (p<0.001; ANOVA); both West Indians and Africans had significantly higher mean serum ferritin concentrations than the Caucasians (p=0.004 and p=0.013, respectively; Scheffe).

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			Ethnicity	¥		Treatme	nt Group	Whole Group
	African	Asian	Caucasian	West Indian	Other	Treatment	Placebo	
Haemoglobin								
Booking	106	42	153	63	26	201	189	390
Mean nmol/l (SD)	11.7 (1.0)	12.4 (1.0)	12.2 (0.9)	11.9 (1.0)	12.1 (1.1)	12.0 (1.0)	12.0 (1.0)	12.0 (1.0)
n<11g/dL (%)	20 (19)	1 (2)	11 (7)	12 (19)	5 (19)	26 (13)	23 (12)	49 (13)
34 weeks n	80	34	121	55	21	159	152	311
Mean nmol/l (SD)	11.0 (1.0)	11.1 (1.1)	11.2 (1.2)	10.9 (1.1)	11.1 (1.2)	11.3 (1.2)	10.9 (1.0)	11.1 (1.1)
n<11 g/dL (%)	37 (46)	13 (38)	52 (43)	28 (51)	10 (48)	57 (36)	83 (55)	140 (45)
PCV								
Booking n	106	42	153	63	26	201	189	390
Mean L/L (SD)	0.347(0.030)	0.367(0.029)	0.361(0.027)	0.355(0.028)	0.360(0.035)	0.357(0.029)	0.357(0.030)	0.357(0.029)
n<0.330 L/L (%)	26 (25)	4 (10)	16 (10)	10 (16)	5 (19)	30 (15)	31 (16)	61 (16)
34 weeks n	80	34	121	55	21	159	152	311
Mean L/L (SD)	0.330(0.027)	0.338(0.028)	0.336(0.030)	0.331(0.027)	0.336(0.034)	0.338(0.029)	0.330(0.028)	0.334(0.029)
n<0.330 L/L (%)	46 (58)	11 (32)	49 (40)	30 (55)	10 (48)	66 (42)	80 (53)	146 (47)
Serum Ferritin								
Booking n	91	41	137	55	26	180	170	350
Mean <sup>a</sup> µg/L (SD)	48.0 (0.41)	42.2 (0.34)	38.7 (0.31)	43.2 (0.34)	39.8 (0.36)	43.5 (0.36)	40.8 (0.34)	42.2 (0.35)
n<15 μg/L (%)	12 (13)	4 (10)	15 (11)	5 (9)	3 (11)	18 (10)	21 (12)	39 (11)
34 weeks n	28	9	40	13	7	55	42	97
Mean <sup>a</sup> 15 µg/L (SD)	17.3 (0.25)	10.7 (0.25)	10.0 (0.27)	21.9 (0.29)	11.0 (0.20)	15.8 (0.28)	10.4 (0.28)	13.2 (0.29)
n<15µg/L (%)	11 (39)	7 (78)	28 (70)	3 (23)	5 (71)	24 (44)	30 (71)	54 (56)

Table 1: Iron status of pregnant women according to ethnicity, treatment group and stage of gestation.

<sup>a</sup>Mean calculated by back transformation from log<sub>10</sub> as data not normally distributed; standard deviation is \*/.

#### DISCUSSION

The levels of low maternal iron status during the first trimester of pregnancy in the current study were similar to a previous study in East London, UK where using the same definitions 9% were anaemic and 10% had low iron stores (Rees *et al.*, 2005). The proportion of participants with low iron stores in the current study (11%) was identical to that found in women aged 19-64 years in UK National Diet and Nutrition Survey (Ruston *et al.*, 2004).

Caucasians and Asians had consistently higher levels of haemoglobin than the Africans and West Indians in the current study. Correspondingly, Steer *et al.* (1995) demonstrated Africans and Afro-Caribbeans had lower haemoglobin than Asians and Caucasians when comparing lowest reported haemoglobin during pregnancy. Earlier work also showed African American women have lower haemoglobin but higher serum ferritin than Caucasian women (Perry *et al.*, 1992). The reasons for these ethnic differences are unknown but it is of clinical significance as women are routinely prescribed iron supplements if their haemoglobin falls below 10 g/dl. This may be acceptable for Caucasian women, however this may be unnecessary for African and West Indian women who may have good iron stores despite low haemoglobin concentrations.

Milman (2006) suggests that women require stores of 500 mg iron at conception in order for dietary iron to be sufficient for requirements. Few women have such stores thus routine prophylaxis of iron during pregnancy is often required. A low dose of iron during pregnancy reduced the incidence of LBW in women without iron deficiency (Cogswell *et al.*, 2003). Women with haemoglobin  $\geq 11g/dL$  and serum ferritin  $\geq 20 \ \mu g$  who were less than 20 weeks of gestation took either 30 mg iron or placebo daily until 28 weeks of gestation. From 28 weeks until delivery all participants received iron supplements. Birth weights of the infants born to iron supplemented women (n=110) were significantly higher by a mean of 206g (p<0.01) than those born to women receiving placebo (n=96). The incidence of LBW (<2.5 kg at birth) in the placebo group was significantly higher than in the treatment group (17% and 4% respectively; p=0.003); this difference was attributed to the higher proportion of preterm, LBW infants in the placebo group compared to the treatment group (10% and 3% respectively; p=0.017).

A U shaped association exists between maternal haemoglobin and birth weight (Rasmussen, 2001); both high and low iron concentrations are associated with adverse pregnancy outcomes. Scanlon *et al.* (2000) showed anaemia early in pregnancy was related to preterm delivery. Haemoglobin and birth outcome of 17,3031 pregnant women were retrospectively analysed. Mothers with low haemoglobin during the first or second trimester (<9.0-10.0g/dl depending on gestation) had an increased risk of preterm birth. Conversely a high haemoglobin level during the first or second trimester (>14.4-14.9g/dl depending on gestation) was associated with an increased risk of SGA (small for gestational age), possibly through failure of the plasma to expand. High haemoglobin levels have been associated with both LBW (Steer *et al.*, 1995) and SGA (Scanlon *et al.*, 2000). Failure of the plasma to expand can result in high haemoglobin, Yip (2000) suggests that it is not the high haemoglobin *per se* which leads to adverse birth outcome; both pregnancy hypertension and smoking elevate haemoglobin and are both direct causes of LBW.

Many of the current participants received additional iron supplements throughout their pregnancy and all participants continuing in the study were asked about iron supplement use. For women who withdrew the information is less clear as reporting of provision of iron supplements in the obstetric notes was patchy and prescription does not mean compliance. It was therefore not possible to account for additional iron supplementation; however, using intention to treat analysis those receiving the multiple micronutrient supplement had higher iron status for all 3 measures at 34 weeks of gestation compared to the placebo group. The treatment provided 20 mg iron per day, higher than the UK Reference Nutrient Intake during pregnancy of 14.8 mg/day (Department of Health, 1991); although much lower than commonly prescribed during pregnancy (up to 300 mg/day). High dose iron supplements are often poorly tolerated due to adverse gastric side effects (Beard, 2000). The lower dose of iron provided by the treatment may have been better tolerated and hence resulted in improved iron status. The current study supports previous work in Australia. Makrides et al. (2003) found that women supplemented with 20 mg iron daily from 20 weeks of gestation until delivery had improved iron status with no difference in gastrointestinal side effects compared to those receiving placebo. Correspondingly, Zhou et al. (2009) demonstrated a daily dose of 20mg elemental iron during pregnancy resulted in reduced occurrence of nausea, stomach pain and vomiting compared to 40 or 80mg iron and yet no difference in the incidence of anaemia.

Low iron status is a problem for this deprived population that increases as pregnancy progresses. Clear ethnic differences exist for haemoglobin and serum ferritin and further investigation is required to fully understand this and to ensure optimal management of iron status. This study adds further weight to the argument that treatment with prophylactic, low dose iron supplements may be preferable to using high doses in improving iron status during pregnancy.

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#### Iron status of female university students living in New Zealand

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# ABSTRACT

**Background:** Female university students may be at risk of iron deficiency due to time constraints, financial issues, and making the transition from home to independent living. **Objectives:** This study aimed to determine the prevalence of iron deficiency in a convenience sample of female students (18-44 years) studying at Massey University in Auckland.

**Design:** 283 female students participated in the study. A venipuncture blood sample was collected to analyse serum ferritin (SF), haemoglobin (Hb) and C-Reactive Protein. Data on ethnicity, medical history and supplement use was obtained using face-to-face interviews.

**Outcomes:** The median (25<sup>th</sup>, 75<sup>th</sup> percentile) age of the women was 22 (19, 28) years. The majority of women (83.3 %) had normal iron stores (SF≥20 µg/L, Hb≥120 g/L). Ten women (3.7 %) had iron deficiency anaemia (SF<12 µg/L, Hb<120 g/L), 3.7 % had iron deficiency without anaemia (SF<12 µg/L, Hb≥ 120 g/L) and 5.2% of women had low iron stores (SF 12-19 µg/L, Hb≥120 g/L). 4.1% had non iron deficient anaemia. Women of non-European descent were more likely to have SF<20 µg/L than Europeans (24.0% *vs.* 8.6%; p=0.001). Similarly, women living in New Zealand for fewer than five years were more likely to have SF<20 µg/L than those born in or living in New Zealand longer than five years (23.4% *vs.* 10.8%; p=0.03).

**Conclusions:** The prevalence of iron deficiency anaemia was similar to that found in other New Zealand studies, but the prevalences of iron deficiency without anaemia and low iron stores were considerably lower. New residents and non-Europeans appear to be more vulnerable to poor iron status. The role of blood loss and dietary factors will be investigated.

#### **INTRODUCTION**

Iron deficiency is the most common nutritional deficiency worldwide. Young women are at particular risk of iron deficiency due to factors including blood loss and dietary factors (Heath *et al.*, 2001). The New Zealand National Nutrition Survey found that low iron stores, iron deficiency and iron deficiency anaemia mainly affected women aged 15-44 years (Russell *et al.*, 1999). Levels of iron deficiency anaemia (SF<12 µg/L, Hb<120 g/L) in females living in New Zealand ranges from 1-2.2% (Fawcett *et al.*, 1998; Russell *et al.*, 1999; Heath *et al.*, 2001). Low iron stores (SF<20 µg/L, Hb $\geq$ 120 g/L) have been found in 23% (Heath *et al.*, 2001) and 15.6% (Fawcett *et al.*, 1998) of New Zealand women aged 18-40 and 21 years respectively.

Female university students may be at further risk of iron deficiency due to factors that impact on their dietary intake such as time constraints, financial issues and making the transition from home to independent living. In addition, they may be more

vulnerable to using low energy diets to reduce weight and heavy alcohol consumption (Hendricks *et al.*, 2004). Only one study has investigated the iron status of university students living in New Zealand. In this study, 10% of 115 women enrolled in their first year of nutrition at the University of Otago, Dunedin had serum ferritin levels <12  $\mu$ g/L (Horwath, 1991). An Australian study in school and university female students aged 15-30 years found that 19.8% of women had a SF<16  $\mu$ g/L, while 4.5% of women had iron deficiency anaemia (SF<12  $\mu$ g/L, Hb<120 g/L, transferrin saturation (TS)<16%) (Rangan *et al.*, 1997). In France, 30.7% of 543 female school and university students aged 17 to 38 years had a SF<20  $\mu$ g/L (Grondin *et al.*, 2008). In the United States, 34% of women had a SF<15  $\mu$ g/L and 6% had iron deficiency anaemia (SF<12  $\mu$ g/L, Hb<120 g/L) (Houston *et al.*, 1997). It is important that up to date information on the iron status of female university students living in New Zealand is obtained.

This study aimed to determine the prevalence of iron deficiency in a convenience sample of female students (18-44 years) studying at Massey University in Albany, Auckland. The effect of ethnicity and time spent living in New Zealand on iron status was also investigated.

### **METHODS**

## Recruitment

This study was called the WISE (Women's Iron Status and Education) Study. Gender and age were the only exclusion criteria. All women aged 18-44 years studying at the Massey University campus in Albany were invited to take part in this study through a centrally distributed email. Flyers and posters were distributed around the campus and in orientation packs. The study was advertised during orientation week for new students. Uni guides (senior students who provide support and guidance to first year students) were informed about the study and asked to bring the orientation stall to students' attention, where information on the study was given to the students. An article about iron deficiency was written for the student magazine and the study was promoted at student lectures. Incentives to take part included a free measure of iron status, a beauty therapy voucher and entry into a draw to win various prizes. An email address was set up so that students could contact the researchers to register interest in the study.

Subjects who registered their interest were sent an information sheet and asked to contact the researchers if interested in taking part. A time was then organised for them to visit the Human Nutrition Research Unit.

#### Subject appointment

All subjects attended one 2-hour appointment on a weekday or weekend morning. A consent form was signed at this appointment. Participants completed a face-to-face interview with a researcher. This included questions on age, ethnicity, country of birth, time spent living in New Zealand, medical history, previous iron deficiency and supplement use in the past year. A fasting venipuncture blood sample was taken by a trained phlebotomist to analyse serum ferritin (SF), haemoglobin (Hb) and C-Reactive Protein (C-RP). Samples were refrigerated at 4°C and sent to an accredited laboratory, Diagnostic MedLab in Auckland, for analysis. A further blood test was taken approximately 3 weeks later if C-RP was >5 mg/L, as a high C-RP is associated with infection which may falsely elevate serum ferritin levels (Hulthen *et al.*, 1998).

#### Statistical analysis

All statistical analyses were undertaken using SPSS software (version 15) (SPSS Inc, 2006). The population was described by using median and interquartile range (IQR,  $25^{\text{th}}$ - $75^{\text{th}}$  percentile). Kolmogorov-Smirnoff test was used to test normality. Subjects were divided into those with a SF<20 µg/L (low iron stores, iron deficiency and iron deficiency anaemia) and those who had a SF≥20 µg/L (normal iron stores). Chi-square analysis was used to investigate categorical variables.

#### Ethics and funding

The study was funded by the Massey University Research Fund and the Institute of Food, Nutrition and Human Health, Massey University. Ethical approval was obtained from the Massey University Human Ethics Committee: (Southern A), Reference No. 07/73.

#### RESULTS

Two hundred and eighty three women took part in this study (9.3% of female students aged 18-44 years at Massey University, Albany). The median (IQR) age of the women was 22 (19-28) years. The majority of women were born in New Zealand (55.5%) with the remainder from 38 different countries. Women with a C-RP >5mg/L (n=12) were excluded from data analysis.

Table 1 shows the majority of women had normal iron stores. Ten women (3.7%) had iron deficiency anaemia, 3.7% had iron deficiency without anaemia and 5.2% of women had low iron stores.

Table 1: Iron status of female university students.

Category	n=271 (100%)
Normal iron stores (SF≥20 µg/L, Hb≥120 g/L)	226 (83.3%)
Iron deficiency anaemia (SF<12 µg/L, Hb<120 g/L)	10 (3.7%)
Iron deficiency without anaemia (SF<12 $\mu$ g/L, Hb $\geq$ 120 g/L)	10 (3.7%)
Low iron stores (SF 12-19 µg/L, Hb≥120 g/L)	14 (5.2%)
Anaemia without iron deficiency (SF $\geq$ 12 µg/L, Hb<120 g/L)	11 (4.1%)

Women of non-European descent were more likely to have SF<20  $\mu$ g/L than Europeans (24.0% *vs.* 8.6%; p=0.001). Women living in New Zealand for fewer than five years were more likely to have SF<20  $\mu$ g/L than those born in or living in New Zealand longer than five years (23.4% *vs.* 10.8%; p=0.03).

#### DISCUSSION

The prevalence of iron deficiency anaemia was slightly higher than that found in other New Zealand studies (Fawcett *et al.*, 1998; Russell *et al.*, 1999; Heath *et al.*, 2001), but lower than that of female school and university students in Australia (Rangan *et al.*, 1997) and the United States of America (Houston *et al.*, 1997). Two of the women who had iron deficiency anaemia in this study were pregnant. In this group of students, the prevalence of low iron stores (SF<20 µg/L, Hb≥120 g/L) was considerably lower than that of females in other New Zealand studies (Fawcett *et al.*, 1998; Heath *et al.*, 2001) and studies in female university students overseas (Houston *et al.*, 1997; Rangan *et al.*, 1997; Grondin *et al.*, 2008). This finding was unexpected, and may be unique to this particular student population.

On the Massey University campus in Albany 38.3% of the female student population aged 18-44 years are of European and 45.3% are of Asian descent. In this study, 71.2% of women identified as European and 19.2% identified as Asian. The percentage of Maori (5.8%) and Pacific Island (1.5%) students in the study was more representative of the student population. Including more Asian students would have made this study more representative of the Massey University student population and would probably have had an influence on estimates of the iron status of this population.

New residents and non-European students appeared more vulnerable to poor iron status than European students or students born in New Zealand or students living in New Zealand longer than five years. A study in Auckland high school students found iron deficiency in Maori, Pacific Island and Asian females to be two to three times higher than that of European females (Schaaf *et al.*, 2000). The reasons for this are unknown. In the third National Health and Nutrition Examination study, iron deficiency was significantly higher in Black and Mexican American women aged 20 to 49 years compared with European women even after controlling for poverty and parity (Looker *et al.*, 1997). In conclusion, further analysis of the WISE Study data will be undertaken to investigate the influence of other factors on iron status including dietary intake and blood loss.

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# Nutrient aberrations in people with coeliac disease after the institution of a gluten free diet

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## ABSTRACT

Coeliac disease (CD) has traditionally been associated with nutrient deficiencies attributed to malabsorption resulting from intestinal inflammation. We analysed dietary intake from food diaries to determine the existence of any nutrient aberrations in subjects newly diagnosed with CD immediately preceding the introduction of a gluten-free diet (GFD) and at monthly intervals for three consecutive months post-diagnosis after the implementation of a GFD. Matched control subjects without symptoms were also recruited. The presence of CD was confirmed by serology and by duodenal biopsy. Subjects completed a comprehensive customised food questionnaire. Dietary data was analysed using Foodworks Professional 2007. The levels of specific macronutrients were determined. Significant differences were identified in gluten, starch and carbohydrate intake but not in other macronutrients. Differences in iron and sodium intake were also noted however, contrary to established literature, micronutrient analyses showed few significant differences in other micronutrients within subjects over the time frame allocated.

## **INTRODUCTION**

Coeliac disease (CD) is an inappropriate immune response to gluten, the dietary protein in wheat, rye, barley and possibly oats. Manifestations range from no symptoms to overt malabsorption of nutrients with multiple organ system involvement. The basis of treatment is strict adherence to a lifelong glute-free diet (GFD), which enables people to remain symptom-free and healthy. We assessed changes in nutrient intake in newly diagnosed subjects prior to commencement of a GFD and subsequent comparison over three consecutive months post-diagnosis in order to determine whether removal of gluten from the diet and ensuing resolution of inflammation is associated with an improved nutrient intake.

A GFD may not be nutritionally balanced as it removes a high proportion of grain-based foods thus reducing essential nutrient intake (Hallert *et al.*, 2002).

Average daily grain-based food intakes (in the US), is around 250g per person; 180 g of this intake is from foods which contain gluten which is removed on a GFD (Koehler 2005). Whilst substitution of wheat, rye, barley and oats with other grains does occur, these alternative grains are usually poorer sources of fibre, B-group vitamins, iron, calcium, magnesium, zinc and selenium (see Table 1). People with CD are susceptible to nutrient deficiencies due to malabsorption which worsens the clinical picture. Untreated CD is associated with deficiencies in iron and folate, vitamins A, D, E, K, B<sub>12</sub>, B<sub>6</sub>, and the mineral calcium (Halfdanarson *et al.*, 2007; Saibeni *et al.*, 2005). Thus, people newly diagnosed with CD should be routinely evaluated for nutrient deficiencies (Presutti *et al.*, 2007).

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Nutrient /100g	Wheat	Oats	Brown Rice	Barley	Maize	Rye	Sorghum	Millet
Fibre (g)	13	11	3.5	16	NA	15	NA	9
Thiamin (mg)	0.4	0.8	0.4	0.2	0.4	0.3	0.2	0.4
Riboflavin (mg)	0.1	0.1	0.1	0.1	0.2	0.3	0.1	0.3
Niacin (mg)	6.7	1.0	4.7	4.6	3.6	4.3	2.9	4.7
Pantothenic acid (mg)	0.9	1.4	1.5	0.3	0.4	1.5	NA	0.9
Pyridoxine (mg)	0.4	0.1	0.5	0.3	0.6	0.3	NA	0.4
Folate (µg)	43	56	20	23	19	60	NA	85
Vitamin E (mg)	1.4	0.7	0.7	0.1	0.8	1.9	NA	0.2
Calcium (mg)	34	54	28	29	7	33	28	8
Iron (mg)	5.4	4.7	1.6	2.5	2.7	2.7	4.4	3
Magnesium(mg)	144	177	143	79	127	121	NA	114
Phosphorus(mg)	508	523	299	221	210	374	287	285
Potassium (mg)	435	429	246	280	287	264	350	195
Sodium(mg)	2	2	6	9	35	6	6	5
Zinc(mg)	4.2	4	2	2.1	2.2	3.7	NA	1.6
Selenium (µg)	89	NA	23	38	16	35	NA	2.7

Table 1: Nutrient content of selected cereal grains (Gendel et al., 2005; Kasarda 2002)

NA: data not available

## **METHODS**

#### **Recruitment** of subjects

Eight subjects (age 24 - 68 years; 3 male, 5 female) with active CD and 8 control subjects matched for age and gender were recruited. The CD group was recruited on presentation to private gastroenterology clinics with symptoms indicative of CD. Diagnosis was confirmed by positive serology and duodenal biopsy. The non-coeliac control subjects were healthy and had no family or personal history of chronic abdominal discomfort, gastrointestinal allergy, coeliac or other chronic inflammatory bowel disease.

The study protocol was approved by the Massey University Ethics Committee (ref: HEC: Southern A Application 06/08) and written informed consent was gained from all participants

#### Sampling Procedures

A customised questionnaire incorporating 3-day dietary recall and a food frequency questionnaire was administered and data analysed using Foodworks Professional 2007 (Xyris®) software.

Intakes of gluten, starch, energy, protein, total fat, saturated fat, carbohydrate, sugars, dietary fibre, sodium, water, vitamins C,  $B_6$  and  $B_{12}$ , and the minerals sodium, iron, and calcium, were determined. It was not feasible to chemically analyse the gluten content of foods consumed so gluten intake was scored based on a devised rating system prior to and after the implementation of a GFD.

#### Rating system

0 = no known gluten

- 1 = trace amounts of gluten
- 2 = small amounts of gluten
- 3 = moderate amounts of gluten
- 4 = significant amounts of gluten
- 5 =large amounts of gluten

#### Statistical analysis

Data from both groups was analysed using the Lillefors test and a *Post Hoc* Bonferroni to allow for comparisons of means while controlling the type-1 error rate (Howell 2002). However, data was not normally distributed, so a Kruskall-Wallis One Way Analysis of Variance (K-W) was performed as it is about 95% as powerful as equivalent parametric tests (Klugh 1986).

#### **Results and discussion**

No significant differences in intake were found for any of the nutrients over the sampling period for the control group. No significant differences in intake of fibre, energy, total fat, saturated fat, protein, vitamin C, vitamin B<sub>6</sub>, vitamin B<sub>12</sub> calcium were found for the CD group. Significant differences were seen between gluten intake at diagnosis and subsequent months (months 1-2; Mann-Whitney U score (MWU)=576.000; p<0.0005; months 1-3; MWU=576.000; p<0.0005; months 1-4; MWU=576.000; p<0.0005 but no significant differences between months 2-3; MWU=259.500; p=0.534, months 2-4; MWU=302.500; p=0.745 or months 3-4; MWU=325.500; p=0.404). This is surprising as inadvertent consumption of gluten was

expected. Most participants were not very adventurous in their food choices and were eating foods known to be gluten-free i.e. meat, fruit and vegetables.

As expected starch intake fell when carbohydrate foods containing wheat, oats, rye and barley were removed. Highly significant differences were found immediately after the diagnosis and over 3 subsequent months (except CSAA who increased consumption) (months 1-2; MWU=505.000; p<0.0005), months 1-3; MWU=544.000; p<0.0005) and month 4; MWU=518.000; p<0.0005). Significant differences between carbohydrate intake at diagnosis (months 1-2; MWU=409.000; p=0.013) were found but no significant differences in consecutive months (month 1-3; MWU=378.000; p=0.063, months 1-4; MWU=325.000; p=0.445). A significant decrease in sodium intake occurred between months 1-2; MWU=392.000; p=0.032 and months 1-3; MWU=394.000; p=0.029 but differences between months 1-4; MWU=374.000; p=0.076 were not significant. Very significant differences occurred for iron intake between months 1-2; MWU=480.000; p=0.000, months 1-3; MWU=486.500; p=0.000 and months 1-4; MWU score= 491.000; p=0.000.

# Comparison of nutrient intake with the 1997 National Nutrition Survey and the Recommended Daily Intakes from the Food and Nutrition Guidelines

The intake of selected nutrients for both groups was compared with the 1997 National Nutrition Survey (Ministry of Health 1999) and the National Nutrition Guidelines for Healthy Adults (Ministry of Health 2003) to assess adequacy of intake in terms of RDIs for New Zealanders, whether subjects consumed a typical New Zealand diet, to compare the two groups against national data and to see if obvious differences exist between the two test groups.

Table 2: Comparison of nutrient intakes with the 1997 NNS and the RDIs or nutrient reference values. Mean or range value indicated

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Nutrient	RDI/NRV	NNS 1997	Coeliac	Control
Energy (kJ/day)	8700	10325	8587	7990
Protein (g/day)	50	88	83	87
Total fat (g/day)	70	91	80	83
Saturated fat (g/day)	24	39	33	34
Carbohydrate (g/day)	310	260	251	207
Sugars (g/day)	90	114	118	79
Fibre (g/day)	30	20	22	22
Water (ml/day)	2200	NA	1948	1661
Vitamin C(mg/day)	45	102	100	110
Vitamin $B_6$ (mg/day)	1.6	1.4	1.6	1.1
Vitamin B <sub>12</sub> (ug/day)	2	4	4.8	4.5
Vitamin A (ug/day)	800	939	993	982
Vitamin E(mg/day)	10	9.7	8.2	9.9
Sodium (mg/day)	920-2300	3473	2242	2447
Iron (mg/day)	12	12	10.5	12
Magnesium (mg/day)	320	309	290	34
Calcium (mg/day)	800 -1000	766	832	746
Zinc (mg/day)	12	12	10	12

## Potential limitations and sources of error with this study

#### (a) Small size of study

Due to difficulty recruiting people newly diagnosed with CD in the time allocated for recruitment. A larger study would provide a more representative sample of people with CD, however as the study was a within subjects design statistical data remains valid. The age and gender matched control ensured that changes over the 4 months of sampling were not simply due to stochastic drift.

#### (b) Limitations in the use of the Foodworks food composition database

Only broad estimates of quantities were given in the questionnaire thus inaccuracies in estimating serving sizes occurred. The Foodworks programme itself is limited as it does not account for seasonal variation of foods, strain variation in nutrient content, ripening variation, or shelf-life variation. It gives a representation based on an average product. Differences often occur between diet composition analysed by food composition databases and chemical analysis. The only totally accurate means of measuring intake is to collect all food and beverages consumed over the sampling period and perform chemical analyses. This was not feasible for this study. Foodworks has limited coverage of unusual food items such as gluten-free food thus these foods were individually entered from the nutritional information on the packaging. Some vitamins and minerals were not listed or ingredients were not recognised by Foodworks thus underestimating the content of some nutrients.

#### (c) Nutrient availability

Nutrient values derived from databases represent maximum amounts of nutrients available not amounts actually absorbed and utilised which is especially relevant in CD as malabsorption of nutrients is often present.

#### (d) Under-reporting of food intake

Under eating and consequent under-estimation of nutrient intake is widespread in self-administered dietary studies (NNS 1997). Using the Goldberg cutoff to identify obviously implausible intake values could minimise this effect. However this method only identifies extreme under-reporting and it has poor sensitivity (Coulston *et al.*, 2008). There is huge variation in type and quantity of food consumed daily over the 3 day sampling periods and foods eaten may not be representative of the typical diet for the person. This could be overcome by sampling over a longer time frame but this could have reduced reliability as it would have increased respondent burden.

#### CONCLUSIONS

Contemporary research finds nutrient deficiencies common in newly diagnosed CD which continues in the subsequent months following diagnosis due to malabsorption and probably alteration in diet. This study found significant decreases in gluten, starch and carbohydrate intake associated with CD following the implementation of a GFD, but no significant changes in B-group vitamins, vitamin C or the fat soluble vitamins.

Whilst iron deficiency is frequently reported in both untreated and treated CD, the significant decrease in iron intake in this study was not expected. The reasons for this can only be surmised and could warrant further investigation. By comparison no changes were found in the control group.

This research has created as many questions as it has found answers and has opened the door to future investigation in this field including carrying out a longitudinal study to charts the nutrient levels in people newly diagnosed with CD and over a prolonged period following the implementation of a GFD, directly measuring serum nutrient levels rather than relying on non-empirical measures or estimates, or implementing a CD national screening programme and assessing adequacy of nutrient intake in this population group.

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# Aspireforlife.com: a research based weight loss programme for Kiwis

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## ABSTRACT

**Background:** Plant & Food Research has developed an Internet-based diet programme, <u>www.aspireforlife.com</u> which offers a comprehensive approach to weight management that is grounded in science.

**Objectives:** To implement an Internet weight loss programme based on University of Otago research and a subsequent Plant & Food Research pilot study of the Aspireforlife.com in 2007.

**Methods:** A participant evaluation and a separate usability study were conducted for Aspireforlife.com in 2007.

**Outcomes:** As a result of this research, a new website was developed with improvements in ease of use, more personalised food information, and an expanded, interactive physical activity component incorporating research on energy modelling and input from Sport and Recreation New Zealand.

**Conclusions:** Aspireforlife.com is now a 24-week programme, implemented in two phases of 12 weeks, ready to be made available to the general public.

## **INTRODUCTION**

Excess body weight is a global health problem (World Health Organization, 2006) to which New Zealand is not immune. More than 62% of New Zealanders are defined as overweight or obese (Ministry of Health, 2008) therefore effective tools to reduce excess body weight are essential for the long-term health of the country. Little consensus exists about the appropriate treatment for those who are overweight and obese, but growing evidence supports the use of low glycaemic load (GL) diets as an effective tool for weight management (Thomas *et al.*, 2007). Structured Internet weight management programmes are also regarded as an option to help people lose weight (Saperstein *et al.*, 2007; Berkel *et al.*, 2005; Tate *et al.*, 2001; Tate *et al.*, 2003) and maintain weight loss (Harvey-Berino *et al.*, 2004).

In the past 4 years, two New Zealand studies have contributed to the body of research substantiating the use of low GL diets and the Internet to reduce body weight. These include the Aspire for Life dietary intervention trial and the Aspireforlife.com pilot study.

In 2004 Plant & Food Research, within their 'Lifestyle Foods' research contract with the Foundation for Research Science and Technology, commissioned the University of Otago to conduct a clinical trial to compare the weight loss effects of a diet based on low GL or sustained-energy foods such as whole grains, legumes, fruits, vegetables, milk and yoghurt, with a traditional low-fat eating plan. One hundred participants completed the 18 month 'Aspire for Life' trial. Both groups lost significant amounts of weight, approximately 7 kg at 6 months and 5 kg at 18 months (unpublished data). However, attrition rates were notably lower on the low GL plan, indicating greater acceptability of it than of the low-fat eating plan. As a result the researchers saw

the potential benefits of promoting the sustained-energy approach to the greater New Zealand public.

Research indicates that the Internet has great potential to deliver cost effective weight loss advice to large groups of people (Saperstein *et al.*, 2007), so Plant & Food researchers chose to assess if a sustained-energy weight loss strategy could be successfully transferred to an on-line application. Researchers expanded Aspire for Life's original nutrition content to include recipes, physical activity guidelines, cognitive behavioural strategies and a chat room for participant interaction. In 2007, Aspireforlife.com was launched for a 6-month pilot study. Seventy participants (67%) completed the study, of whom 75% lost a clinically significant amount of weight, averaging 3.5 kg (unpublished data). Most participants reported lower levels of self-rated hunger after the study (unpublished data).

The pilot study demonstrated that the Internet could be an effective medium to deliver a low GL weight loss method. We report here on the next stage of the site's development, in which results from the pilot study's participant questionnaires were combined with a usability study commissioned to develop the site's ease of use.

## **METHODS**

#### Participant questionnaires

Researchers used two participant questionnaires during the pilot study to evaluate the Aspirediet.com website tools (such as the chat-room, weigh-ins, food check sheets, etc) and their correlations with weight loss. Participants completed questionnaires at 3 and 6 months designed to measure the usability of the Internet site, and to give information on diet, energy, satiety and exercise habits. The 3-month questionnaire was completed electronically; the 6-month survey was completed in person at each participant's final weigh-in.

Associations between weight change and measures of programme engagement and subjective outcome ratings were investigated using statistical measures where appropriate. Measures of programme engagement included number of times participants used chatrooms, completed food checksheets, or entered their own weights into the Internet site.

#### Usability study

After the completion of the pilot study in 2007, Plant & Food Research contracted the Christchurch Polytechnic Institute of Technology to conduct a discount usability testing method to further evaluate the site. The discount usability testing method is used to uncover problems that real users experience when using a website (Barnum, 2002; Travis, 2003). A small number of test subjects, who fitted a user profile, were asked to complete a set number of tasks on the website, then answer questions about their impressions and experiences.

The test group comprised of seven Internet-experienced, overweight adults interested in weight loss and/or lifestyle change. Test subjects were asked to spend 1 hour using the website to complete a series of tasks designed to evaluate the following: how well the public pages informed and inspired users, how easily users could access, understand and use information in the members' web pages, and how easily users could find and accomplish tasks using the interactive tools (Wilson, 2007).

#### RESULTS

#### Participant questionnaires

Statistical analyses revealed that participants who lost a clinically significant amount of weight were more likely to enter self-reported weights and use the chat-room than those participants who lost less than 5% of their initial body weight (unpublished data). In other words, those who engaged with the site lost more weight. Subjective data indicated that a more complex site was needed to meet the needs of a diverse range of individuals. Other feedback included recommendations to discontinue the diet sheets, change the chat-room to give it a wider appeal, and improve the physical activity component.

#### Results of the usability study

Overall the seven users reported satisfaction with the site, but several technical issues were revealed (Wilson, 2007). Most of the users were not adequately motivated by the public pages to want to subscribe although once they interacted with the website, their attitudes were more positive. Participants struggled to find key information on the site and inadequate instructions led to further confusion. Long web pages with too much text were difficult to read, and a lack of search functions or a site map reduced user efficiency.

#### DISCUSSION

The pilot study of Aspireforlife.com verified previous research on Internetbased weight loss sites, indicating that those who engage in a programme more frequently lose more weight (Womble *et al.*, 2004), therefore efforts to engage participants more fully will have long-term benefits for the user. Applying feedback from participant questionnaires and a usability study of Aspirediet.com, recent efforts have targeted three major areas for improvement: ease of use, a personalised food plan and an expanded physical activity component.

To make the website more 'user-friendly' a new interface was designed with shorter navigation paths, a search function was installed for recipes, and a clearer classification of topics was developed. The site changed to evolve over weeks so as to keep the user engaged but not overwhelmed. A searchable database of over 100 recipes was installed. The web pages were made more readable with highlighted text and bullet points. The content in the nutrition, physical activity and recipe areas was also expanded to meet the tastes and needs of a broader population.

In previous Aspire studies, research participants received one standard food pyramid, regardless of their sex, age or physical characteristics. Responding to the call for more individualised information, the food pyramid was updated to change according to a person's specific energy requirements. Web designers employed the Mifflin St. Jeor equation, considered the most accurate for estimating resting energy metabolic rate in non-obese and obese adults (Frankenfield *et al.*, 2005), to adjust the pyramid for each person while maintaining the programme's low GL principles.

Participant feedback indicated a need for improved physical activity information, so Plant & Food Research partnered with Sport and Recreation Canterbury to build a more comprehensive physical activity section. An interactive component was added to the site to guide participants to develop a physical activity plan that promotes weight loss and health. The content was also expanded to include information on safety, injury, illness and more advanced topics such as resistance training and flexibility. Other improvements designed to engage participants included regular reviews with a dietitian and physical activity coach, a participant forum instead of a chat-room, weekly newsletters that address the psychological issues that individuals face when changing their diet and exercise behaviour, and online tools to set personal goals and monitor progress.

## CONCLUSION

Aspireforlife.com now offers a 24-week programme, implemented in two phases of 12 weeks. Using feedback from participant questionnaires and a usability study, Aspireforlife.com has expanded into a more engaging and interactive website with shorter navigation paths and more information and tools available to users, thereby improving their chances of achieving long-term weight-loss.

## **ACKNOWLEDGEMENTS**

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# Plasma PUFA and liver enzymes in HIV-infected subjects: The PURE Study

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### ABSTRACT

**Background:** Omega-6 PUFA intake was reported to be adversely related to liver function in HIV-infected compared to HIV-uninfected subjects in a black population in South Africa. It was speculated that the use of heavily oxidized vegetable fats (abused fats) could have been responsible.

**Objectives:** To investigate the relationship between total plasma PUFA levels (a marker of PUFA intake) and liver enzymes in HIV-infected asymptomatic compared to HIV-uninfected black South Africans; to investigate the re-use of oil and the use of abused oils.

**Design:** Case-control study nested in an epidemiological study on 305 HIV-infected (cases) and 301 HIV-uninfected matched controls (matched according to location, gender and age), as part of the PURE study, a prospective cohort study including a representative sample of 2000 apparently healthy black volunteers, aged between 36 and 60 years, from the North West Province of South Africa.

**Outcomes:** Total plasma omega-6 PUFA levels were significantly (p<0.05) negatively associated with liver enzymes (GGT, ALT, AST, ALP) in both HIV-infected and HIV-uninfected subjects (*r* ranged from -0.22 to -0.56). 99% of subjects reported that they do not buy oil that has been used before. Oil was only used  $2.23 \pm 0.85$  times for deep-frying before being discarded.

**Conclusions:** The adverse relationships between omega-6 PUFA intake and liver enzymes previously shown could not be confirmed in this study. In contrast, plasma omega-6 PUFA was inversely related to liver enzymes in both HIV-infected and HIV-uninfected subjects. A possible explanation could be that subjects in this study did not use abused fats.

## **INTRODUCTION**

South Africa continues to be plagued with a high prevalence of HIV-infection that has a profound impact on the workforce and human and financial resources required to provide care for people living with AIDS. In addition, the needs of orphans will profoundly affect all aspects of social and economic development (Benatar, 2004; Dorrington *et al.*, 2006; UNAIDS, 2008). Optimizing nutritional status is a key objective in the care, management and treatment of people infected with the virus and suffering from AIDS (Mbewu, 2003). Very little research has been done on the role of dietary fat and fatty acids in HIV-infected people. Oosthuizen and colleagues reported that omega-6 polyunsaturated fatty acid intake was adversely related to liver function in HIV-infected compared to HIV-uninfected subjects in a cross-sectional epidemiological study of the black population in the North West Province of South Africa in 1996 and

1998. The authors speculated that PUFA intake may promote liver damage through its susceptibility to attack by free radicals and consequently contributes to inflammation and oxidative stress in HIV-infected subjects who are already vulnerable to these conditions. They further speculated that the use of heavily oxidized vegetable fats (abused fats) could be partly responsible for the adverse effects seen (Oosthuizen *et al.*, 2006). It has been reported that large amounts of heavily oxidized vegetable fats (abused fats), rich in omega-6 PUFA, have been distributed for many years by frying establishments as waste to the poor black communities across South Africa for use in food preparation (Kock *et al.*, 2002). Unfortunately, Oosthuizen did not investigate whether subjects actually consumed abused fats. South Africa has traditionally high intakes of omega-6 PUFA rich vegetable oils, and it is therefore important that these findings are confirmed and the reasons or mechanisms responsible are investigate the

relationship between plasma PUFA levels (as marker of PUFA intake) and serum liver enzymes in HIV-infected compared to HIV-uninfected black South Africans and to investigate the re-use of oil and the use of abused oils in this population.

# MATERIALS AND METHODS

#### Study design and subjects

The study design used was a case-control study nested in an epidemiological study on HIV-infected (cases) and HIV-uninfected matched controls, as part of the PURE (Prospective Urban and Rural Epidemiology) Study. The PURE Study is a prospective cohort study including a representative sample of 2000 apparently healthy black volunteers, aged between 36 and 60, recruited from two urban and two rural areas in the North West Province of South Africa.

Subjects were recruited from four different locations in the North-West Province of South Africa, two urban locations (Ikageng and Ikageng informal settlements) and two rural locations (Ganyesa and Tlakgameng). Urban represents people living in established townships and rural represents people still living under tribal law (Vorster *et al.*, 2005). A family census questionnaire of 6000 households in total was carried out in the four communities (2000 in each community) through filling out questionnaires from door to door. Out of these 6000 households, 2000 volunteers were invited to take part in the study. Inclusion criteria were: aged between 35 and 60 years, not using any medication for chronic disease and no known conditions or diseases and not intending to move away from the area in the foreseeable future. Of the 2000 volunteers 305 were newly identified to be infected with the HIV virus and 305 matched control subjects that were not infected were identified. The cases and controls were matched according to location, gender and age ( $\pm$  1year). Blood samples were available for 301 control subjects.

#### Measurements and biochemical analyses

The following relevant information, which has been obtained as part of the core PURE study, was used for this particular investigation. Standardized, interviewerbased questionnaires were administered by trained fieldworkers in the language of the subject's choice to collect demographic information. Dietary information was obtained by using a quantitative food frequency questionnaire, designed and validated for this population (MacIntyre *et al.*, 2001). A questionnaire was designed, tested for comprehensibility in this population and used to investigate the re-use of oil and the use of abused oils. These questionnaires were administered by trained fieldworkers. Height and weight were measured and body mass index (BMI) calculated.

Registered nursing sisters collected venous blood samples. The subjects were asked to fast overnight (at least 8 hours with no food or beverage, excluding water). For the determination of plasma fatty acids, blood was collected in 5 ml tubes containing EDTA. Blood was left to clot to determine liver enzymes. Plasma and serum were prepared by centrifugation of the blood for 15 minutes at 2000g at 4°C. Aliquots of the plasma and serum were then frozen on dry ice, stored in the field at -18°C and after 2-4 days in the laboratory at -82°C until analysis. HIV status was determined on the day of the data collection by a first response HIV card test (PMC Medical) using whole blood. In case of a positive outcome a second test (the Pareeshak test) was performed to confirm the result. Serum liver enzymes (gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotranferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)) were analysed with Sequential Multiple Analyzer Computer (SMAC) using the Konelab<sup>TM</sup> analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Plasma fatty acids were analysed using the GC-MS.

#### Ethical considerations

The study was approved by the ethics committee of the North-West University, Potchefstroom, South Africa (approval no 04M10). All subjects were fully informed about the objectives and procedures of the study in their own language and signed an informed consent form. Pre- and post HIV testing counseling was provided to all participants by two researchers trained to give HIV counseling. Pre-counseling was conducted in groups of 10 people and post-counseling sessions were conducted on an individual basis.

#### Statistical analysis

The data were analysed using the SPSS package version 15 (SPSS Inc., Chicago, IL, USA). Data that were not normally distributed were normalised by logarithmic transformations. Variables that were not normally distributed are expressed as median [25, 75 percentile]; normally distributed variables are expressed as mean [95% confidence interval (CI)]; variables that were logarithmically transformed are expressed as geometric means [95% CI] and categorical data are expressed as frequencies or percentages. Differences between HIV-infected and HIV-uninfected subjects were tested using the Mann-Whitney U test for non-parametric data, the independent t-test for parametric data and the chi-square test for categorical variables. Pearson's correlation coefficients were calculated to assess the relationships between plasma PUFA levels and serum liver enzyme concentrations, stratified for HIV-status. These relationships were also assessed while controlling for confounding factors (gender, alcohol use and rural/urban location) using partial correlations.

## RESULTS

The HIV-infected subjects had a significantly lower BMI than the HIVuninfected subjects, but did not differ with regard to other characteristics including education, use of tobacco products and alcohol use. The two groups did not differ with regard to location (urban or rural), gender and age as expected since the two groups were matched according to these variables (Table 1).

	HIV-infected	HIV-uninfected	р
	(n=305)	(n=301)	value
Men/women	111/194	105/196	0.381
Rural/urban	159/146	156/145	0.503
Age (years)	42 [38, 48]	42 [38, 48]	0.598
Body mass index (kg/m <sup>2</sup> )	21.3 [18.6, 25.1]	23.5 [19.4,	< 0.001
		29.0]	
Education (%)			0.260
None	27.9	30.5	
Primary school	38.3	40.4	
Secondary/trade school	33.6	27.7	
College/University	0.3	1.4	
Use of tobacco products (%)			0.777
Formerly used	3.3	4.4	
Currently using	54.5	53.0	
Never used	42.2	42.6	
Alcohol use (g/day)	0.41 [0, 15.4]	0 [0, 15.7]	0.598

Table 1: Characteristics of HIV-infected and HIV-uninfected subjects

Results expressed as median [25, 75 percentiles] or frequencies

Only serum AST concentrations were significantly higher in HIV-infected compared to the HIV-uninfected subjects (Table 2). All liver enzyme concentrations were within the normal reference ranges (Kumar andClark, 2002).

Table 2: Serum liver enzymes in HIV-infected compared HIV-uninfected subjects

	HIV-infected (n=303)	HIV-uninfected	р
		(n=294)	value
GGT (U/L)	53.5[48.4, 58.6]	50.4 [45.6, 55.7]	0.43
ALT (U/L)	17.6 [16.3, 18.9]	16.6 [15.3, 17.8]	0.28
AST (U/L)	28.8 [25.8, 31.8]	24.8 [22.4, 27.4]	0.04
ALP (U/L)	120 [114, 125]	118 [112, 123]	0.51
LDH (U/L)	245 [235, 255]	233 [224, 240]	0.06

Results expressed as geometric means [95% CI]

Total plasma omega-6 PUFA levels were significantly (p<0.05) negatively associated with liver enzymes (GGT, ALT, AST, ALP) in both HIV-infected and HIV-uninfected subjects (Table 3). The same significant relationships were seen when controlling for confounding factors (gender, alcohol intake and rural/urban location).

This population did not use abused oils and the frequency of re-use of oils for deep-frying appeared to be adequate. Almost all the subjects (99%) reported that they do not buy oil that has been used before. Most subjects (95%) used oil for deep frying on average two times per week. Those that used oil for deep-frying discarded the oil after it has been used twice (mean  $\pm$  SD; 2.23  $\pm$  0.85).

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	HIV-infected (n=305)		HIV-uninfected (n=301)	
	r	p value	r	p value
GGT	-0.50	< 0.01	-0.56	< 0.01
ALT	-0.31	< 0.01	-0.23	< 0.01
AST	-0.22	< 0.01	-0.25	< 0.01
ALP	-0.26	< 0.01	-0.31	< 0.01
LDH	-0.10	0.10	-0.01	0.86

Table 3: Pearson correlations between total plasma omega-6 PUFA and serum liver enzymes

## DISCUSSION

Similarly to the study by Oosthuizen (Oosthuizen et al., 2006) the HIVinfected subjects in this study were newly diagnosed with HIV and probably at an early state of infection. The HIV-infected subjects had a significantly lower BMI but liver enzyme concentrations were not affected by HIV-status in this group. The adverse relationship between omega-6 PUFA intake and liver enzymes previously shown by Oosthuizen et al. (2006) could not be confirmed in this study. In contrast, total plasma omega-6 PUFA was inversely related to liver enzymes in both HIV-infected and HIVuninfected subjects. The current study used plasma PUFA, a short-term marker of PUFA intake, which may be superior to measuring PUFA intake using self-reported dietary assessment methods (Arab, 2003; Arab and Akbar, 2002). A possible explanation why we could not confirm the adverse relationship between omega-6 PUFA intake and serum liver enzymes could be that subjects in this study did not use abused fats. Ninety nine percent of subjects reported that they do not buy oil that has been used before. It was proposed by Das that essential fatty acids and their metabolites could be useful in the prevention and management of AIDS (Das, 2005), but further studies are needed. This study showed that total plasma PUFA were not adversely related to liver function in HIV-infected subjects at the early stages of HIV-infection. Until research demonstrates otherwise the use of omega-6 PUFA rich vegetables oils by HIV-infected subjects in the quantities and the way that it is currently being used seems to be safe.

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# What are the midlife health concerns of NZ women

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# ABSTRACT

As the population ages, women will require a proportionally greater share of health resources due to declining function, disability and reportedly poorer "health related quality of life". This study's aim was to identify New Zealand midlife women's concerns about ageing, particularly the nutrition and health behaviours affecting ageing and specifically practices believed to promote health, alleviate functional decline and prevent incapacity or disease. Focus groups were conducted with 55 women aged between 40 and 65yrs from a wide variety of backgrounds and ethnicities. Grounded theory was the method of analysis. The Basic Social Process identified was "Delimiting the decline" and is how the women dealt with the various health concerns including memory loss/cognitive decline, physical mobility, cancer, heart disease, diabetes and weight issues. Lifestyle behaviours considered important to "Delimiting the Decline" included eating more "natural" food (e.g. growing their own vegetables/fruit) and exercise for mobility, flexibility, bone strength, muscle tone and to elevate mood. Family histories and the concept of personal vulnerability also have an influence in determining health related behaviours. Midlife women have a wide range of health concerns and state they want good quality of life in their later years. They are aware of risk factors and the aetiology of decline with many women implementing strategies to enhance ageing and forestall functional decline and disease.

## **INTRODUCTION**

Women outlive men and with a longer life expectancy will require a proportionally greater share of health resources due to declining function, disability and reported poorer "health related quality of life" (Wilson and Cleary, 1995, Tennenbaum *et al.*, 2007). Understanding women's perception of their health needs at this time can help health care providers and other health professionals improve services in areas most likely to enhance women's health related quality of life (Gaudreau, 2007, Tannenbaum *et al.*, 2003 and 2007). The menopause transition can be a critical point in a women's life when health choices can significantly influence ageing. Women who have a family history of a particular illness or condition have usually identified their susceptibility and made plans earlier than most to counteract this illness especially if they have lost a parent or close relative (Lindenmeyer *et al.*, 2008).

This study looked at what New Zealand women regard as the most important health and wellbeing issues during this time. It focuses on what women believe should be done by themselves and health providers to maintain health, prevent illness and limit functional decline as women age.

#### METHODS

Midlife women between 40 and 65 years were recruited by word of mouth predominantly from the Hawke's Bay region to participate in focus groups (Barbour and Kitzinger, 1999, Barnett, 2002). The participants were recruited from a range of workplaces, organisations and community settings and included women from a variety of ethnic groups: European, Maori, Pacific Islander, Asian and South African. Some ethnicities were difficult to recruit especially Polynesian women as traditionally they don't like to discuss health issues. While most of the focus groups were of mixed ethnicities a few groups were predominantly one ethnicity e.g. a Chinese church group, a Maori health provider group. Several of the focus groups were conducted in the workplace for convenience of the participants. Participants provided written informed consent and the study had been "Peer reviewed" for ethical purposes. A trained facilitator introduced the topics and ran the sessions to encourage sustained contributions from all the participants. A note taker was present at all the sessions and took notes in addition to a taped recording made of all discussion. The women were asked to discuss a series of open ended questions pertaining to current and future health concerns and lifestyle behaviours. There was an emphasis in the questions on healthy lifestyle behaviours the women thought necessary to prevent illness and functional decline as well as to enhance their wellbeing. The women were also asked to confidentially provide their personal summation of the issues by filling in a sheet stating their three main health concerns and the three behaviours they considered most important to their health and ability to age well. All recorded discussion was transcribed verbatim and then coded and analysed using Grounded Theory (GT) which is an inductive method of analysis predominantly used with qualitative data that aims to understand the action in a substantive area from the points of view of the people involved. This understanding revolves around their main concern and how they continually resolve it (Glaser, 1998). Using constant comparison (Glaser, 1992) the categories and codes that were being built up were then compared with each new transcripts analysis for categories and codes and in this way the overarching categories were developed for the individual codes. This process continued until saturation was reached i.e. when no new codes or categories emerged from new transcripts and coding was only producing the same themes (Glaser, 1978). A theory concerning ageing and women was built from the perspectives revealed in the focus groups.

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# Figure 1: Grounded Theory Findings

Main Health concerns in midlife women





Health Strategies for Ageing Well

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#### RESULTS

The focus group's main health concerns are depicted in Figure 1 with cognitive decline including memory loss, dementia and Alzheimer's featuring significantly among other health conditions associated with midlife. These concerns were continually resolved by the process of "Delimiting the Decline" whereby the women endeavoured to influence how ageing would evolve for them by implementing a variety of strategies to avoid major disease, maintain function and enhance their quality of life as they age. The women personalized the ageing process by acknowledging their role in preventative care and in their comments, e.g. "we want a better quality of life if we look after ourselves now". They acknowledged their parents and grandparents may not have had the opportunities, information and health screening available that they now have to take this control of their health at this time. Having a family history with a genetic predisposition to a particular illness was seen as a major motivator for healthy lifestyle choices while racial susceptibility could also influence behaviours for some but to a lesser extent. The category "Concept of Vulnerability" personalised the process and included the subcategories "Family History" which was a major determinant of the strategies used, "Previous Lifestyle Choices" (e.g. poor diet, smoking) and "Perceived Racial Susceptibility" (mental illness, asthma, diabetes) whereby the women determined susceptibility to ill health e.g. obesity as being the result of previous poor choices by either themselves if they were obese or else this condition was attributed to the poor choices by other less informed people or a racial characteristic due to lifestyle choice.

## DISCUSSION

The Basic Social process "Delimiting the Decline" resulted in strategies for preventative care of health and wellbeing. These strategies were based around exercise and healthy diet with exercise (particularly walking) seen as addressing a range of the women's health concerns. Walking was well supported by a majority of the women because it provided multifunctional benefits such as de-stressing, companionship, enjoyment as well as physical health benefits such as weight control and load-bearing for muscles and bones. As a woman's concept of vulnerability to particular illnesses developed it appeared that conscious decisions were made to engage in certain strategies or not depending on their perceived susceptibility. Many of the women wanted information on what they could do to stay healthy as they aged and what motivated them to seek that information out was often their family history or someone else who they identified with, for example, a close friend who had experienced a particular illness such as breast cancer if similar ethnicity, however the familial link was deemed more significant.

Personal intrafamilial experience of serious illness with significant functional decline such as osteoporosis, dementia or diabetes focussed a woman's awareness of susceptibility and gave impetus to the use of exercise or diet as a means of avoiding or delaying onset of these diseases. Other determinants of vulnerability were past behaviours such as smoking (e.g. lung cancer, emphysema) and race (e.g. renal disease, asthma, mental illness). Conversely, health messages such as nutritional recommendations for consuming more dairy products to ward off osteoporosis were considered but if determined to lack validity or be "hyped" were discarded. For example, the Asian women interviewed in the focus groups knew the nutritional recommendations regarding osteoporosis and consumption of dairy products but several

questioned its validity to them "how many Asian women have osteoporosis that we know?" Maintenance of mental fitness and good relationships with family and friends was noted as fundamental to healthy ageing strategies. Strategies to maintain mental fitness involved learning new skills, reading, Sudoku and doing crosswords. A positive outlook was considered essential at this time to prevent both physical and mental decline and strategies encompassed activities that released stress and brought balance to hectic working and family lives. A healthy diet was seen as pivotal and the focus was traditional, wholesome food and growing vegetable gardens. The Maori women discussed their specific racial health needs and vulnerabilities and covered a broader range of health problems encompassing both physical and mental health issues. A supportive partner and whanau was seen as crucial to Maori women ageing well.

Figure 1 outlines the concerns discussed in the focus groups and which women summarised individually and confidentially at the end of each session. They are not intended as a statistical representation of all midlife women's health concerns.

The model developed below (Figure 2) to represent the Grounded theory developed from the women's discussions on the topic could be used for behavioral nterventions aimed at reducing ill health and disability in this age group. Interventions such as formalized group advice on diet and exercise to reduce functional decline at this time in a woman's life with particular reference to maintenance of memory and cognitive function, bone health, CVD and obesity as well as prevention of serious ill health through cancer and diabetes prevention and weight control would be well received with the women open to learning more and better ways of preserving health at this time of their lives.

Figure 2: Grounded TheoryModel

Midlife Women, Wellbeing and Ageing.



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# Identifying schoolchildren at risk of overweight / obesity – development of a screening tool

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# ABSTRACT

**Background:** Overweight prevalence is increasing rapidly among adolescents worldwide.

**Objectives:** To describe a screening tool developed in the THUSABANA study to identify 10-15 year old school children at risk of overweight/obesity in the North West Province, South Africa.

**Design:** A random, systematically selected sample (n=1336) of school children (both genders, all ethnic groups) was recruited to collect data on demography, family circumstances, habitual physical activity, dietary intake and anthropometry to assess weight status and percentage body fat (%BF).

**Outcomes:** The overweight prevalence rate was twice as high in females and white children, and more apparent in urban areas, smaller households, and parents with both low and high incomes. Female gender and post-menarche age were determinants of overfatness, but not energy or fat intakes. Discriminant analysis confirmed small household size and inactivity as determinants for overweight and high %BF; girls tended to be more overweight as they aged. The tool was developed to screen children by applying these identified determinants in a particular order: age, socioeconomic status, gender, physical measurements, BMI assessment, dietary intake and physical activity, suggesting appropriate management at various stages throughout.

**Conclusions:** This tool could effectively screen adolescents at schools, healthcare clinics or by general practitioners to identify risk and to verify the level of healthcare involvement or the need for referral to prevent overweight/obesity. A similar model could be used to develop a screening tool for identifying New Zealand children at risk of overweight/obesity.

## **INTRODUCTION**

Many researchers consider obesity as a global epidemic carrying with it an increased prevalence of other chronic diseases and complications with a major public health impact (Rippe *et al.*, 1998; Styne, 2001; Wang, 2001; James, 2008). Obesity is now considered a modern, chronic multisystem disease with various underlying causes including genetic, behavioural, environmental, and cultural influences. It remains untreated in most instances and is associated with a variety of co-existing chronic diseases, especially coronary heart disease, diabetes mellitus, and hypertension. The prevalence of overweight/obesity among adolescents is increasing rapidly due to the increasingly obesogenic environment where more time is spent on sedentary activities such as watching television and less time is spent on physical activities. Higher consumption of high fat / high sugar-fat foods may also contribute to the problem (Rippe *et al.*, 1998; Eriksson *et al.*, 2001; MacAulay and Newsome, 2004; James,

2008). A high body mass index (BMI) in childhood tracks into adulthood with a profound influence on morbidity and mortality in adult life. Nutritionists should focus on identifying and planning suitable preventive strategies to halt the global obesity epidemic (Wang, 2001; Popkin, 2001; Ebbeling *et al.*, 2002).

As the treatment of obesity in adults generally has poor results, the attention should rather be focused on identifying children prone to overweight/obesity so that the public health problem of obesity could be addressed at an earlier stage and preventive measures could be implemented sooner. The THUSA BANA study investigated the weight status of school children, aged 10 to 15 years, in a population in the transitional phase in the North West Province (NWP) of South Africa (SA). The aim of this research report is to describe a screening tool that was developed to identify 10-15 year old school children at risk of overweight/obesity, using their weight status and determinants that was identified as being related to obesity (dietary intake, eating habits, physical activity, anthropometric results, and socioeconomic status).

# **METHODS**

# Subjects and design

A cross-sectional, descriptive research design was used to investigate the weight status of the children and the determinants related to obesity. The Ethics Committee of the North-West University approved the study protocol. A single, random sample, stratified for age (10-15 years), gender (male/female), type of school (primary/secondary), and ethnic group (black, white, coloured (mixed ancestory) and Indian), was drawn from 44 schools in the five regions in the NWP, using a 2-digit random number. For statistical significance, at least 100 children per age and gender group and at least 60 children per ethnic group with equal numbers per age group were required, resulting in a planned sample of 1336 children. Girls and boys of each age group were randomly selected systematically in each school from class lists (n=1336). The research was conducted during school hours at the respective sampled schools after informed consent was given by the schools' headmasters, the children and their parents (information sheets and consent forms were given beforehand). Trained interviewers used a structured questionnaire, specifically designed to gather information regarding demographics, socioeconomic, environmental and health factors of each child individually and his/her family and circumstances. Trained field workers gathered dietary intake data with a multi-pass 24-hour recall method in the children's home languages. The dietary data were evaluated for adequacy of energy intake and fat content using the Recommended Daily Allowance (1989) and the 2000 Dietary Guidelines for Americans. Patterns of intake were used to identify poor eating habits/dietary practices impacting on the weight status of the children. Four different anthropometrical measurements (weight, stature, skin folds, and circumferences) were used to evaluate the weight status and body fat content of the children. The prediction equations of Boileau et al. (1985) and the Lohman classification (1984) were used to calculate and classify children according to %BF. Different indicators of overweight/obesity were compared. Children also gave information about their physical activities over the previous 24 hours and one previous weekend day; "Previous day physical activity recall" (PDPAR) (Trost et al., 1999).

#### Statistical Analysis

One-way analysis of variance (ANOVA), generalised linear models (GLM) procedure of SAS, and Tukey Post Hoc Honest Significant Difference (HSD) test were

used to investigate the relationships between the demographic, anthropometric and dietary variables and also to identify the most important determinants of overweight/obesity in this population.

# RESULTS

The final sample comprised 1257 subjects (94% response rate) including 919 black, 191 white, 78 Indian, and 69 coloured children (608 boys, 649 girls). The results revealed that the child cut-off points/standards for overweight/obesity, set up by the International Obesity Task Force (IOTF), linking absolute cut-offs for adult obesity with corresponding centiles of BMI-for-age, are useful for determining the weight status of children and adolescents (Cole *et al.*, 2000).

The overweight prevalence rate was twice as high in females and white children, and more apparent in urban areas, smaller households, and parents with both low and high incomes. Female gender and post-menarche age were determinants of overfatness, but not their dietary energy or fat intakes. Girls tended to be more overweight with increasing age; the post-menarche girls had a moderately high mean %BF (26.04%) (Lohman *et al.*, 1984). Discriminant analysis confirmed small household size and inactivity as determinants for overweight and high %BF. Few variables showed a significant association with high %BF. In boys however, the number of household members and weekend physical activity, and for girls, their age showed significant associations. In the overweight or obese group, most of the boys lived in households with the fewest members, and post-menarche girls were most inactive both on weekdays and weekends, also becoming more overweight with increasing age. A more complete report on the research results has been published by Kruger *et al.*, (2006).

These results were used to compile a screening tool to identify children at risk of overweight/obesity by using the identified determinants of overweight/obesity as well as trends influencing the development of overweight/obesity. This screening tool applies only to the assessment of the weight status of children aged 10–15 years and the subsequent decisions are based on this assessment. No other disease conditions are assessed simultaneously. The tool was developed to screen children by applying these identified determinants in a particular order:

- o Contact via suitable health professional
- o Assessment of age
- Identifying determinants (residential area, parental occupation, family size) mainly regarding the socioeconomic status of the children's situation and how it may impact on their weight status.
- Weight history assessment
- o Gender assessment
- o Physical measurements (weight, height, %BF)
- BMI assessment; the relation between weight and mortality is J-shaped, and evidence suggests that the right side of the "J" begins to rise at a BMI of 25. Therefore, the child cut-off points have been compiled to resemble the adult cut-off points (NHLBI expert panel, 1998)
- Additional risk factor assessment (dietary intake (specifically energy and fat intakes) and physical activity)
- Refer those with high risk to a dietitian/nutritionist to devise goals and treatment strategy for weight control and risk factor control
- o Various follow-up assessments combined with periodic weight and BMI control.

Screening should take place at least once for children in this age group to identify those exposed to high risk. School-based interventions have proved to be successful at preventing or minimising obesity and obesity-related factors and should be the focus of the monitoring process (Jerum and Melnyk, 2001; James, 2008). Children identified as being at different levels of overweight risk, should have a second screening approximately two years after their weight control or maintenance counselling was implemented. This time span is a reasonable compromise between the need to identify weight gain at an early stage and the time, effort, and costs involved of repeated measuring.

## DISCUSSION

A high body mass index in childhood seems to track into adulthood, having a profound influence on morbidity and mortality in adult life. Early life factors play an important role in promoting adult obesity. The age of puberty or the adolescent growth spurt (one of the three critical periods during childhood) is central to the persistence of obesity into adulthood (Styne, 2001; Wang, 2001; Eriksson *et al.*, 2001). BMI changes during adolescence are the most critical indicators of adult adiposity (Styne, 2001).

As the children are our future adult population, it is of utmost importance to attend to the increasing problem of childhood obesity. The need to estimate the prevalence of overweight in children and adolescents is recognised as a critical step in the process of identifying high-risk groups (Wang, 2001; Ebbeling *et al.*, 2002; James, 2008). As the treatment of adult obesity is mostly unsuccessful, health care providers should implement assessment strategies (such as screening tools) to identify children that are prone to overweight/obesity to reduce the prevalence of overweight/obesity at an earlier stage (Sallis *et al.*, 2000; Jerum and Melnyk, 2001; James, 2008). This screening tool could be used effectively to screen children at schools, healthcare clinics or by general practitioners to identify risk and to verify the level of healthcare involvement or the need for referral to prevent overweight/obesity. Weight status could be addressed efficiently as a preventive measure aimed at lowering chronic diseases of lifestyle later in life. A similar model could be used to develop a screening tool for identifying New Zealand children at risk of overweight/obesity.

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# **Obesity prevention strategies in Counties Manukau**

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# ABSTRACT

**Background:** The Let's Beat Diabetes (LBD) Benchmark Survey 2007, of 2520 residents aged 16 years and over in Counties Manukau, was undertaken to inform obesity and diabetes prevention strategies, including planning the allocation of the Nutrition Fund to support more healthy food environments in schools and early childhood education services (ECES).

**Objectives:** To provide accurate information for programme planning for LBD about beliefs, attitudes and practices about nutrition, physical activity, health and diabetes within demographic groups and included questions about how to reach the many different ethnic groups in this area.

**Outcomes:** Most respondents were interested in eating more healthily, including those in high deprivation areas and those responsible for children. The survey data have influenced programme planning for Nutrition Fund grants in the education sector to improve food environments, knowledge about food and food preparation practices to increase consumption of more healthy foods and drinks.

**Conclusions:** The LBD Benchmark Survey information has shaped interventions across the LBD action areas in Counties Manukau. These include disbursement of the Nutrition Fund to improve knowledge and practices of healthy eating to prevent the development of overweight and obesity in children and young people in one hundred and nine schools and early childhood education services.

## **INTRODUCTION**

The Let's Beat Diabetes Programme (LBD) is a community partnership in the Counties Manukau region drawing on the active involvement of health promotion, primary health care, secondary health services, local and central government, the food industry, schools and early childhood education services (ECES), sports organisations, workplaces, churches and community organisations. Let's Beat Diabetes involves more than 70 different initiatives and integrated services in primary and secondary care. LBD aims to reduce, prevent or delay of onset of diabetes and increase the quality of life for people with diabetes for people in Counties Manukau.

LBD has a strong focus on Maaori, Pacific and South Asian peoples and those affected by high deprivation, as the prevalence rates for Type 2 Diabetes for these groups are significantly greater than the general population.

The Nutrition Fund, available to each District Health Board from the Healthy Eating, Healthy Action programme of the Ministry of Health, offers funding to create supportive environments and facilitate skill development in food and nutrition for schools and ECEs (including teacher release costs to facilitate attendance for professional development in food, nutrition and related curriculum as well as the direct provision of grants).

#### METHODS

The LBD Benchmark Survey 2007 surveyed 2520 residents about their beliefs, attitudes and practices around nutrition, physical activity, health and diabetes across Pacific, Maaori, Asian and Other/European groups. The research was undertaken to inform planning for prevention strategies around obesity and Type 2 Diabetes. The method used to gather information was by computer assisted telephone interviews (CATI), with an average call time of 24 minutes per call.

Language used in the telephone interviews was carefully framed to be comprehensible for those with English as a second language. Maaori, Pacific (Samoan or Tongan) or Chinese people were offered an interviewer from their ethnic group. 2520 Counties Manukau residents (59% female; 41% male) were interviewed. This included 594 Maaori, 712 Pacific (295 Samoan, 173 Tongan, 186 Cook Island Maaori, 62 Niuean and 58 'other Pacific'), 599 Asian (299 South Asian) and 988 Other, including New Zealand European.

Maaori and Pacific groups were over-sampled to ensure that the findings would be statistically reliable. Counties Manukau region comprises of higher percentages per capita of people identifying as Maaori, Pacific and South Asian when compared other regions in New Zealand. Ethnic group comparisons were based on agestandardised data, as some ethnic groups have younger populations.

LBD Benchmark Survey 2007 data have been used to inform the criteria for allocation of funds to education settings in Counties Manakau District Health Board (CMDHB) to prevent obesity in children in schools and ECES. Key outcomes of the Benchmark Survey provide information about beliefs about food, cooking methods and food choices within ethnic groups and across the Counties Manukau region. When developing the criteria for funding nutrition action plans of schools and ECES, opportunities for community outreach through the involvement of schools and ECES was noted, as well as the opportunities to modify knowledge and practice around food choices and food preparation within educational settings.

## RESULTS

Results from the Benchmark Survey indicated that the majority of people are interested in eating more healthily, including those in high deprivation areas and those responsible for children. Support for healthy eating was important to the majority of respondents. Many households were positively supported in healthy eating behaviour by children.

The results also indicated that even though there was an interest in eating more healthily there was generally a low awareness of what foods were healthy choices, and a perception that healthy choices were the more expensive option. This result suggested that there is still much work to educate children and parents on healthy choices and cost effective ways of making healthy foods more accessible for parents.

Fruit and vegetable knowledge equated to fruit and vegetable consumption. Respondents who mentioned knowledge of 5+ fruit and vegetable intake a day tended to consume at least that amount. Approximately 50% of respondents mentioned that fruit and vegetable consumption was related to a healthy weight. Some of the key outcomes are listed in the Tables 1-5.

Difficulty in eating more healthily	Sample interested in eating more healthily	Maaori	Pacific Peoples	Asian	Other
	(*1694)	(386)	(561)	(440)	(548)
	%	%	%	%	%
Interested, don't find it difficult	41	34	46	49	36
Interested, find it a little difficult	33	32	29	29	38
Interested, find it somewhat difficult	17	20	13	16	20
Interested, find it very difficult	8	13	12	6	6

Table 1: Difficulty in eating more healthily, by ethnicity

\*Based on those who are interested in changing (n=1694)

Table 2: Possible barriers to eating more healthily

Possible barriers to eating more healthily	Total*	Maaori	Pacific Peoples	Asian	Other
	(979)	(253)	(315)	(225)	(342)
	%	%	%	%	%
Can't afford the cost of healthier					
Agree	24	39	31	20	18
Disagree	43	36	32	47	49
I don't know enough about which foods are healthy for you		10	•	10	0
Agree	15	18	20	19	9
Disagree	56	59	46	44	65
There is not enough healthy food available in the places where I eat or shop					
Agree	12	12	17	16	7
Disagree	64	66	57	54	70

\*Note this question was only asked of those who had difficulty in eating more healthily (n=979)

Tuble 5. Tossible sources of si	аррон ю е	ai neainni y			
Sources of support to eat	Total	Maaori	Pacific	Asian	Other
healthily			Peoples		
	%	%	%	%	%
Other adults in your	72	65	75	77	70
household					
Children in your household	54	52	65	56	48
Your wider family/whanau	57	53	67	68	49
and close friends					
Your employer	34	31	52	38	24
People you work with	42	38	60	50	30
People at your church or	54	61	67	53	38
place of worship					
People at your marae in	NA	53	NA	NA	NA
Counties Manukau					
Doctor/medical centre staff	57	59	75	62	47

Table 3: Possible sources of support to eat healthily

Table 4: How	felt about	children's	food
10000 11 11000		0	10000

	*				
How felt about children's	Total	Maaori	Pacific	Asian	Other
food			Peoples		
	(1442)	(365)	(521)	(357)	(416)
	%	%	%	%	%
It could be a lot healthier	20	22	29	23	10
It could be a bit healthier	41	43	33	30	50
It is healthy enough	39	34	37	46	38
Don't Know/Refused/ Not	0	1	0	1	1
relevant					

\*This was only asked of those who are responsible for their children (n=1442)

Table 5a: Allocation of	funds to schools	from June	2007 to June	2008 = \$366,191
	J			

Decile of School	Number of schools funded 2007 /2008	Number of schools in this decile in CMDHB	% of schools funded within each decile	% of funds allocated
1	47	53	88.7	73%
2	13	30	43.3	11.5%
3	4	15	26.7	2.5%
4	2	16	12.5	5.7 %
5	2	6	33.3	2.2 %
6	2	8	25	1.5 %
7	4	13	30.8	1.8 %
8	4	10	40	1.1 %
9	-	11		
10	-1	30	3.3	0.7 %

Table 5	b. Allocation	of funds to EC	ES from June	2007 to Jun	$e\ 2008 = \$98\ 544$
1000000		of fundo to Lo		2007 10 Jun	$c_{2000} = \phi / 0, 0 m$

		,		
ECES funded	Numbers of the	% of ECES	Decile of	% of funds
from 2007 to	organisation	funded within	ECES	allocated
June 2008, by	type in CMDHB	the category		
type	area			
12 Te	40	30 %	1-3	43.4%
Kohanga Reo				
8 PI	38	21%	1-3	14.5%
Language				
Nests				
14	48	29%	1-8	29.4 %
kindergartens				
1 cluster of 12	27	44.4 %	1-10	3.3 %
Play Centres				
8 Daycare	203	2 %	5+	10.2%
ECES				

# DISCUSSION

The LBD Benchmark Survey 2007 information has informed programme planning for allocation of funds in the education sector to reduce the development of obesity in children by improving food environments, knowledge and practices to increase consumption of healthier food and beverages.

The finding that parents were interested in supporting their children to eat more healthily, especially those in lower socio-economic areas, offered schools and ECES the evidence to support 'a whole school approach' for nutrition education.

The finding from the LBD Benchmark Survey 'readiness to eat more healthily' has strongly influenced the Nutrition Funding Grant Process to support Nutrition Education Outreach programmes which connect healthy eating knowledge and behaviours learnt in Early Childhood settings with family and whanau.

Some initiatives funded to date include food preparation and cooking skills development in both schools and ECE settings; books and toys (ECES) about healthy

eating; development of edible gardens (a strategy to encourage participation and learning about the growth, harvest and preparation of plant food by children and their families and whanau); improving access to drinking water as the healthy beverage; support for Breakfast Clubs; student lead initiatives for peer education and support for ethnic specific food and nutrition outreach education for parents.

# CONCLUSION

Supporting changes towards more healthy food choices in educational environments such as schools and early childhood education services is a key strategy to reduce the risk of obesity and diabetes in vulnerable groups such as children, young people and their families. Information derived from the LBD Benchmark Survey has informed the criteria for funds allocation within this strategy in Counties Manukau District Health Board. This includes opportunities for strengthening outreach to parents, so they can support their children and young people.

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# The knowledge and use of sports drinks in talented adolescent athletes

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## ABSTRACT

This study investigated the nutritional knowledge and practices of talented adolescent athletes from five sports; basketball, football, netball, rugby, and underwater hockey. The study involved 98 participants (39 males, 59 females, mean age 16.64  $\pm$ 1.94 years). The participants' knowledge of sports drinks and hydration were investigated through a questionnaire. 96.9% of subjects agreed that dehydration affects sporting performance and 52.6% agreed that "a sports drink helps the body to retain fluid". 68% of subjects knew that a sports drink contain carbohydrates, although only 58.8% stated that a sports drink "replaced carbohydrates." 63.3% of participants knew that sports drinks contained sodium. 85.7% of the athletes reported using a sports drink, 26.8% of who reported using more than one sports drink; 82.9% used PowerAde and 23.2% used Horley's Replace. The reasons for use varied widely; subjects referred to being performance enhancing, but do not directly refer to their role in hydration, and prevention of dehydration. A number of participants reported they used a particular sports drink because: they "were told to"; the drink had "a good reputation"; "you think you are doing something good"; or referred to a drink's role in preventing cramps. The availability and advertising of different sports drinks also contributed to choice of drinks. More research is needed to identify the influences on use of sports drinks in this group and to determine the potential benefits and disadvantages of using sports drinks within this group.

# **INTRODUCTION**

It is known unequivocally that nutrition has an effect on sports performance (Croll et al., 2006, Burke, 2000). Sports nutrition is a rapidly growing field and is the science of improving sporting performance by tailoring specific food and fluid recommendations to an athlete. It is known that factors such as dehydration and a lack of energy supply can limit performance (Burke, 2000). Although there is an abundance of literature on sport nutrition practices in adults there is a dearth of research on sports nutrition in adolescent athletes (Bar-Or, 2001). In terms of sporting performance, dehydration is generally seen to be more harmful in young athletes compared to adults as the thermoregulatory strain is higher on the young athlete than that which is seen in an adult (Bar-Or, 2001). Although it is commonly suggested that a 2% decrease in bodyweight reduces physical performance by up to 20% in adults (Howe et al., 2002), it is thought that endurance performance is decreased in children by a 1% decrease in bodyweight, however exact levels of dehydration and their effect on a young athletes performance are yet to be established (Petrie et al., 2001). A young athlete will usually produce more heat when exercising than an adult, but the shift of heat to the skin for further transfer is less efficient in the young athlete and therefore the sweat rate is lower than that be seen in an adult (Bar-Or, 2001, Petrie et al., 2004). Sweat composition in

younger athletes is also different to that of mature adults (Bar-Or, 2001). There is less sodium and chloride loss seen in children and young adolescents compared to adults (Bar-Or, 2001, ADA, 1996) and consequently recommendations set for adults may not be suitable for this group.

# METHODS

#### **Participants**

Letters were sent to ten national sporting organisations (NSOs) describing the study and asking if they would give permission for their athletes to take part in the study. The ten sports were chosen as they encompassed a range of both team and individual sports, and sports which are more generally related to gender. Sporting organizations were asked to provide contact details of athletes who met the entry criteria for the project. The entry criteria for participants were that athletes needed to be between the ages of 13 and 20 years old as of the 1<sup>st</sup> of January 2007, and be considered a talented or elite athlete in their chosen sport by being a carded athlete, or part of a High Performance Academy Group, Regional Talent Identification Group or an equivalent Development group. Five sporting organizations agreed to take part in this research; Basketball New Zealand, Netball New Zealand, New Zealand Football (NZF), New Zealand Rugby Union and New Zealand Underwater Hockey (UWH). Sporting organisations that agreed to participate in the study were asked to forward contact details of athletes who met the criteria to an independent third party researcher. This preserved confidentiality of contact details. The study involved 98 participants (39 males, 59 females, mean age  $16.64 \pm 1.94$  years). The study was given ethical approval by the Massey University Committee of Ethics (ref 07/52).

#### Questionnaire

The questionnaire was developed to address four different aims. Section A aimed to assess the basic nutritional knowledge of the participants. Section B aimed to investigate influences on food choice and food availability in the participants. Section C was designed to assess the basic sports nutrition knowledge and practices of the participants and section D gathered basic demographic information related to the participants.

A pilot trial of the questionnaire was conducted with athletes of the same age and who participated in the same ten sports as the targeted population and changes were made to questions which were unclear before sending to the actual participants. Changes included rewording four questions so that they were appropriate for the age group in this study. To identify those athletes who had correct knowledge as opposed to those who had incorrect knowledge, and those who did not have any knowledge the questionnaire used 'yes', 'no' and 'unsure' answers.

#### Procedure

The independent researcher used the contact details from NSOs to create address labels for each contact. The contact details were used to send the initial questionnaire pack which included a participant information sheet, a participant consent form, a parental information sheet, a parental consent form, a questionnaire and a return envelope. The questionnaires were coded to identify which participants had returned their questionnaire. The independent third party researcher followed up the athletes who had not returned the questionnaire with a phone call. When the independent researcher received the unnamed questionnaires they were removed from the envelopes and passed on to the student researcher to ensure confidentiality of the participants. Analysis of the questionnaires was carried out using SPSS version 14.0

# RESULTS

79% of the participants considered sports nutrition to be very important in their sporting plans, while 16% considered it to be slightly important (2% considered it to be both very and slightly important), and 2% stated that it was not important. 88% of the participants stated that sports nutrition strategies could definitely improve their performance, whilst 12% answered that sports nutrition strategies may help in improving performance. No participants stated that sports nutrition strategies would not improve performance. The average rating of sports nutrition knowledge was  $5.87 \pm 1.58$  (out of 10; variance, 2.51). UWH participants had the highest rating of knowledge (7.0), followed by NZF (6.50).

When questioned about the role of water in the body, 48% of the participants correctly identified that the water does not make up 50% of the human body, while 48% knew that water was used as a transport system in the body. 83% knew that if you are thirsty you are already dehydrated, and 93% knew that the colour of urine is a good indicator of hydration status.

Tuble 1. Responses to do these help improve sporting perjormance								
Do these help improve sporting	Agreed YES	Agreed	Unsure					
performance?		NO						
Drinking water immediately before an	60.6%	32.3%	7.1%					
event								
Drinking a caffeinated energy drink	14.1%	76.8%	9.1%					
before an event								
Drinking a sports drink during the event 87.8% 7.1% 5.1%								
Drinking Coca Cola during the event	5.1%	87.8%	7.1%					
Drinking fruit juice during the event	21.6%	64.9%	13.4%					

Table 1: Responses to "do these help improve sporting performance"

Table	2.	Resp	onses	to	true/	false	auestions
IGUICI	<b>~.</b>	$\mathbf{r} \mathbf{c} \mathbf{b} \mathbf{p}$	Uniber	w	i i vi c/	Juise	question

Are these statements True or False?			Agreed True	Agreed False	Unsure	
Dehydration	can	affect	sporting	96.9%	1.0%	2.0%
performance						
Blurry vision is a sign of dehydration			75.8%	5.3%	18.9%	
The 'stitch' is a	a sign o	of dehydrat	tion	27.1%	46.9%	26.0%
Sports drinks c	ontain	carbohydr	ates	68%	15.5%	16.5%
Sports drinks contain salt			63.3%	12.2%	24.5%	
Sports drinks contain fat		22.4%	56.1%	21.4%		
					* (	2 values missing

85.7% of the athletes reported that they use a sports drink; 82.9% used PowerAde, 23.2% used Horley's Replace, 1.2% used Endura, and 8.5% used Gatorade. 26.8% of the athletes reported using more than one sports drink. No rugby players reported using Horley's Replace Reasons for using specific sports drinks were as follows; PowerAde "because it has a good reputation in sports drinks and tastes good", "told [it was] good by coach", its "nice, think you're doing something good", "thirst quenching and tastes nice", its "a good source of electrolytes for faster rehydration", "retain the losses of water in your body", "fast rehydration and recovery", and "don't know", and "because they tell us too". Horley's Replace "because I know it helps and they tell us too", "stops cramps", "for vitamins and minerals", "it tastes good", "to not get dehydrated, replace the electrolytes lost", to replace sodium etc that you lose". Endura "replaces my electrolytes". One subject answered with V\* "helps me personally". [\*N.B. V is not regarded to be a sports drink as it contains more than 8% carbohydrate].

Subjects were also asked to identify the functions of a sports drink. Over 50% of the subjects knew that a sports drink makes the body retain fluid and that it replaces carbohydrates. Only 37.1% knew that a function of a sports drink was to replace sodium, yet 50.5% agreed that a sports drink replaces sweat. When asked in another question 68% of the subjects stated that sports drinks contain carbohydrates, 63.3% stated that they contain salt (compared to 37.1% saying it replaces sodium), and 22.4% thought that they contained fat (8.2% of the subjects thought that a function of a sports drink was to burn body fat). 24.5% were unsure whether sports drinks contained salt, whereas 16.5% were unsure if sports drinks contained carbohydrates. Just over half of the subjects knew that sports drinks did not contain fat (56.1%).

## DISCUSSION

Sports drinks contain carbohydrates, water and electrolytes. Carbohydrates function to replace muscle and liver carbohydrate, which are otherwise limited stores of this muscle fuel. Water in sports drinks functions to replace water lost through sweating. The addition of carbohydrates to water increases the absorption of water in the digestive system (namely the small intestine), provided it is at the correct concentration (Maughan, 1998).

85.7% of the participants used a sports drink, with 26.8% reporting that they use more than one sports drink. There is limited research on the use of sports drinks in adolescent athletes. O'Dea (2003) found that adolescents regularly consumed supplements including sports drinks and caffeine and stimulating energy drinks. The adolescents had varying reasons for using these supplements. In terms of sports drinks the participants often had these instead of soft drinks and used them as "thirst quenchers and [as] drinks that taste good" (O'Dea, 2003, pg 103). The participants in O'Dea's research also stated that the sports drinks could be used to enhance sports performance, although none of them identified using a sports drink as a rehydration tool. Many of the athletes in this research identified sports drinks as being performance enhancing but limited numbers acknowledged that they were performance enhancing because they were a rehydration tool. When asked about the functions of sports drinks 58.8% of the athletes agreed "it replaces carbohydrates". 52.6% agreed that "it makes the body retain fluid", while 50.5% agreed that "it replaces sweat". Only 37.1% agreed that a function of a sports drink was to "replace sodium" and worryingly, 8.2% thought that a function was "it helps to burn body fat" and 12.4% said, "it replaces protein". Energy drinks were found to be very popular among the participants in O'Dea's (2003) research. As O'Dea (2003) states "they talked enthusiastically about the perceived beneficial effects that energy drinks have on their bodies and their sports performance" (pg. 103). Males especially were using the energy drinks as stimulants for sport. This study found that

one participant referred to an energy drink being a sports drink and this may highlight the need for education about the differences in these drinks. The results of this study mirrored the work of both Pratt and Walberg (1988) and Chapman and Toma (1997) by showing that many athletes believed that a sports drink was more effective for rehydration compared to water.

There is little research on what sports drinks adolescents choose to use, and why, especially in the New Zealand population. In this study 82.9% reported using PowerAde as a sports drink, which was much higher than the next ranked sports drink that was Horley's Replace used by 23.2% of athletes. While products like Horley's Replace are available in most New Zealand supermarkets, some chemists and specialist sporting shops, PowerAde is widely available in local dairies, shops, takeaway outlets as well as supermarkets. This may be part of the reasoning behind why such a high percentage of the adolescent athletes surveyed reported using PowerAde. As well as the easy availability of PowerAde, adolescents are known to be impressionable (Croll et al., 2006) and this may be a potential reason for the high usage of this sports drink in this research. Many adolescents are swayed by advertising. In New Zealand the national rugby team The All Blacks are vigorously sponsored by PowerAde and this is advertised widely. No participants from rugby reported using Horley's Replace, but virtually all used PowerAde. Many coaches were sighted as having recommended that the athlete use a sports drink, although 64.3% of the participants in this study noted that the coach should be involved in their nutrition education, recent research (Jacobsen et al., 2001) suggests that often they do not have high levels of nutritional knowledge themselves. This could be an issue when coaches promote sports drinks.

Whilst it is clear that sports drinks, that contain carbohydrates and sodium can enhance performance in adults by delaying dehydration and carbohydrate (muscle and liver glycogen) depletion (Maughan, 1998), the fact that younger athletes exhibit differences in sweat rate (Bar-Or, 2001, Petrie *et al.*, 2004), composition (Bar-Or, 2001) and the fact that they seem to exhibit a lower capacity for carbohydrate oxidation (Bar-Or, 2001, Petrie *et al.*, 2004), sports drinks that are formulated for adults may not be ideally suited to these younger athletes. There is a need for further research in this area as it has been shown that sports drinks are a popular ergogenic aid for younger athletes (O'Dea, 2003).

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# Does consuming berries reduce blood pressure in a hypertensive rat model?

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# ABSTRACT

Dietary flavonoids are thought to protect against cardiovascular diseases by acting as antioxidants and vasodilatants. The present study aimed to determine if dietary antioxidants from blackcurrants, boysenberries or blueberries reduce blood pressure in a hypertensive rat model. Forty Spontaneously Hypertensive Rats (SHR) male rats were fed diets containing 10% freeze-dried blackcurrants, boysenberries or blueberries for 35 days. Ten Wistar Kyoto (WKY) rats were fed a control diet for 35 days. Liveweight, food intake and systolic blood pressure were recorded weekly for each rat. The WKY rats gained more weight than the SHR rats. There was no significant difference in food intake between dietary treatments. The systolic blood pressure for the WKY rats did not change over the course of the study. The SHR rats had increasing systolic blood pressure during the study. The SHR rats fed blackcurrants and blueberries had lower blood pressure measurements (p=0.095) than the rats fed the control and boysenberry diets. The SHR rat is good model for hypertension, and in this study feeding diets rich in blackcurrants and blueberries reduced systolic blood pressure in rats.

# **INTRODUCTION**

Polyphenols (flavonoids, phenolic acids, tannins) are found in tea, wine, fruit and vegetables, and have been shown to be protective against cardiovascular disease, some forms of cancer, brain degeneration as well as having anti-inflammatory effects (Steer, 2006). Epidemiology studies have shown an inverse correlation with dietary flavonoid intake and long term mortality from coronary heart disease (Hertog *et al.*, 1993; Stoclet *et al.* 2004). Berries are a rich source of polyphenols, and also contain other bioactive substances, such as vitamin C, folate, potassium and soluble fibre. The flavonoids occurring in commonly consumed berries are different. For example, blueberries and cranberries contain predominantly proanthocyanidins, whereas blackberries, raspberries and strawberries contain predominantly elligitannins and these differences may result in different biological properties (Seeram, 2008). The consumption of berries could affect pathways related to cardiovascular health by several different mechanisms including antioxidation, enhanced production of vasodilating factors, and inhibition of pro-angiogenic factors (Stoclet *et al.*, 2004).

The aim of the present study was to determine the effect of daily berry fruit consumption on blood pressure using the spontaneously hypertensive rat (SHR) as an animal model. This strain of rats is used for studies in cardiovascular research and hypertension (Vaziri *et al.*, 2000; Duarte *et al.*, 2001; Takaki-Doi *et al.*, 2009) due to the development of high (greater than 200 mm Hg) systolic blood pressures with age. Wistar Kyoto (WKY) rats are used with this animal model as the normotensive control animals.

## MATERIALS AND METHODS

Forty male SHR rats and 10 WKY rats were purchased from Animal Resources Centre, Australia at 5 weeks of age. They were housed in groups of 3 rats per cage in shoebox cages and fed commercial pelleted feed for 14 days. At 7 weeks of age they were transferred to individual hanging cages and fed diet AIN-76A (American Institute of Nutrition 1977, 1980). The study was carried out with ethics approval (Application number 10728) from the AgResearch Grasslands Animal Ethics Committee, Palmerston North, New Zealand. The four experimental dietary treatments were based on diet AIN-76A and contained either no berries (control), freeze-dried and finely ground blackcurrant, boysenberry, or blueberry (Table 1).

At 8 weeks of age the rats were removed once a week from their cage and had the tail cuff placed over their tails and briefly inflated to pressure to accustom them to the blood pressure recording procedure. The rats were randomly assigned to the experimental diets with 10 rats per dietary treatment. At 10 weeks of age the rats were fed the experimental dietary treatments. The rats were offered 20 g per day for 35 days. Systolic blood pressure was recorded for each rat once every seven days. Systolic blood pressure was measured using a tail pressure cuff attached to ML125 NIBP controller (ADInstruments, Bella Vista NSW, Australia) as per the manufacturer's instructions. Live weight and food intake for each rat were recorded every 7 days.

The data for all endpoints were analysed by analysis of variance to examine the effect of diet. Levels of significance were calculated from F statistics for the various treatment effects and the least significant differences (LSDs) calculated at the 5% level to compare means.

Tuble 1. Ingreatent compositions (70) of experimental aless.								
Ingredient	Control	Blackcurrant	Boysenberry	Blueberry				
Lactic casein <sup>1</sup>	20.0	20.0	20.0	20.0				
Methionine <sup>2</sup>	3.0	3.0	3.0	3.0				
Sucrose <sup>3</sup>	37.5	27.5	27.5	27.5				
Fructose <sup>2</sup>	5.0	5.0	5.0	5.0				
Glucose <sup>2</sup>	5.0	5.0	5.0	5.0				
Wheaten cornflour <sup>4</sup>	15.0	15.0	15.0	15.0				
Cellulose <sup>5</sup>	5.0	5.0	5.0	5.0				
Corn oil <sup>6</sup>	5.0	5.0	5.0	5.0				
Mineral mix <sup>7</sup>	3.5	3.5	3.5	3.5				
Vitamin mix <sup>7</sup>	1.0	1.0	1.0	1.0				
Blackcurrant <sup>8</sup>		10.0						
Boysenberry <sup>8</sup>			10.0					
Blueberry <sup>8</sup>				10.0				

Table 1: Ingredient compositions (%) of experimental diets.

<sup>1</sup> Alacid, Lactic casein 30 mesh, NZMP Ltd, Wellington, New Zealand

<sup>2</sup> Sigma, Sigma-Aldrich Inc, St Louis, MO, USA

<sup>3</sup> Caster sugar, Chelsea, New Zealand Sugar Company Limited, Auckland, NZ

<sup>4</sup> Wheaten cornstarch, Golden Harvest, Primary Foods Ltd, Auckland, New Zealand

<sup>5</sup> Arbocell B600, J. Rettenmaier & Söhne GmbH + Co, Rosenberg, Germany

<sup>6</sup> Tradewinds, Davis Trading, Palmerston North, New Zealand

<sup>7</sup> For details see American Institute of Nutrition (1977, 1980)

<sup>8</sup> "Freeze dried and finely ground

# RESULTS

The rats were healthy and all gained weight. There was no significant effect of diet (p=0.455) on percent weight gain but there was a significant effect of rat genotype (p<0.001) with the SHR rats having a lower weight gain than the WKY rats (Figure 1). There was no significant difference in food intake between treatments (data not shown). The WKY control rats had stable systolic blood pressure during the 35 day experimental feeding period, while the SHR rats had increasing systolic blood pressure over the 35 days (Figure 2). SHR rats fed the blackcurrant and blueberry diets had significantly lower blood pressure measurements (p=0.095) than those fed the control and boysenberry diets (Figure 3).





Figure 2: Effect of dietary berryfruit on systolic blood pressure (mm Hg) in rats during a 35 day experimental feeding period. Values are means  $\pm$  SEM, n=10.



*Figure 3: Percent systolic blood pressure change (%) in rats fed berryfruit over a 35 day experimental feeding period \* Average LSD between groups within days* 



# DISCUSSION

In the present study, we demonstrated that SHR rats have higher systolic blood pressure compared with the WKY rats at 10 weeks of age onwards, and the systolic blood pressure in the SHR rats increased with age over the 35 day experimental feeding

period. The systolic blood pressure measurements in the present study were similar to those found by Vaziri *et al.* (2000), Duarte *et al.* (2001), and Takaki-Doi *et al.* (2009). The SHR rats fed the blackcurrant and blueberry diets showed a lower percentage change in systolic blood pressure than the SHR rats fed the control and boysenberry diets. In other studies using SHR rats, Maruyama *et al.* (2009) found flavonoids in propolis reduced blood pressure, and Duarte *et al.* (2001) found that the flavonoid quercetin reduced heart rate, systolic, diastolic, and mean arterial blood pressure. A human clinical study found that the daily consumption of moderate amounts of berries resulted in favourable changes in platelet function, HDL cholesterol and blood pressure (Erlund *et al.*, 2008).

Animal studies on antihypertensive effects of drugs or food use the normotensive WKY rats as controls and the hypertensive backcross of WKY, the SHR rat. The SHR rat develops hypertension between 12 and 20 weeks of age and shows symptoms of hypertension and hypertension-related organ damage as seen in human patients. There has been concern in the use of this model due to the restrainers required during the tail cuff measurement of blood pressure (Grundt *et al.*, 2009). In the present study, we did not use restrainers but allowed the rats to move freely under a towel on the bench during blood pressure measurements to reduce this possible source of stress to the animals.

These results provide evidence that diets high in blackcurrants and blueberries could prevent or reduce hypertension in humans. Future feeding trials using SHR rats and whole foods should extend the feeding and recording beyond the 35 days used in the present study.

## ACKNOWLDEGEMENTS

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## Harakeke (*Phormium tenax*) seed oil

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# ABSTRACT

Harakeke seeds and oil are a resource that has not been investigated in detail. The seeds contain 20% oil which contains 70.9% linoleic acid (C18:2). The seed composition of 25 different cultivars of harakeke were also characterised. In addition, bulk samples of harvested in 2004 and 2005 were stored until 2006 to evaluate storage effects on seed composition. The linoleic content ranged from 67.3 to 70.9% and the oleic acid content ranged from 12.1 to 21.7%. The fatty acid content remained constant during storage while the total tocopherol and total phytosterol content were considerably reduced during storage.

## INTRODUCTION

New Zealand flax, Phormium spp., grows wild in virtually all regions of the country and can grow in almost any type of soil (Hector, 1889). There are two species of New Zealand flax, *Phormium tenax* and *Phormium cookianum*, commonly known by their respective Maori names harakeke and wharariki (Scheele, 1994). Phormium has considerable morphological and genetic variability and Maori selected and cultivated forms with superior leaf and fibre properties. The leaves and extracted fibre of both *P. tenax* and *P. cookianum* were extensively used by Maori people to make ropes, baskets, fishing lines and were woven and dyed into matting and garments (Scheele, 1994). Early European colonists recognized the fibre qualities of harakeke and developed an industry exporting fibre processed into cordage, wool-bales, carpeting and coarse textile for the domestic market (Jones, 2003). However, no evidence exists to suggest that the seeds were used as a source of food or oil (Scheele and Walls, 1994), apart from a 19<sup>th</sup> century reference to harakeke seeds forming "an excellent substitute for coffee" (Taylor, 1870).

Both *P. tenax* and *P. cookianum* are widely referred to as New Zealand flax, flax, phormium flax, native flax and New Zealand hemp (McIvor, 1980; Macdonald, 1973; Critchfield, 1951). The use of the name New Zealand hemp has led to some confusion with hemp (*Cannabis sativa*), which can provide a similar fibre material to harakeke. The use of the word flax or flax seed oil can also lead to confusion. Harakeke seed oil is very different from true flax seed oil, which is derived from the seeds of the plant *Linum usitatissimum* L. Flax seed oil (*Linum usitatissimum* L.) is better known as linseed oil.

Morice (1962) found that harakeke seeds contained 29% oil that consisted of 10-15% oleic and 75-81% linoleic acids, similar to the composition of sunflower, safflower and maize oils. Therefore, Phormium lipids contain approximately 70% of polyunsaturated fatty acids (PUFA) such as linoleic (18:2) and small amounts of alpha-linolenic (18:3) acids. No studies have been carried out to identify differences in fatty acid contents between the different cultivars. Morice (1969) estimates that a potential

yield of 155-163 kg of linoleic acid/hectare from harakeke may be possible and commented that the harvesting of the seed should not present any difficulties.

In a later study, Morice (1971) evaluated the nutritional value of harakeke seed oil by feeding the oil as part of the diet to weanling rats. She showed that the weanling rats had excellent growth rates and no adverse or ill effects where observed when either harakeke oil or whole seeds were fed over a 70 or 90 day feeding trial. Recently, Wharemate (2003) evaluated the potential for commercial and industrial uses of 46 different native New Zealand species of plant seed oils. Harakeke seed oil was again identified as a potential source of linoleic acid. Unfortunately, the origin and the species of the harakeke seeds were not identified in this study.

Harakeke seed oil warrants further investigation into potential use as an edible seed oil, based on previous studies, with potential functional food status and possible nutritional benefits from consumption. Hence the objective of the study was to determine shelf life stability, year to year variation and chemical constituents of the oil.

# MATERIAL AND METHODS

All chemicals and solvents used in the following studies were of analytical grade and purchased from VWR International Ltd, Leicestershire, U.K. unless otherwise stated.

#### Characterization of harakeke seed oil produced from three consecutive years

Bulk samples (>5kg fresh weight) of harakeke seed were harvested from mature plants in a plantation consisting of more than 1,000 individual bushes of harakeke (*P. tenax*), of mixed maturity, at Matapihi Road, Wairarapa, New Zealand (S  $40^{\circ}$  54.1', E  $175^{\circ}$  41.8'), in March of 2004, 2005 and 2006. Samples were stored in hessian sacks in a cool dry storeroom. In October 2006 samples were evenly mixed prior to analysis and sub-sampled for total fat, fatty acid profile, tocopherol, phytosterol and phytosterol oxidation product analysis. In addition, *P. cookianum* seeds were harvested from a site in the Manawatu Gorge area (S  $40^{\circ}$  20.2', E 175 48.1') approximately 98 km North of the Matapihi road site. This sample was stored under the same conditions and sampled at the same time. Harakeke seed from the 2005 harvest was also cold pressed by a commercial seed oil extractor (Glenn Vile, BayOils Ltd., Blenheim, New Zealand).

## **Rene Orchiston Collection**

Seed from twenty five identified cultivars of the species *P. tenax* and *P. cookianum* were harvested in March 2006 from the Rene Orchiston Collection of weaving cultivars held at Landcare Research, Lincoln, Canterbury, New Zealand. This collection contains fifty selected forms of harakeke and a few hybrids as characterized by their seed pod morphology (Scheele and Walls, 1994). The fatty acid profile of each cultivar was determined following extraction of the oil.

## Lipid analysis

Lipid samples were extracted from harakeke seed using a modified method (Savage *et al.*, 1997). In brief, approximately 10 g of seed were extracted in 30 mL of hexane/isopropanol (3:2 v/v) at room temperature, in steels tubes containing four steel ball bearings, for 1 hour. The homogenate was then centrifuged and the supernatant washed with 20 ml of 6.7% w/v aqueous sodium sulphate. The supernatant was then

transferred into 100 ml round-bottom flasks and evaporated to dryness using a Büchi Rotovapor-R (Postfach, Switzerland) set at 40°C, samples were stored under  $N_2$ (oxygen free) at -20°C prior to analysis.

Total lipid content was determined gravimetrically, using an automated soxhlet extraction (Tecator Soxtec 1043, Foss Tecator AB, Höganäs, Sweden) in accordance to AOAC methods (2002).

Lipid samples were methylated prior to GLC analysis according to the method described by Azadmard-Damirchi and Dutta (2008). GC analyses were performed with a Chrompack CP 9001 gas chromatograph (Chrompack, Middelburg, The Netherlands). The GC was equipped with a flame ionisation detector and split/splitless injector. A 50 m 9 0.22 mm, 0.25 mm film thickness fused-silica capillary column BPX70 (SGE, Austin, TX, USA) was used for analysis. Injector and detector temperatures were 230 and 250°C, respectively. Oven conditions were 158°C increasing to 220°C at a rate of 2°C/min and maintained for 5 min. Helium was used as a carrier gas and nitrogen as a make-up gas at a flow rate of 30 mL/min. FAMEs were identified by comparison of their retention time with standard FAMEs. The peak areas were integrated by Maestro version 2.4 (Chrompack, Middelburg, The Netherlands) and reported as a percentage of total fatty acids.

# Tocopherol and phytosterol analysis

Tocopherols and tocotrienols were analysed by high pressure liquid chromatography (HPLC) according to the previously published method Dutta et al., (1994) with slight modification by Azadmard-Damirchi and Dutta (2008). A 7725 Rheodyne Injector fitted with a 20 µL loop and connected to a 510 HPLC pump (Waters, Milford, CT, USA) was used. The column used was LiChroCART 250-4 packed with LiChrosphere 100 NH 2,5µm particle size and coupled to a guard column LiChroCART 4-4 (Merck KGaA, Darmstadt, Germany). Approximately 10 mg fats were dissolved in 1 mL n-heptane and 10 µL was directly injected into the HPLC column. The isocratic mobile phase was а mixture of nheptane/tertbutylmethylether/tetrahydrofuran/methanol. Tocopherols and tocotrienols were detected by fluorescence detector Varian 9070 (Walnut Creek, CA, USA) at a wavelength of 294 and 320 nm for excitation and emission, respectively. Identification of peaks was performed by the comparison of retention times to a standard reference mix of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  to copherols and  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  to cotrienols (Merck, Darmstadt, Germany).

Phytosterol analysis of extracted oil samples was performed according the method described in Azadmard-Damirchi *et al.*, (2005). Quantification was performed by using  $\alpha$ -cholestane (Sigma-Aldrich, St Louis, USA) as an internal standard and results calculated relative to  $\alpha$ -cholestane.

## Statistical analysis

All Statistical analysis and calculations were performed using Minitab version 15.1 (Minitab Ltd, Coventry, UK) and Microsoft® Office Excel 2003.

## **RESULTS AND DISCUSSION**

#### Inter-species comparison

The total fat and fatty acid composition of the seeds harvested in 2004, 2005 and 2006 are shown in Table 1. The mean total fat content of *P. tenax* was 27.4%, which is lower than the value for *P. cookianum* (32.0%). These values are comparable to an early study by Morice (1965) who reported a total fat content of 29% for *P. tenax*.

Table 1: Fatty acid profile of P. tenax seed harvested from the same location, for three consecutive years and P. cookianum seed harvested in 2005.

Year	Fatty acid composition (%)							
	Fat (%)	C16:0	C18:0	C18:1 (n-9)	C18:1 (n-7)	C18:2 (n-6)		
2004	26.3	9.8	2.7	15.2	1.0	70.7		
2005	26.7	9.6	2.5	15.4	1.1	70.7		
2006	29.1	9.2	2.7	15.6	0.9	71.2		
Mean	27.4	9.5	2.6	15.4	1.0	70.9		
2005*	32.0	7.4	3.5	19.8	0.8	67.3		

\* P. cookianum

The individual fatty acid content of pooled samples from the same harvest year (2005) for P. tenax and P. cookianum are shown in Table 2. This is the first time a fatty acid profile for the two different species of Phormium has been performed. It would appear there are minor differences and repeated analysis from a larger sampling range of identified species and locations would have to be performed to confirm this. Linoleic acid (C18:2) was the major fatty acid (mean 68.8%) with a range from 67.3 to 70.9% for P. tenax and P. cookianum, respectively.

Differences between the two species for the four major fatty acids present, palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) were 2.8, 0.5, 4.9 and 3.0%, respectively. The fatty acid profile in this study compares well with the results of Wharemate (2003). Wharemate (2003) reports 10.0, 3.9, 17.0 and 68.0 % for C16:0, C18:0, C18:1 and C18:2, respectively, on an unidentified provenance of harakeke. The fatty acids C14:0, C16:1 and C18:3 were detected, but at levels less than 0.1% (data not shown). Harakeke seed oil, with linoleic acid content greater than 60%, is comparable to safflower and sunflower seed oils, which have approximately 77 and 68% linoleic acid respectively (Wharemate, 2003). Recent work on safflower seed oil by Rahamatalla *et al.*, (2001) reports between 54.7 to 70.5% linoleic acid and Perretti *et al.*, (2004) reports between 42.33 to 63.45% linoleic acid.

Individual fatty acid analysis of 25 different cultivars from the Rene Orchiston collection showed no large differences in fatty acid profile (Table 2).

Cultivars	C16:0	C18:0	C18:1	C18:1	C18:2
			(n-9)	(n-7)	(n-6)
Aohanga	8.4	2.2	19.0	1.3	68.4
Atewhiki	8.6	2.1	15.6	1.1	71.6
Awahou	7.8	2.3	12.5	0.9	75.6
Kōhunga	7.4	2.4	14.6	0.7	74.4
Māeneene	9.0	2.6	19.8	1.2	66.8
Motu-o-nui	10.0	2.2	15.1	1.4	70.8
Ngutunui	9.7	2.1	14.1	1.3	72.1
Opiki	9.5	2.0	14.1	1.3	72.8
Oue	8.1	2.2	13.3	1.1	74.4
Pango	8.5	2.5	14.2	1.0	72.8
Rauhine	9.0	1.9	15.7	1.1	71.9
Raumoa	8.7	2.3	11.2	1.1	75.4
Rehutai	9.8	2.2	12.9	1.1	72.9
Ruapani	9.1	2.1	13.8	1.2	72.8
Ruawai	8.8	2.7	16.2	1.1	70.4
Taeore	8.2	2.3	12.1	1.2	76.1
Tukura	8.5	3.1	21.7	1.0	64.7
Tāpoto	9.4	2.3	17.3	1.2	68.8
Taumataua	10.7	2.4	17.4	1.4	66.0
Te Mata	7.9	2.1	12.7	1.0	75.6
Te Tatua	9.5	2.4	13.1	1.2	72.4
Tūtaewheke	8.8	2.7	18.8	1.2	67.8
Wharariki- 62*	8.5	2.0	19.3	1.4	68.2
Wharariki- 41*	9.0	2.3	14.0	1.1	71.9
Whareongaonga	8.7	2.8	12.2	1.1	74.5
Mean	$8.9\pm0.1$	$2.3\pm0.1$	$15.2\pm0.6$	$1.1\pm0.1$	$71.6\pm0.6$

Table 2: Fatty acid profile of harakeke seed harvested from the Rene Orchiston Collection.

#### \* A cultivar of *P. cookianum*

The predominant fatty acid for all cultivars was linoleic acid (C18:2), with *Tukura* having the least amount (64.7%) and *Taeore* the most (76.1%), with the overall mean being 71.7% (Table 2). The other most abundant fatty acid was oleic acid (C18:1), with a mean content of 15.2%. Cultivars *Raumoa* and *Tukura* had the least (11.2%) and most (21.7%) oleic acid (C18:1), respectively. The fatty acids myristic (C14:0) and palmitoleic (C16:1) were detected, but in levels less than 0.1% (data not shown).

## Characterisation of harakeke seed oil produced from three consecutive years.

The total fat which ranges from between 26.3 to 29.1% (mean 27.4%) is low when compared to walnuts, hazelnuts, macadamia nuts and almonds grown in New Zealand, which have reported total fat contents of 66.1, 58.4, 73.8 and 54.3% respectively (Savage, 2000). From Table 2, it can be seen that over the three years the fatty acid profile remains stable with no changes occurring during storage.

Stránský et al., (2005) stored blackcurrant seeds for 10 years and upon fatty acid analysis observed no loss or degradation of linoleic acid. The harakeke seed oil

results are consistent with this observation; however other factors such as tocopherol content were not explored by Stránský *et al.*, (2005).

All four forms of tocopherol were detected in the samples and one  $\beta$ -trienol (Table 3). Harakeke seed oil consists mainly of  $\beta$ -tocopherol (mean 77.7%). The total tocopherol content ranges from 52.4 to 117.3 mg/100 g oil, which is high, compared to pumpkin seed oil, which ranges from 33 to 91 mg/100 g oil and grape seed oil reported to be 45 mg/100 g oil (Vanhanen *et al.*, 2005; Vanhanen and Savage, 2000). The total tocopherol content declined by 55% over the three years storage. The presence of tocopherol in an oil seed aids the resistance of the oil to oxidation. The decline in total tocopherol content indicates the importance of correct storage conditions and timely storage to maximise the potential of the positive functional properties of the oil.

The presence of phytosterols in plant seeds is often reported as the percentage of the unsaponifiable matter. This has the disadvantage that an assumption is made that all the unsaponifiable content are sterols. Using the modified method of Savage *et al.*, (1997) four phytosterols were correctly identified. Table 4 shows sitosterol as the most abundant (mean 63.7%), with the total phytosterol content ranging from 311.1 to 516.7 mg/100 g oil. There was also a reduction of 39% in total sterol content from 2006 to 2004. In comparison, Rahamatalla *et al.*, (2001) reports an unsaponifiable fraction of between 1.0 and 1.3% for safflower seed oil, which is more than twice as much as harakeke seed oil. However, Rahamatalla *et al.*, (2001) also reported that 52.8 to 56.1% of the unsaponifiable fraction was identified as sterols, which is comparable to the harakeke seed oil in this study.

In a recent survey of the phytosterol composition of commonly consumed nuts and seeds (Philips *et al.*, 2005) sunflower seeds are recorded to have an average value of 271 mg phytosterols/100 g. The highest recorded seed was wheat germ with an average of 413 mg phytosterol/100 g. This is comparable to 516.6 mg phytosterol/100 g oil obtained from fresh harakeke seed oil harvested in 2006 (Table 3). Even after loss of total phytosterol content due to storage, the sterol content of harakeke seed oil was high compared to other nuts and seeds.

Increased daily consumption of phytosterols has been shown to be beneficial to health by blocking the absorption of cholesterol and hence helping to reduce levels of blood cholesterol (USFDA, 2000). With the promotion of increasing the consumption of phytosterols in the diet, there is also the potential to increase the consumption of oxyphytosterols (oxidised forms of phytosterols). It is postulated that since the structure of phytosterol oxidation products (POPs) may have detrimental effects, similar to cholesterol oxidation products (COPs), which have been implicated in atherogenesis, cytotoxity, mutagenesis and carcinogenesis (Hovenkamp *et al.*, 2008). The study of the development of POPs in oils has centred around oil production quality issues, such as, during accelerated aging by heat (Johnsson and Dutta, 2006) and interesterification (Azadmard-Damirchi and Dutta, 2008).

# CONCLUSIONS

This study clearly shows that harakeke seed oil remains stable over three years of storage, even though the seed oil contains high levels (68.8%) of linoleic acid. There were no major differences in the fatty acid content among 25 different cultivars evaluated. Compared to other seeds and seed oils, harakeke seed oil has high total tocopherol and total phytosterol contents, with the predominant tocopherol being  $\beta$ -
to copherol (77.7%) and the predominant phytosterol being  $\beta$ -sitosterol (63.7%). The reduction in total sterol and total to copherol contents over storage for three years was modest.

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Year Tocophe mg/100 g oil (9			rol 5 of total)		Total tocopherol	Phytosterols mg/100g oil (% of total)				Total phytosterol	
	α	β	γ	δ	β- trienol	(mg/100 g oil)	Campesterol	Stigmasterol	Sitosterol	$\Delta^5$ - Avenasterol	(mg/100 g oil)
2004	1.4 (2.9)	41.2 (78.6)	0.8 (1.5)	6.6 (12.6)	2.4 (4.5)	52.4	22.1 (7.1)	37.4 (12.1)	198.2 (63.7)	53.4 (17.2)	311.1
2005	12.9 (11.6)	86.3 (77.8)	2.0 (1.8)	6.5 (5.9)	3.2 (2.9)	110.9	29.8 (7.7)	44.7 (11.6)	248.5 (64.5)	62.1 (16.1)	385.1
2006	11.6 (9.9)	90.1 (76.8)	2.7 (2.3)	9.3 (7.9)	3.6 (3.0)	117.3	40.3 (7.8)	63.5 (12.3)	323.5 (62.6)	89.4 (17.3)	516.7
Mean	8.6 (8.1)	72.5 (77.7)	1.8 (1.9)	7.5 (8.8)	3.1 (3.5)	93.5	30.7 (7.5)	48.5 (12.0)	256.7 (63.6)	68.3 (16.9)	404.3

Table 3: Tocopherol and major phytosterol composition of harakeke seed oil, harvested from three consecutive years.

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# Predicting glycaemic responses from *in vitro* digestion of food carbohydrates using dose-sensitive baselines for glucose homeostasis

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# ABSTRACT

The aim of this work was to produce a valid *in vitro* method for measuring the glycaemic impact of foods that would accurately mimic *in vivo* responses. Baselines representing cumulative glucose disposal were generated from the rates of decline in blood glucose concentrations after postprandial blood glucose surges in response to three intakes of each of five foods and glucose references. Cumulative sugar release from the same foods was also measured, by *in vitro* digestion of 2.5 g of the chewed foods, and the results extrapolated to the quantities of test foods consumed *in vivo*. The sugar release curves were converted to glycaemic glucose equivalents (GGE), the baselines of cumulative glucose disposal were inserted, and the net glycaemic impact measured as the area between the GGE release and glucose disposal curves, and converted to GGEs by comparison with a reference (one slice of white bread) of known area per GGE. This approach allowed *in vitro* digestive analysis to mimic, and provide an accurate prediction of, blood glucose responses:

GGE *in vivo* = 0.999GGE *in vitro* + 0.65;  $R^2 = 0.88$ 

# **INTRODUCTION**

Glycemic responses to foods reflect the balance between blood glucose loading and blood glucose disposal. Current *in vitro* digestion methods for predicting relative glycaemic responses to foods measure carbohydrate that is rapidly available for glycaemic loading, but ignore the homeostatic role of insulin-driven glucose disposal, and its dependence on the amount of blood glucose loading. With the need for *in vitro* methods that predict the relative glycaemic effect of realistic intakes of food, such as the *recommended amount customarily consumed* (RACC), or servings, methods that take account of the homeostatic response to dose are required.

The aim of the work described here was to develop a dose-sensitive *in vitro* method for measuring the relative glycemic impact of food intakes customarily consumed by humans that reflected the net balance between *in vitro* digestive release of glycemic glucose equivalents (GGE) from food quantities consumed and the rate at which the consumed GGEs would be disposed of in the body.

# **METHODS**

Five foods, white bread, fruit bread, muesli bar, mashed potato and chick peas, were each consumed at three intakes by 20 volunteers. Blood glucose response (BGR) curves were obtained, and by comparing incremental areas under the curves (iAUC) for the foods with those from glucose references, the iAUCs were converted to glycaemic glucose equivalents (GGE) (Venn *et al.*, 2006). Glucose disposal baselines could then

be generated from rates of glucose (GGE) disposal as a function of GGE intake (equation in **Figure 1**), which had been calculated from the rate of iAUC loss from under the declining *in vivo* BGR slopes, converted to GGE loss per minute. Timed release of carbohydrate during *in vitro* digestion of the foods was also measured as glucose equivalents (Mishra *et al.*, 2008), and by adjusting for the glycaemic potency of the constituent sugars, were converted into *in vitro* GGE/g. These were then multiplied by the weights of foods consumed to provide values for GGE loading against time (**Figure 2**). Relative glycemic responses could then be determined as the area between the *in vitro* GGE loading curves and dose-dependent glucose (GGE) disposal baselines (**Figure 2**). By comparing these areas with the area for a reference (white bread) with a clinically predetermined GGE per serving, the areas could be converted to GGE per weight of food consumed.

# RESULTS

The prediction of relative glycaemic response *in vivo* (GGE) from digestive release of glycaemic carbohydrate *in vitro* improved markedly when the glycaemic potency of the sugars and the rate at which the GGEs would be disposed of *in vivo* were taken into account (compare Figures 3A and 3B). An almost 1:1 correspondence was achieved; the relationship between *in vitro* and *in vivo* determinations of GGE was GGE*in vivo* = 0.99GGE*in vitro* + 0.75 ( $R^2 = 0.88$ ) when based on areas between GGE release and glucose disposal curves.

An improved prediction is achieved by inserting a dose-sensitive baseline for glucose disposal is seen in the Bland Altman plots (Figures 4A *vs.* 4B). The difference between GGE release and GGE disposal with time mimicked the *in vivo* glycaemic response profiles for the three intakes of the five test foods consumed is shown in Figure 5.





Figure 2: Curves of in vitro digestive release of glucose equivalents per gram, adjusted to glycaemic glucose equivalents (GGE) per gram, extrapolated to food intakes of 50, 100, and 150 g, and with straight-line baselines representing glucose disposal in vivo as a function of GGE intake (Figure 1) inserted. The glucose disposal lines were calculated (equation in Figure 1) from GGE disposal rates based on GGE released up to 60 min digestion. The GGE curves are displaced to the right by 10 min compared with in vitro to allow for an approximately 10 min lag between food consumption and initiation of the glycaemic response to food intake.





Figure 3: Comparison of GGE in vitro alone (A) with GGE in vitro adjusted for glucose disposal (B), as predictors of glycaemic response in vivo.



Figure 4: Bland-Altman plots showing the improved correspondence between in vitro and in vivo values when cumulative glucose disposal is taken into account (B) compared with prediction of glycaemic responses from digestive release of GGE at 20 min (A).

Figure 5: Profiles for in vitro GGE release minus glucose disposal (A. In vitro) and for clinical blood glucose responses (B. In vivo), for five foods at three intakes, showing the ability of in vitro digestive analysis to mimic in vivo responses when homeostasis is allowed for



#### DISCUSSION

By accounting for homeostatic control of blood glucose, and its dependence on the amount of blood glucose loading, the results of *in vitro* digestion can be made to mimic the human blood glucose response and provide a more accurate *in vitro* prediction of the *in vivo* glycaemic response to foods. Because we have expressed the rates of glucose disposal relatively, as GGEs, the values obtained here and plotted in Figure 1 should be generally useable. Indeed, when the results are normalised to the response to the common intake of 50 g glucose, the effects of homeostasis on blood glucose responses to glucose have been extremely consistent across a number of doseresponse studies (Monro and Shaw, 2008).

The area between the GGE intake and glucose disposal curves was used here to show that the *in vitro* results mimicked *in vivo* measurements. However, our results (not shown here) have also revealed that in practice an equally accurate prediction is obtainable by measuring the net GGE release (i.e. release minus disposal) at 20 min, based on 20 min (rapid) GGE release minus cumulative disposal at 20 min, the latter calculated from the rate of GGE disposal measured at 70 min when the highest measureable rate of blood glucose decline occurs.

#### CONCLUSION

Relative glycemic impacts of foods may be accurately measured in grams per quantity consumed, and in a way that mimics the human postprandial response, as the difference between cumulative GGE release from foods during in vitro digestion, and theoretical cumulative glucose disposal. The method accurately measures the relative glycaemic impact of realistic food intakes such as recommended amounts customarily consumed, and multiples of servings, without the need for equal carbohydrate portions.

## ACKNOWLEDGMENTS

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# Absorption and metabolism of red lettuce phenolics in rats

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# ABSTRACT

The absorption and metabolism of phenolic compounds (namely quercetin, luteolin and cyanidin glycosides) from a single meal containing a red lettuce extract was determined in rats over a 48-h period. Plasma, urine and faeces were collected at nine timepoints and analysed for flavonoid metabolites by LC-MS<sup>n</sup>. Several isomers of a mixture of methyl, glucuronide and sulfate conjugates of quercetin and/or luteolin were observed in plasma and urine. Free quercetin and luteolin were also present in urine and/or faeces. Neither anthocyanidins nor their glycosides were detected in plasma or urine but glycosides were detected in faeces. The majority of metabolites peaked at 8–12 h after ingestion of the meal in both plasma and urine. Although anthocyanins in red lettuce contribute to significantly higher antioxidant activity *in vitro* than in green lettuce, it appears that little or no absorption of anthocyanins occurred in these rats. However, both absorption and metabolism of quercetin and luteolin glycosides were observed.

## **INTRODUCTION**

Salads, and lettuce as their major component, have long been considered healthy foods. Lettuces are known to contain a diversity of antioxidant compounds, including phenolics, carotenoids, vitamin E and ascorbic acid (Nicolle *et al.*, 2004; Serafini *et al.*, 2002). However, anthocyanins, a subclass of colourful flavonoids with powerful antioxidant properties, are only found in red varieties. There has been much debate about the efficacy of antioxidants, such as flavonoids, including questions regarding their absorption, the effects of glycosylation, metabolism differences between subclasses of flavonoids, and the bioactivity of their metabolites.

Food flavonoids were assumed not to be absorbed because they are bound to sugars as  $\beta$ -glycosides and it was thought that only the free flavonoid aglycones (i.e. without a sugar molecule) could pass through the gut wall. However, recent research has shown that flavonoid glycosides are metabolised during absorption and the products of human flavonoid metabolism are glucuronides and sulfates of the aglycone and methylated derivatives. Flavonoid metabolism occurs predominantly in the intestine (gut epithelial cells and colonic microflora) and the liver. Deglycosylation of flavonoids to their aglycone occurs in the small intestine while there is evidence for *O*-methylation, sulfation and glucuronidation of hydroxyl groups in the liver. Circulating metabolites present in plasma are usually different from those ingested. For most flavonoid subclasses, these are glucuronidated, sulfated and methylated derivatives of the aglycone. However, anthocyanins appear to be absorbed and circulate in the plasma as the intact glycosidic form. Bacterial ring fission of flavonoids occurs in the colon and the subsequent degradation products are phenolic acids, which are consequently absorbed and found in urine (Hollman and Katan, 1997; Kroon, 2006).

Understanding the metabolism of these compounds in the body and elucidating their structures will allow their activity, bioavailability, efficacy and potential health benefits to be assessed. The objective of this study is to determine the absorption and metabolism of phenolic compounds from a single meal containing a red lettuce extract in rats over 48 h.

# **METHODS**

Following a screen of lettuce varieties, lollo rosso (*Lactuca sativa crispa* L.), a red loose-leaf lettuce, was selected for its high phenolic levels and *in vitro* antioxidant activity, compared to other varieties analysed. An extract of lollo rosso lettuce was prepared, which contained glycosides, acylated glycosides, and/or glucuronides of three major subclasses of flavonoids, namely quercetin (flavonol), luteolin (flavone) and cyanidin (anthocyanidin), as well as several phenolic acids (e.g. caffeoylquinic acids and caffeoyltartaric acids) as identified by LC-MS analysis.

Thirty-six healthy, male Sprague-Dawley rats (~200 g) were fasted for 16 h, and then offered a single meal containing an extract from lollo rosso lettuce (0.8 g) mixed with their normal lactic casein diet (1.2 g). Animal ethics approval was obtained from AgResearch Grasslands Animal Ethics Committee (No. 08/04). Plasma, urine and faeces were collected at nine time points over 48 h and analysed for flavonoid metabolites by LC-MS<sup>n</sup> following extraction (Day *et al.*, 2001; Gee *et al.*, 2004; Mullen *et al.*, 2006).

An LC-MS ion-trap (model LTQ, Thermo Finnigan, San Jose, CA) was used, with electrospray ionisation (ESI) in negative and positive ion modes.  $MS^n$  data were collected based on parent masses from 210–1500 m/z after elution via a PDA detector scanning 220–600 nm. Separation was performed on a Synergi-Fusion RP, 4 µm, 150 x 2.1 mm column with a 4 x 2 mm guard cartridge (Phenomenex Ltd, Torrance, CA) with a flow rate of 300 µL/min. The mobile phase consisted of a water/acetonitrile gradient acidified with formic acid.

# **RESULTS AND DISCUSSION**

Several isomers of methyl, glucuronide and sulfate conjugates of quercetin and/or luteolin were observed in plasma and urine (Table 1), indicating that deglycosylation and extensive conjugation (methylation, glucuronidation and sulfation) had occurred. Free quercetin, methylquercetin, luteolin and methyl-luteolin were also present in urine and/or faeces, indicating further deconjugation.

The majority of metabolites peaked at 8–12 h in both plasma and urine. In plasma, mono- and diglucuronides of quercetin, methylquercetin and luteolin peaked 8 h post-feeding, whereas sulfoglucuronides of quercetin and methylquercetin peaked 12–24 h post-feeding, indicating slower uptake into the bloodstream. Due to limited data, peaks could not be formally identified for faecal metabolites. However, no metabolites were observed in faeces prior to 8 h, and few metabolites were observed after 24 h.

No anthocyanins (free, glycosylated or conjugated forms) were detected in plasma or urine, but intact cyanidin glycosides were detected in faeces (Table 1) 12–24 h post feeding, indicating low bioavailability and differences in metabolism from other flavonoid subclasses. The literature reports that <0.1% ingested anthocyanins are excreted in urine (Manach *et al.*, 2005) and therefore the low levels present might have been difficult to detect in plasma and urine.

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Compound	Extract	Plasma	Urine	Faeces
Flavonols				
Quercetin			•	•
Quercetin glucoside	•			
Quercetin rutinoside or quercetin	•			
coumaroylglucoside				
Quercetin malonylglucoside	•			
Quercetin malonyldiglucoside	•			
Quercetin glucuronide	•	•	•	
Quercetin diglucuronide		•	•	
Quercetin glucuronide sulfate		٠	٠	
Methylquercetin			٠	
Methylquercetin glucuronide		•	٠	
Methylquercetin diglucuronide			٠	
Methylquercetin glucuronide sulphate		•		
Flavones				
Luteolin				•
Luteolin glucoside	•			
Luteolin glucuronide	•	•	٠	
Luteolin diglucuronide			٠	
Luteolin glucuronide sulfate			٠	
Methyl-luteolin glucuronide			•	
Methyl-luteolin diglucuronide			•	
Anthocyanidins				
Cyanidin glucoside				•
Cyanidin diglucoside	٠			
Cyanidin rutinoside or cyanidin	•			
coumaroylglucoside				
Cyanidin malonylglucoside	•			•
Phenolic acids				
5-Caffeoylquinic acid (chlorogenic acid)	•			
Dicaffeoylquinic acid	•			
Dicaffeoyltartaric acid	•			
Syringic acid			٠	
Vanillic acid			•	
3-Hydroxybenzoic acid			٠	
Dihydroxybenzoic acid				•
3-Hydroxyphenylacetic acid			•	
3-Methoxy-4-hydoxyphenylacetic acid			•	
(homovanillic acid)				
3-Hydroxyphenylpropionic acid			•	
Ferulic acid			•	
Catterc acid			•	
<i>p,m</i> -Coumaric acid			•	

 Table 1: Polyphenol metabolites identified in rat samples by LC-MS after ingestion of lollo rosso lettuce extract.

A number of phenolic acids were present in urine and faeces (Table 1) as a result of microbial degradation, which causes ring fission and thus breakdown of the flavonoid backbone. Metabolites of caffeoylquinic acids and caffeoyltartaric acids were also observed.

## CONCLUSIONS

Both absorption and metabolism of quercetin and luteolin glycosides were observed. The presence of conjugated derivatives of quercetin and luteolin in the plasma and urine of these rats indicated that deglycosylation (usually in the small intestine) and extensive conjugation (primarily in the liver and small intestine) occurred. Further deconjugation produced aglycones and methyl derivatives of quercetin and luteolin in urine and/or faeces. The presence of phenolic acids in both urine and faeces indicated that microbial degradation of quercetin and luteolin also occurred.

Although anthocyanins in red lettuce contribute to significantly higher antioxidant activity *in vitro* than in green lettuce, little or no absorption or metabolism of anthocyanins occurred in these rats and thus these compounds are not readily bioavailable in rats. Findings also confirm differences in absorption, metabolism and bioavailability between anthocyanins and other flavonoid subclasses found in red lettuce. Further studies are planned to assess the fate of anthocyanins labelled with a stable isotope at a physiologically relevant dose.

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# Effect of extraction method of grape seed on the protection from oxidative processes in beef patties

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# ABSTRACT

Grape seed extracts (GSE) are becoming very popular health supplements and their high antioxidant activity has been demonstrated in several food systems. This study compared the antioxidant activity of Pinot Noir grape seed extracts following extraction with water, ethanol or acetone. The model system used was the change in oxidative status of beef patties during a simulation of 7 days of display at 4 °C. All of the grape seed extracts delayed lipid oxidation in the patties. The acetone extract was the most effective followed by the water extract while the ethanol extract had very little effect (p<0.001). After 7 days of display, acetone extracted grape seed fraction had the highest lipid oxidation inhibition % (p<0.01). However the synthetic antioxidant, butylated hydroxytoluene (BHT), was more effective than any of the grape seed extracts in inhibiting lipid oxidation of beef patties. Water GSE and acetone GSE did not have any effect on the redness values despite lower metmyoglobin % during display time in water GSE treated patties and at the end of display in acetone GSE treated patties. Ethanol GSE was not effective in inhibiting the oxidation of myoglobin derivatives. These results demonstrate that the antioxidant activity of grape seed extracts is determined by the extraction method and that while grape seed extracts could slow lipid oxidation they were not effective in preserving the colour of fresh beef patties.

# **INTRODUCTION**

Studies have demonstrated that grape seeds contain high concentrations of polyphenols and advocated their use as dietary supplements and food additives (Jayaprakasha *et al.*, 2003; Yilmaz and Toledo, 2006; Luther *et al.*, 2007; Brannan and Mah, 2007). Different methods have been suggested for optimal grape seed extraction which aim to maximize the yield of polyphenols and antioxidant capacity as measured with standard methods. Commercial grape seed extracts have been suggested to inhibit oxidative processes and improve the shelf life of fresh red meat (Bañón *et al.*, 2007; Brannan and Mah, 2007). Oxidation in fresh red meat has been linked to both lipid oxidation and auto-oxidation of the meat pigments. The method used to extract grape seeds will alter the composition of the extract and determine its efficacy in preventing the oxidative processes. The present study was to investigate the effects of extraction methods on the effectiveness of GSE in inhibiting lipid and red meat pigments oxidation in a "real-world" simulation, the display of meat patties at  $4^{\circ}$ C.

# **METHODS**

All solvents/chemicals were of analytical grade. BHT was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

#### Extraction of grape seed

Grape seeds were obtained from Pinot Noir vines grown in Nelson, New Zealand. The seeds were hand-picked from the berries and dried to constant weight at room temperature (20 °C). Three groups of ground grape seeds (60 g each) prepared as described by Jayaprakasha *et al.* (2003) were extracted with 200 ml of solvent (water, ethanol or acetone) overnight at room temperature. The extracts were centrifuged and the supernatant was either freeze-dried (water extract) or dried under vacuum (ethanol and acetone extracts).

#### Sample preparation

One gram of grape seed extract was dissolved in 1 ml of corresponding extraction solvent (water, ethanol or acetone) and diluted with 4 ml of deionised H<sub>2</sub>O. Each diluted extract was mixed with 150 g minced beef obtained from *Longissimus dorsi* muscle and formed into 5 patties. Patties containing all of the materials except the extracts were run in parallel as control for each treatment and reference patties were made with the synthetic antioxidant (butylatedhydroxytoluene, BHT). The increase in lipid oxidation and colour changes in beef patties were investigated during a simulation of 7 days of display at 4 °C.

### Thiobarbituric acid reactive substances (TBARS) analysis

Lipid oxidation of the samples was determined at 3, 5 and 7 days of display time by the method of Witte et al. (1970) with modification. A 5 g meat sample was mixed with 7.5 ml of 10% ice-cold perchloric acid (BDH, Poole, England) and 10 ml of cold deionized water and homogenized (on ice) for 45 s (15,000 r.p.m.). The homogenizing container was rinsed with 2.5 ml deionised water and added to the homogenate. The homogenate was then filtered through Whatman No.2 filter paper. Filtrate (5 ml) was transferred to a screw cap test tube containing 5 ml of 0.2 M TBA reagent (Sigma, St. Louis, MO, U.S.A.). The test tubes were capped, vortexed and heated in temperature controlled bath water at 95 °C for 10 minutes to develop the pink colour. The absorbance of the resulting solution was measured at 529.5 nm using a Unicam UV4 spectrometer (Unicam Ltd, U.K.). TBARS were calculated from a standard curve (8-50 nmol) of malondialdehyde (MDA), freshly prepared by acidification of 1,1,3,3tetraethoxypropane (TEP) from Sigma (Sigma, St. Louis, MO, U.S.A.). TBARS was calculated as mg MDA/kg sample. Triplicate extracts were prepared for each sample and measurements were performed in triplicate for each extract. The mean of the triplicate measurements of each extract was used for the statistical analysis.

#### Colour measurements

Objective colour measurements were obtained for patties light-exposed surfaces using MiniScan XE (Model 45/0-L, Hunter Associates Laboratory, Inc, Reston, VA). The unit was calibrated using a black standard plate and a white standard C2-36852. A red standard plate (C2-17107) was used as a reference for daily measurements. Measurements were CIE L\*, a\* and b\* values and spectral reflectance (400 to 700 nm) using illuminant C and a 10° observer. The relative % of myoglobin derivatives (myoglobin, oxymyoglobin and metmyoglobin) were determined following the procedures in meat colour evaluation guidelines (AMSA, 1991).

## Statistical analysis

The effect of treatment and display time on colour measurements and relative % of colour pigments for each treatment was determined by analysis of variance (ANOVA) using the GLM protocol in Minitab 15. The inhibition of lipid oxidation (%) compared to the corresponding control was calculated for the three grape extracts and the reference BHT. The data was analyzed using ANOVA using GLM routine.

## **RESULTS AND DISCUSSION**

#### Lipid oxidation

The effects of water, ethanol and acetone grape seed extracts (GSE) on lipid oxidation of beef patties during storage at 4 °C are shown in Figure 1. GSE extracted using ethanol exhibited weak antioxidant activity, as reflected by the inhibition of lipid oxidation, compared with GSE extracted with deionised water over the 7 days of storage at 4 °C. At 7 days of storage, acetone-extracted GSE exhibited higher lipid oxidation inhibition compared with deionised water-extracted GSE (p<0.001). However, the synthetic antioxidant BHT was more effective than any of the GSE in inhibiting lipid oxidation (p<0.001). Our results support studies that demonstrated the efficacy of commercial GSE in inhibiting lipid oxidation of raw beef (Bañón et al., 2007; Brannan and Mah, 2007). Numerous organic solvents and mixtures have been used to optimize the extraction of phenolics from grape seeds (Jayaprakasha et al., 2003; Yilmaz and Toledo, 2006; Baker et al., 1995) with the criterion being the yield. Generally, acetone was found to have the best yield of procyanidins and total phenols while ethanol or deionised water alone were ineffective in extracting the phenolics from grape seeds (Yilmaz and Toledo, 2006; Baker et al., 1995). These studies focused on yield and the antioxidant capacity measured using standard methods; meanwhile the efficacy in food applications received little attention. The range of polarities of the commonly used solvents for grape seeds means that they will have differing affinities toward particular phenolics and the ability to extract those individual phenolics will therefore vary. This will lead to extracts with varying compositions and efficacies as antioxidants; especially in food matrixes where the oxidative mechanisms are more complex than in the standard antioxidant assays. Thus, it is not surprising that the effectiveness of acetone, deionised water and ethanol extracts varied widely despite the use standard GSE: meat ratio. Luther et al. (2007) found that ethanolic grape seed extract was effective in inhibiting lipid oxidation of fish oil during accelerated testing at 80°C, which indicate that the system in which GSE is used is important.

#### Oxidation of myoglobin

The colour of fresh meat changes during storage from purple (the colour of native myoglobin) to red (the oxygenated form myoglobin, oxymyoglobin) and then to brown (the oxidised form metmyoglobin). Thus, the changes in the red hue of red meat reflect the oxidative changes in meat pigments. For retail display it is desirable that the meat retains its red "fresh" colour. The effects of water, ethanol and acetone grape seed extracts on pigment oxidation of beef patties during storage at 4°C are shown in Figure 2. There were no differences between water and acetone GSE treated beef patties and their respective controls over the display period. Ethanol GSE treated beef patties exhibited lower (p<0.001) redness values compared with control patties from the beginning of the display period but the rate of change in redness was higher in control. This indicates that myoglobin oxidation was not the cause for the observed differences

in redness (Figure 2 B). The deoxymyoglobin % in water GSE was not different from control, whereas it was lower (p<0.001) in ethanol and acetone GSE and BHT treated beef patties compared with their controls. Water GSE had higher oxymyoglobin % than its control at day 3, 4 and 6 display time. Acetone GSE had higher (p<0.001) oxymyoglobin % than its control whereas ethanol GSE had lower oxymyoglobin % initially and no difference was found after 74 hours of display compared with the control. This means that lower reduced and oxygenated myoglobin derivatives were found in ethanol GSE treated beef patties compared with its control. The oxidised myoglobin derivative (metmyoglobin) is a better indicator for the oxidative changes in the meat pigments. Both water GSE and BHT inhibited the formation of metmyoglobin (Figure 3A). Metmyoglobin % in water GSE and BHT treated patties were not different except after 7 days of display where water GSE treated patties exhibited lower metmyoglobin % compared with BHT treated patties. Thus the redder colour exhibited by BHT treated patties (Figure 2A) compared with water GSE treated patties seems to be caused by the higher oxymyoglobin % rather than differences in metmyoglobin %. Ethanol GSE treated patties had higher metmyoglobin % during the display time. Metmyoglobin % in acetone GSE treated patties was not different from control during the first 2 days of display but lower (p<0.001) metmyoglobin % was found in acetone GSE patties after 7 days of display. The redness values (Figure 2B & 2C) seems not to be reflecting the oxidative status in beef patties (e.g. metmyoglobin %) accurately but it represent the status of the 3 myoglobin derivatives collectively. The present results indicate that water GSE and acetone GSE were successful in inhibiting lipid and myoglobin oxidation in beef patties while ethanol GSE exhibited weak protective effect against these oxidative processes. Despite the protection against metmyoglobin formation, water GSE and acetone GSE did not improve the colour of beef patties due to their influence on myoglobin and oxymyoglobin % which masked the lower metmyoglobin % found in these treatments.

Figure 1 Effect of water GSE, ethanol GSE, acetone GSE and BHT on the inhibition of lipid oxidation (%) in raw beef patties during display at 4°C.



\* = not determined

Figure 2. Effect of water GSE and BHT (A), ethanol GSE (B) and acetone GSE (C) on the redness values of raw beef patties during display at 4°C.



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Figure 3. Effect of water GSE and BHT (A), ethanol GSE (B) and acetone GSE (C) on the percentage of colour pigments (deoxymyoglobin, oxymyoglobin and metmyoglobin) of raw beef patties during display at 4°C.

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# Oxalate content of purslane regrowth is unaffected by differing repeat harvesting regimes

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# ABSTRACT

Purslane (Portulaca oleraceae) is an herbaceous, fleshy small plant with succulent leaves and stems. Purslane plants are repeatedly harvested for their succulent leaves and stems and is one of the most widely used medicinal plants. Published nutritional values indicate positive nutritional properties such as good antioxidant potential and elevated fatty acid content relative to other green salad plants. However it is known that the plant contains appreciable levels of oxalates. Established purslane plants were either given a heavy or light harvest and then the plants were harvested in the same way on a further three occasions. The total soluble and insoluble oxalate contents of the whole material, leaves, stems and buds were determined using HPLC analysis. The oxalate contents of the harvested plant parts were unaffected by the harvesting techniques but significant differences were found between the total oxalate content of the three different plant parts (leaves, stems and buds). The leaves contained a mean of 832.1 mg total oxalate/100 g fresh weight (FW), while the buds contained the lowest amounts (mean 376.4 mg total oxalate/100 g FW). The stems contained the highest levels of soluble oxalate mean 477.8 mg soluble oxalate/100 g FW, which was significantly higher (p<0.001) than the levels found in the buds and leaves (184.8 mg soluble oxalate/100 g FW and 335.6 mg soluble oxalate/100 g FW respectively). The soluble oxalate content of the stems and the buds were significantly lower (p<0.01) for the plants exposed to the light harvest. The mean insoluble oxalate contents of the stems and the buds (132.5 and 185.6 mg insoluble oxalate/100 g FW, respectively), were significantly lower (p<0.001) than the leaves (mean 506.5 mg insoluble oxalate/100 g FW). There were no significant differences in insoluble oxalate contents between the light and heavy harvesting methods for all plant parts investigated.

# **INTRODUCTION**

Purslane (*Portulaca oleraceae* L.) belongs to the Portulacaceae family which includes over 120 species of succulent herbs and shrubs (Liu *et al.*, 2000). *P. oleraceae*, often called "common purslane", is an herbaceous, fleshy small plant, having succulent obtuse-shaped leaves which taper toward the base. The stem is also succulent with colours ranging from pink, flesh-coloured to maroon. The flowers only open during the morning (Rashed *et al.*, 2003) and its common name in Papua New Guinea is "*Morning Glor*" (Sollie Kareno, pers. comm.).

*P. oleraceae* is widely distributed around the world and is classed as the eighth most abundant plant (Lui *et al.*, 2000). Purslane can be found growing from warm Caribbean climates (Rashed *et al.*, 2003) to the colder regions of Canada (Fontana *et al.*, 2006). Purslane has been used as an edible and medicinal plant for a considerable time although historical records are comparatively few (Fontana *et al.*, 2006; Yazici *et al.*, 2007). Anecdotally, purslane has been widely used as a food, reflecting its wide geographic distribution. In the Mediterranean, China, Philippines, Southeast Asia,

Eastern and Central Africa and Central Europe, the stems and leaves of purslane are eaten as a salad or leaf vegetable and are used to make stock for use in soups or simply used as a pot herb (Lim and Quah, 2007; Mohamed and Hussein, 1994; Obied *et al.*, 2003). Purslane is also dried and used to make soups and tea in certain parts of Asia. The Aborigines of Australia grind the dried plant into flour and use it in mush and breads. Purslane plants are repeatedly harvested or sampled for their succulent leaves and stems and established plants are capable of re-growth after repeated harvests.

Purslane is listed by the World Health Organisation as one of the most widely used medicinal plants (Lim and Quah, 2007a; Yazici *et al.*, 2007). Published nutritional values indicate positive nutritional properties such as good antioxidant potential and elevated fatty acid content relative to other green salad plants (Simopoulos *et al.*, 2005). The use of wild purslane plants is limited in modern societies because limited herbal knowledge hinders the identification of the plant and little information is available about its potential uses (Liu *et al.*, 2000). There appears to be limited cropping of purslane in European countries except in Portugal and Turkey (Özlem Tuncay, pers. comm.). Besides the collection of wild plants, there is also commercial production of purslane in Turkey. According to the Turkish Statistical Institute (2008) commercial purslane in Turkey is 0.5 USD/kg so it is one of the most expensive leafy vegetables, more expensive than spinach, about the same as dill, parsley and rocket which are not used for cooking, therefore smaller amounts are used.

Purslane has a number of positive nutritional components but the plant also contains phyto-chemicals such as oxalic acid, alkaloids and anthraquinone glycosides, which can limit its nutritive value (Obied et al., 2003). Oxalic acid is of particular concern because the edible portions of the plant, especially the leaves, are known to contain very high levels of this compound (Poeydomenge and Savage, 2007). Palaniswamy et al. (2004) showed that the oxalic acid concentration in the leaves of plants grown in hydroponic solutions could be reduced by 40-50% when the plants were grown in solutions containing ammonium ions rather than nitrates. Palaniswamy et al. (2004) went on to suggest that young purslane leaves should not be harvested as the oxalic acid content decreased with the age of the leaves. These results are in direct contrast to Poeydomenge and Savage (2007) who showed that the total, soluble and insoluble oxalate content of soil grown purslane leaves increased on a dry matter basis as the leaves increased in size and age. Repeated harvesting may promote the development of a larger number of small leaves which could contain lower levels of oxalate compared to older larger leaves. Alternatively, as one of the possible functions of oxalate in plant tissue may be as a protective against predation (Libet and Franceschi, 1987) repeated harvesting of a plant may encourage increased synthesis of oxalate in the re-growth tissue. However, the synthesis of oxalate appears to be relatively slow and there is a gradual build up of oxalates as the tissue ages. For instance, Sing and Saxena (1972) showed that there was a gradual increase in the oxalate content of spinach as the leaves aged.

As increased intake of soluble oxalate reduces mineral availability by forming insoluble iron and calcium oxalates it is important to establish which parts of the plants can be eaten regularly. This is particularly important if the plants are harvested repeatedly over the growing season, which encourages the plants to make further new shoots which would contain a higher proportion of smaller leaves. This study was undertaken to investigate whether the repeated harvesting of established plants would have an effect on the oxalate content of the re-growth tissue of the plants.

## **METHODS**

Golden purslane (*Portulaca oleracea* L.) seeds (Koanga Gardens, Maungaturoto, N.Z.) were sown on the  $18^{th}$  October 2006 into 60% peat and 40% pumice seedling mix. The young plants were grown under glasshouse conditions then hardened off and planted in the field on the  $23^{rd}$  of November 2006 (early summer in New Zealand) in Wakanui silt loam at the Horticultural Research Area, Lincoln University, Canterbury ( $43^{\circ}38'$  S,  $172^{\circ}$  27' E) 19 meters above sea level. The plots were surrounded by shelter trees. The Wakanui silt loam had a good base fertility and no manures or chemical fertilisers were used. The plants were irrigated as required. Two paired rows of 24 purslane plants per row were planted with silverbeet plants as guard rows on the outside and in between the paired rows. The paired rows were divided in half producing four plots for each paired row. Ten plants in the split rows were repeat harvested and plants at either end of the row were left as guards.

On the 3rd January 2007, the plants were trimmed with hand-held secateurs to leave either half the initial plant diameter (the heavy harvest) or three quarters of the initial plant diameter (the light harvest). Successive repeat harvests of vegetative regrowth maintained the plant diameters instituted at the beginning of the experiment.

The re-growth of all the plants were harvested on the  $30^{\text{th}}$  January,  $28^{\text{th}}$  February and  $27^{\text{th}}$  March 2007. Three fractions of the plants were analysed, leaves, stems and buds along with a sample of the whole plants. Representative samples were freeze dried in a Cuddon Freeze Dryer (Model No. E.D.5.3) and ground to a fine powder using a Sunbeam multi grinder (Model no. EMO 400 Sunbean Corporation Limited, NSW, Australia). The oxalate content of each sample was determined in duplicate using the method described by Savage *et al.* (2000). The data were analysed using General Linear Model (GLM) in Minitab version 13.0 (Minitab Ltd., Coventry, UK).

# RESULTS

The oxalate content of each sample harvested from each treatment was determined and the mean results for all three harvests are presented in Table 1 There were no significant differences between the total oxalate content of the heavy or light pruned purslane plants in any of the four different plant parts harvested. However, there were significant differences in the total oxalate contents of the plant parts sampled (p<0.001). The leaves contained a mean of 832.1 mg total oxalate/100 g fresh weight (FW), while the buds contained the lowest amounts (mean 376.4 mg total oxalate/100 g FW).

The stems contained significantly higher (p<0.001) levels of soluble oxalate (mean 477.8 mg soluble oxalate/100 FW) compared to the soluble oxalate levels found in the other two plant parts (ranging from 184.8 mg soluble oxalate/100 g FW for the buds to 335.6 mg soluble oxalate/100 g FW for the leaves). The soluble oxalate content of stems and the buds were significantly lower (p<0.01) for the plants exposed to the light harvesting method. This was not evident in the leaves.

Plant part	Type of pruning	Total oxalate	Soluble oxalate	Insoluble oxalate <sup>1</sup>
		(mg oxa	late/100 g FW ±	= SE).
Whole plant	Heavy Light	$553.7 \pm 27.7$ $590.3 \pm 86.4$	$\begin{array}{c} 234.4 \pm 16.9 \\ 221.9 \pm 28.9 \end{array}$	$\begin{array}{c} 328.0 \pm 96.3 \\ 368.5 \pm 99.4 \end{array}$
Stems	Heavy Light	$\begin{array}{c} 610.3 \pm 57.8 \\ 601.5 \pm 49.6 \end{array}$	$\begin{array}{c} 497.3 \pm 54.9 \\ 458.2 \pm 38.8 \end{array}$	$\begin{array}{c} 121.8 \pm 37.3 \\ 143.2 \pm 19.1 \end{array}$
Leaves	Heavy Light	$\begin{array}{c} 821.5 \pm 67.6 \\ 842.7 \pm 49.7 \end{array}$	$\begin{array}{c} 312.3 \pm 21.8 \\ 338.8 \pm 17.7 \end{array}$	$\begin{array}{c} 509.0 \pm 59.9 \\ 503.9 \pm 47.2 \end{array}$
Buds	Heavy Light	$376.9 \pm 49.5$ $375.9 \pm 30.5$	$\begin{array}{c} 205.8 \pm 14.9 \\ 163.7 \pm 39.2 \end{array}$	$\begin{array}{c} 171.1 \pm 50.8 \\ 200.0 \pm 54.9 \end{array}$
Analysis of variance	d.f.	Significance		
Harvest time	2	ns	ns	ns
Type of pruning	1	ns	*	ns
Plant part	3	***	***	***

Table 1: Mean oxalate content of the whole plant, stems, leaves and buds harvested on three separate occasions following either a light or heavy harvesting of the plants (mg oxalate/100 g FW  $\pm$  SE).

<sup>1</sup>Insoluble oxalate = total oxalate - soluble oxalate (Holloway et al., 1989).

# DISCUSSION

The intensity of trimming at harvest had no effect on the total oxalate contents of the tissue sampled at the following harvests. There was, however, a significant difference between the total oxalate contents of the entire plant tissue and the three different fractions of the plants harvested. The leaves contained the highest levels of total oxalates mean (832.1 mg/100 g WM) while the buds contained the lowest levels of total oxalates (mean 376.4 mg/100 g WM). The differences in the total oxalate contents were mainly influenced by differences in the insoluble oxalate fraction of the plant parts sampled. The leaves contained significantly higher levels of insoluble oxalate (mean 506.5 mg/100 g WM) compared to the stems and buds (overall mean 159.0 mg/100 g WM).

There were no differences observed in the insoluble oxalate contents between the two types of pruning. Comparison of the results obtained in this experiment for plants harvested in late summer 2007 with the same cultivar of purslane grown in 2006 in the same location showed that there was considerable variation in the oxalate contents of the different plant parts. However, both experiments confirmed that the leaves always contained the highest levels of total oxalates and these largely consisted of insoluble oxalates. In this experiment the stems contained the highest proportion of soluble oxalates (mean 78.9  $\pm$  3.2% for both harvests) while the leaves and buds contained similar proportions of soluble oxalate (39.4  $\pm$  1.9 and 42.6  $\pm$  9.7% of the total oxalate content, respectively). The whole plant's soluble oxalate content was 43.7  $\pm$ 7.3% of total oxalates. Poeydomenge and Savage (2007) reported that the raw leaves, stems and buds contained 27.5, 78.6 and 71.1 % soluble oxalate, respectively. In contrast, Moreau and Savage (2009) showed that the leaves of the same cultivar of purslane grown in a greenhouse in the late summer of 2007 contained 56.9 mg oxalate/100 g WM which contained 53% soluble oxalates. Overall, the data suggests that the oxalate content of purslane is affected by the growing conditions but is not affected by harvesting techniques.

These results show that if the plants are harvested repeatedly over the growing season, a procedure that encourages the plants to make further new shoots containing a higher proportion of smaller leaves does not have any effect on the overall oxalate content of any of the tissues.

# CONCLUSIONS

The results show that the harvesting of purslane has no effect on the oxalate content of the re-growth tissue. Comparisons of the data obtained in this experiment with earlier experiments suggest that the plants synthesise variable amounts of oxalates and this appears to be in response to changes in environmental conditions. Since purslane plants contain high levels of oxalates, especially in the leaves and stems, compared to many other common food plants (Noonan and Savage, 1999; Savage *et al.*, 2000), it is recommended that purslane should be eaten with a high calcium food to minimise the potential absorption of soluble oxalates, consumption of the leaves only would lead to a reduction in soluble oxalate intake but the leaves still contain appreciable levels of soluble oxalates. As a high intake of oxalate leads to increased risk of kidney stone development, regular consumption of purslane is inadvisable for people prone to this problem.

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# An exploration of consumer preference for different formulations of tomato jam

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# ABSTRACT

In New Zealand, a small range of traditional fruits are commonly used to make single fruit jams commercially. There is, however, interest in making jams from mixtures of different fruits. Speciality shops and farmers markets have encouraged this use of a wider range of fruits and this led to our investigation of older jam recipes. In this study five different formulations of tomato jam were made (control, low and high acid and low and high sugar) based on a standard basic recipe for tomato jam (tomato and sugar in equal proportions). The raw tomatoes had an average dry matter of 4.2% and for the jams it ranged from 50.9 to 67.0%. The pH of the jams ranged from 3.0 to 3.3. Measurements were also taken of the composition and CIE colour of the raw tomatoes and the jams produced. All jams were acceptable in terms of their physicochemical properties and food safety. The colour co-ordinates of the raw tomatoes were L\* 41.54, a\* 40.28 and b\* 23.82 and the colour of the jams ranged from L\* 30.1 to 41.2, a\*19.9 to 27.2 and b\* 12.5 to 22.1. The a\*/b\* ratio ranged from 0.9 to 2.5, hue values from 22.1 to 48.1 and chroma from 25.7 to 29.7 and showed that the jams were, in general, darker, less red and slightly more yellow than the raw tomatoes used to make them. Sensory evaluation was undertaken using a consumer-type panel. Jams were evaluated for preference for purchase, colour, acceptability of sugar and acid contents, intensity of tomato flavour and overall acceptance. A preliminary exploration of the data revealed that there were differences in response to jam parameters based on age and gender. Further data analysis of the jam will determine a baseline jam recipe to be used for the production of a jam with tomato and other fruits.

# **INTRODUCTION**

Before home freezers became common and/or tomatoes were produced throughout the year people used a variety of preservation techniques to cope with the autumn glut of home grown tomatoes. Common preservation techniques included bottling the tomatoes whole, preserving and eating them fresh as soup and with spaghetti, and using the tomatoes in a range of pickles and chutneys. Less commonly, probably because of the ready availability of other fruits, tomatoes were also used to make jam. In New Zealand tomato jam recipes were popular in the 1930s but have fallen out of favour over recent years, although a recent search of food magazines (Mary Browne, pers. comm., 2008) reveals a recent interest in the home production of small amounts of gourmet jams (e.g. 500 g batches), unlike the bulk jam making common in earlier centuries. The growing number of farmers' markets throughout New Zealand has increased interest in the use of a wider range of fruits and fruit mixtures for jam making.

A database kept by Professor Helen Leach from Otago University revealed an early recipe for pineapple and tomato jam from the Colonial Everyday Cookery (1901), and the first pure tomato jam recipe was in Cookery Book published by the Ashburton Plunket Society in 1926. There are six other NZ books dating from 1933 to 1939 containing tomato jam recipes. The recipes were thought to have originated in the USA and then come to New Zealand via Australia. An internet recipe website had several early examples dating from 1873 to 1918 and Google Books produced four recipes from Australia dated 1886 to 1902 (Helen Leach, pers. comm. 2008).

Most of these recipes involved boiling the tomatoes and sugar for considerable lengths of time so the jams produced would have been more sauce-like rather than jellied like a jam. This may have been another instance of a food being overcooked for no particular reason just as cabbage was in earlier years.

Factory jam making is a highly complex operation with strict quality control procedures are employed to ensure a uniform product, but the manufacturing operations employed are in essence the same as those employed at home (Dauthy, 2008). One of the first producers of jams commercially in New Zealand was the St George Company of Dunedin, established in 1864 by James Irvine, an immigrant from Scotland, who later formed a partnership with his son-in-law, William Stevenson in 1882. In 1955 English-made jams began to be imported into the country and gained favour. However, a challenge by the North Island Housewives' Association that New Zealand jam was inferior was quickly dispelled by a blind taste testing that ranked the locally produced St George strawberry jam as most preferred (Stevenson, 1964).

Currently, a range of traditional fruits are used to make single flavour jams commercially but there is a growing interest in making jams from different fruit combinations, encouraged by the rise in speciality shops and farmers' markets throughout New Zealand. There is a ready supply all-year of locally grown tomatoes containing high levels of antioxidants and lycopene and having an attractive bright colour that makes them a good choice for producing a single fruit jam as well as in combination with other local fruits such as quince and rhubarb. When made into jams they would increase the purchasing choices available to consumers from these retail outlets. Interest is increasing in the use of cooking methods that preserve the nutritional status of the raw ingredients so more products are being made using shorter cooking times facilitated by the use of setting products such as pectin. Jam makers in earlier times would have used apples to achieve the same effect.

This paper reports on an investigation into some physico-chemcial properties of different tomato jam formulations and age and gender related responses to some jam parameters from a sensory evaluation of the jams.

# **METHODS**

Five different formulations of tomato jam were made (control, low and high acid and low and high sugar) based on a standard basic recipe (tomato [Galaxy] and sugar [Chelsea Sugar, Auckland] in equal proportions) (Goodman Fielder, 2001) with the addition of citric acid (Hansells NZ Ltd) and pectin (Citrico 7030) to aid gel production. The low acid and low sugar jams had 50% lower contents of acid and sugar, respectively, and the high acid and high sugar jam had 50% higher content of acid and sugar, respectively, than the control jam (Table 1).

	Control	High sugar	Low sugar	High acid	Low acid
Tomatoes	500 g	500 g	500 g	500 g	500 g
Sugar	500 g	750 g	375 g	500 g	500 g
Citric acid	7 g	7 g	7 g	9 g	5 g
Pectin	2.5 g	2.5 g	3.0 g	2.5 g	2.5 g
Ginger <sup>1</sup>	0.4 g	0.4 g	0.4 g	0.4 g	0.4 g

Table 1: Jam formulations

<sup>1</sup>Cerebos Gregg's ground ginger

#### Jam preparation

The tomatoes were weighed and then cut into 1 cm cubes and placed in a cooking container where they were heated until they had softened and the juices extracted from the cubes. Sugar was added together with the citric acid, pectin and ginger and the mixture was bought to a rolling boil and boiled until a jam thermometer (and the common "teaspoon" test) indicated that the jam had reached a consistency and temperature to set on cooling ( $105^{\circ}C$ ). The jam was placed into heated jars and sealed.

# Physical and chemical analysis

The pH was monitored using a Mettler Toledo, InLab® 427 spear probe attached to a Suntex, SP-701 pH meter. The dry matter content of the tomato jam samples were determined, in triplicate by drying in an oven at 105°C for 24 h (AOAC, 2002). The water activity was measured on the final product by using Water Activity Meter, Aqua Lab, Model CX-2 (Decagon Devices, Inc, USA). The tomato jam sample was placed in small plastic cups. The water activity was measured in triplicates when the temperature of the sample had equilibrated to the temperature of the instrument. Soluble solids were determined using a few drops of the most liquid part of each jam were put on a Bellingham + Stanley Ltd eclipse refractometer prism (Kent, UK) reading in °Brix taken at 20°C.

CIELAB colour measurements (in triplicate) were performed in triplicate on the flesh of representative samples of the fresh and processed tomatoes using a Minolta Chroma Meter (model CR-210, Minolta Camera Co. Ltd. Osaka, Japan), consisting of an 8 mm diameter measuring area with a diffuse illumination/0° viewing angle. A standard white tile (L\* 98.07, a\*-0.23, b\*1.88) was used to calibrate the colorimeter. The three dimensional colour model CIE L\*a\*b\* (CIELAB) was used to analyse the colour. L\* represents the lightness (L\* = 0 is equal to black, L\* = 100 represents white), a\* gives information about red and green (a\*, negative values indicate green while positive values indicate red), the hue blue-yellow is described by b\* (b\*, negative values indicates blue and positive value indicates yellow). Chroma gives information about how pure the colour is.

#### Sensory evaluation

Sensory evaluation was undertaken using a consumer-type panel of 80 participants comprising 80 staff and students from Lincoln University. There were 40 males and 40 females, aged between 19 and 55+ years. One teaspoon of each jam sample was placed on identical white plates coded with a 2-digit random number (Urbaniak, 1997) and served at room temperature. Five different jam samples were presented to panellists in a random order. Panellists evaluated the samples in individual

white booths. Between each sample, panellists were encouraged to rinse their mouth out with water. Taste testing took place in the morning and afternoon of the same day.

The jams were evaluated for preference for purchase, colour, acceptability of sugar and acid contents, intensity of tomato flavour and overall acceptance. Two types of scale, intensity and preference, were applied for rating either (a) the preference (liking) for skin and flesh colour and overall acceptability, or (b) the intensity of an attribute (Meilgaard, Civille and Carr, 1987). The preference rating was scored on a hedonic scale of 5 with 1 = dislike very much and 5 = like very much for skin and flesh colour, and for overall acceptability on a 7 point scale with 1 = dislike strongly and 7 = like strongly. The intensity of flavour was recorded on a 10 centimetre line with the individual measurements subsequently placed in five groups of increasing intensity of flavour (1-20, 21-40, 41-60, 61-80 and 81-100). A standard response sheet was used and the tasting was approved by the Lincoln University Human Ethics Committee. Demographic data about the age and gender of participants were also collected and explored to determine whether there were any age or gender specific responses to the questionnaire.

## **RESULTS AND DISCUSSION**

The raw tomatoes had an average dry matter of 4.2% and for the jams it ranged from 50.9 to 67.0%. The pH of the jams ranged from 3.0 to 3.3. The results for pH, moisture, and water activity are within the acceptable limits for jam from the food safety point of view (FAO, 2008). Brix readings were close to the Australia New Zealand Standard (2.3.2) for jams (ANZ Food Standards, 2004).

Table 2 Physico-chemical data for jam formulations

Tuble 2 Thysico chemical aata jor jam jormalations								
Jams	Water activity	Dry matter	pН	Soluble solids (°Brix)				
	$(A_w)$	%						
Control	$0.7\pm0.002$	$63.4 \pm 1.0$	$3.3\pm0.01$	$68.9 \pm 0.4$				
High sugar	$0.7\pm0.004$	$67.9\pm0.6$	$3.2\pm0.04$	$80.6\pm0.2$				
Low sugar	$0.8\pm0.002$	$51.0\pm0.5$	$3.3\pm0.02$	$70.1\pm0.03$				
High acid	$0.8\pm0.001$	$61.0\pm0.2$	$3.1\pm0.02$	$73.0\pm0.4$				
Low acid	$0.7\pm0.001$	$64.0\pm0.5$	$3.3\pm0.02$	$72.1\pm0.1$				

Measurements were also taken of the composition and CIE colour of the raw tomatoes and the jams produced. The colour co-ordinates of the raw tomatoes were L\* 41.54, a\* 40.28 and b\* 23.82 and the colour of the jams ranged from L\* 30.1 to 41.2, a\*19.9 to 27.2 and b\* 12.5 to 22.1. The a\*/b\* value reflecting the red-green balance of the tomatoes, ranged from 0.9 to 2.5 and the hue values, reflecting red colour of the jams ranged from 22.1 to 48.1 and the chroma, reflecting the intensity of colour, ranged from 25.7 to 29.7 (Table 3).

Tuble 5. Colour	valuses jor ja					
Jams	L*	a*	a* b*		Chroma	Hue
Raw tomatoes	$41.5 \pm 0.3$	$40.1 \pm 0.2$	$23.6 \pm 0.2$	1.70	$46.5 \pm 0.2$	$30.5 \pm 0.2$
Control	$32.2 \pm 0.3$	$23.4 \pm 0.3$	$13.0 \pm 0.2$	1.80	$26.8 \pm 0.4$	$29.0\pm0.1$
High sugar	$41.2\pm0.3$	$19.8\pm0.6$	$22.1\pm0.3$	0.90	$29.7\pm0.6$	$48.1\pm0.6$
Low sugar	$35.5\pm0.4$	$24.4\pm1.0$	$16.3\pm0.8$	1.50	$29.3 \pm 1.2$	$33.7\pm0.3$
High acid	$30.1\pm0.5$	$27.2 \pm 1.1$	$11.0\pm0.5$	2.46	$29.3 \pm 1.2$	$22.1\pm0.2$
Low acid	$34.1 \pm 0.3$	$22.5\pm0.9$	$12.5\pm0.4$	1.80	$25.7\pm1.0$	$29.1\pm0.2$

*Table 3: Colour valuess for jam formulations*  $(L^* a^* b^*)$ 

Overall, processing the tomatoes into jams caused all colour parameters to change with L\*, a\* and chroma showing the largest differences between the jams and the raw tomatoes (except for the high sugar formulation). The jams were, in general, darker, less red and slightly more yellow than the raw tomatoes used to make them.

The data were explored to see whether the panellists could distinguish between the different jam formulations; if there were any age and gender differences in their choices, and whether their choices were influenced by the composition of the jams.

Figure 1: Preference to purchase the five jams



Using the mean data from the sensory evaluation, there were differences in age (mature and young) and gender (female and male) preferences for some jam parameters

(Figure 1), for example, preference to purchase. Previous studies have shown that as the acid content of a jam increases it tends to mask the perception (Schifferstein and Frijters, 1990) of the sugar content in the jam so that changes in sugar levels cannot be distinguished. This have may have affected some of the results for other jam parameters, except for overall acceptability (data not shown).

# CONCLUSIONS

All jams had acceptable moisture, pH and water activity levels from a food safety point of view; the tomatoes retained much of their colour when processed into jam; sensory evaluation revealed of the jams were less red than the raw tomatoes but not markedly so; there were differences in response between some panellists for some jam parameters. Further analysis of the data will determine a baseline jam recipe that will be used for the production of new jams with tomato and other fruits, such as quince and rhubarb.

# **ACKNOWLEDGEMENTS**

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# Vacuum fried jackfruit: effect of maturity, pre-treatment and processing on the physiochemical and sensory

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# **INTRODUCTION**

Jackfruit (*Artocarpus heterophyllus*, Lam.) is one of the most widely grown fruit crops in the tropical countries and also one of the most famous in the world because it produces the largest edible fruit that may weigh as much as 50 kg. It is known for its large edible bulbs of yellow, very sweet aroma, pineapple and banana-flavoured flesh that enclose a smooth, oval, light-brown seed. In addition, jackfruit is considered to be very nutritious due to its vitamins and mineral content. A number of food products have already been developed for jackfruit including vacuum fried products coming from Thailand and Vietnam. The vacuum fried jackfruit utilizes half ripe fruit to produce a bright yellow and crunchy product. However, the present vacuum fried jackfruit lacks the characteristic aroma and taste of ripe jackfruit. There is a need to develop new food products from jackfruit by overcoming the disadvantages of the present products. The aim of this study was to develop vacuum fried jackfruit from ripe pulp.

# **MATERIALS AND METHODS**

#### Equipment

Figure 1 shows the vacuum frying system comprising of the frying chamber (1), the LPG tank (2), the 3-pass condenser (3), the control panel (4), the liquid ring vacuum pump (5), the cooling water tank (6), and the cooling tower (7). The frying chamber consists of stainless steel frying tank (8), oil sight glass (9), tank swing cover (10), 2 chimneys (11), basket raising handle (12), tank cover outlet (13), and a tank body outlet with check valve (14). The condenser consists of condenser body (15), cooling water inlet (16), cooling water outlet (17), and vapour inlet (18). The control panel consists of vacuum pump switch (19), fan and water pump switches (20), vacuum gauge (21), and the thermometer and thermostat panel (22). The cooling tower consists of cooling water column (23), and motor and fan assembly (24).


Figure 1. Vacuum frying system used in the experiments.

#### Vacuum frying of jackfruit pulp

Several studies were carried out on the acceptable frying temperature range and duration, loading density of the frying basket and pretreatment methods on vacuum frying of ripe jackfruit. The amount of oil and the duration that the oil can be used for frying were also established in these preliminary studies.

After the frying temperature range and duration had been established in the preliminary studies, formal experiments were carried out on vacuum frying of jackfruit pulp using different maturity and pretreatment as shown in Table 1.

Treatments	Maturity	Pre-treatment			
T1	Half-ripe	Fresh			
T2	Half-ripe	Chilled			
Т3	Half-ripe	Blanched and Frozen			
Τ4	Ripe	Fresh			
T5	Ripe	Chilled			
Τ6	Ripe	Blanched and Frozen			

Table 1. Treatments used in the vacuum frying experiments.

#### Physico-chemical and sensory evaluation

The moisture content, fat content and percent yield of the different vacuum fried products obtained with different maturity and pretreatment were determined using standard methods. The data were statistically analysed using the Analysis of Variance based on F-test and Tukey's Test.

The different vacuum fried jackfruit products obtained with different maturity and pretreatment were evaluated for their sensory qualities using a 9-point Hedonic score. This data were statistically analyzed using the Friedman Analysis of Variance and Multiple Comparison between Groups.

Based on the results of the various experiments, the optimum conditions for processing of vacuum fried ripe jackfruit were determined.

The vitamin A and C contents of the fresh jackfruit pulp and the vacuum fried jackfruit pulp were determined using AOAC methods.

## **RESULTS AND DISCUSSION**

#### Physio-chemical qualities of vacuum fried products

The mean moisture content for vacuum fried jackfruit from half-ripe pulp were generally less than the vacuum fried jackfruit from ripe pulp with the range of 2.62 to 2.66% dry basis and 5.85 to 6.19% dry basis, respectively. The products from ripe jackfruit had significantly higher moisture content than the products from half-ripe jackfruit. The higher moisture content of products from the ripe jackfruit was probably due to inadequate frying time which was made constant at 2 hours for all products. Due to inadequate frying time, less moisture evaporated from the products from ripe jackfruit which resulted to significantly higher moisture content than the products from half-ripe jackfruit. It was found that the riper the jackfruit pulp the higher its initial moisture content.

The mean fat content of the vacuum fried products from half-ripe jackfruit (23 to 34%) was generally lower than the products from ripe jackfruit (24 to 41%). The products from blanched and frozen ripe and half-ripe samples had significantly higher fat content than the products from fresh ripe and half-ripe samples. This is probably due to the more porous structure of blanched and frozen samples which retained more oil.

The percent yield of the products from half-ripe jackfruit (28 to 34%) was generally higher than the products from ripe jackfruit (27 to 30%). The product from fresh half-ripe jackfruit sample had significantly higher percent yield than the product from blanched and frozen ripe jackfruit sample while the rest of the products were not significantly different from each other.

#### Sensory qualities of vacuum fried products

The colour description of the different vacuum fried jackfruit for the half-ripe jackfruit ranged from very light yellow to yellow and ranged from golden yellow to very golden yellow for the ripe jackfruit. The texture description of the different vacuum fried jackfruit ranged from moderately crunchy to crunchy for half-ripe samples while the ripe samples ranged from slightly crunchy to moderately crunchy. The aroma description of the different vacuum fried jackfruit aroma to moderately pronounced jackfruit aroma while for ripe samples ranged from moderately pronounced jackfruit aroma while for ripe samples ranged from moderately pronounced jackfruit aroma to pronounced jackfruit aroma. The sweetness description of the different vacuum fried jackfruit ranged from slightly sweet for half-ripe samples while for ripe samples ranged from slightly sweet for half-ripe samples while for ripe samples ranged from slightly oily to moderately oily. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly oily to moderately oily. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly is not of the slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly is not of the slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly is no slightly. The general acceptability scores of the different vacuum fried jackfruit ranged from slightly is no slightly.

corresponds to "like moderately" to "like very much" for the products from ripe samples.

Statistical analyses of the colour, texture, and oiliness acceptability of the products from half-ripe samples showed that these products were significantly better than the products from some ripe samples. While the aroma, sweetness and general acceptability of products from ripe samples were significantly better than the products from some half-ripe samples. Because of the contrasting sensory qualities of the half-ripe and ripe samples, the consumers will also have a hard time deciding on which products have better qualities. Hence the use of samples between half-ripe and ripe samples would give a product of optimum sensory qualities.

#### Implications of the results for vacuum frying

The different results imply that in processing of vacuum fried jackfruit halfripe and ripe samples can be used without any pre-treatment methods (fresh) or with chilling pre-treatment since the resulting products have favourable physio-chemical and sensory qualities. However, half ripe vacuum fried jackfruit products are already available in the market coming from Thailand and Vietnam. Processing of vacuum fried ripe jackfruit from fresh and chilled pulp will not be practical in factory situation. Fresh ripe jackfruit pulp will only last for days while chilled ripe jackfruit pulp will only last for weeks after which the pulp samples will deteriorate. However, blanched and frozen ripe jackfruit pulp will last for months. As a result, the blanched and frozen ripe jackfruit should be used in vacuum frying.

Using the results from this study, a recommended technology for processing of vacuum ripe jackfruit was obtained as shown in Figure 2.

#### Vitamin C contents of the vacuum fried jackfruit

Using the recommended technology, vacuum fried jackfruit was processed for vitamin analysis. The initial vitamin A and C contents of fresh ripe jackfruit were 540 I.U. and 9 mg, respectively. After vacuum frying, the vitamin A and C contents of the fried product were 30 I.U. and 3.4 mg, respectively. The retention of vitamin of A in the vacuum fried jackfruit of about 6% is quite low which is probably due to being fat soluble. However, the retention of vitamin C in the vacuum fried jackfruit of about 38% is quite considerable. The jackfruit pulp is frozen prior to vacuum frying at a temperature of 90°C for 2 to 2.5 hours. These conditions probably lessened down the destruction of vitamin C during the vacuum frying process.

Figure2. Recommended technology for processing vacuum fried ripe jackfruit.



Packaging of Vacuum Fried Jackfruit in Metalized Laminate Bag

#### CONCLUSIONS

Vacuum fried products from ripe jackfruit had significantly higher moisture content than the products from half-ripe jackfruit. The vacuum fried products from blanched and frozen ripe and half-ripe samples had significantly higher fat content than the products from fresh ripe and half-ripe samples. The vacuum product from fresh half-ripe jackfruit sample had significantly higher percent yield than the product from blanched + frozen ripe jackfruit sample while the rest of the products were not significantly different from each other. The colour, texture, and oiliness acceptability of the vacuum products from half-ripe samples showed that these products were significantly better than the products from ripe samples. While the aroma, sweetness and general acceptability of vacuum products from ripe samples were significantly better than the products from half-ripe samples. The initial vitamin A and C contents of fresh ripe jackfruit were 540 I.U. and 9 mg, respectively. After vacuum frying using the recommended technology, the vitamin A and C contents of the fried jackfruit product were 30 I.U. and 3.4 mg, respectively.

#### Maintenance energy requirements of grazing ruminants

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## ABSTRACT

Although maintenance is a theoretical concept for animals in a productive state, its calculation is essential to the factorial approach for estimating metabolisable (ME) energy requirements. The most recently published set of ME requirements (CSIRO, 2007) propose that in addition to adding the energy needed for grazing and other activity to basal metabolism, 10% of the ME used directly for production be added to account for support of metabolically active tissues and increased blood flow. A different approach is described here in which the costs of harvesting grazed pasture and tissue support are attributed to individual factors of production (lactation, growth) rather than to maintenance (Nicol and Brookes, 2007). Maintenance costs are therefore independent of productivity, but ME requirements for production are increased. Although the estimated total ME requirement does not change, marginal efficiencies (net energy retained as a percentage of ME required above maintenance) are decreased by approximately 10%. This has implications when estimating production responses to increased levels of feeding.

#### **INTRODUCTION**

Livestock producers need information on the amounts of nutrients that animals require to meet specified production targets, in order to plan their feeding strategies. In New Zealand grazing systems, the main limitation to production is metabolisable energy (ME) intake. It is therefore common practice to express requirements as MJ ME, using a factorial approach. The requirements for the separate processes of maintenance, liveweight gain, pregnancy and milk production are estimated and then summed. Values for energy requirements have been published in authoritative national publications incorporating the results of numerous experiments, *e.g.* those from the United Kingdom (AFRC, 1993), United States of America (NRC, 2000; NRC, 2001; NRC, 2007), France (INRA, 1989) and Australia (CSIRO, 2007). Estimates of total energy requirements for lactating dairy cows were compared against measured intakes (Yan *et al.*, 2003), and the Australian and French systems in current use at the time gave the most accurate predictions. For this reason, CSIRO (2007) was used as the basis of the most recent New Zealand estimates of ME requirements (Nicol and Brookes, 2007).

## DEFINING METABOLISABLE ENERGY REQUIREMENTS

The requirements of animals for energy may be expressed in a number of alternative units (*e.g.* digestible organic matter, digestible energy, net energy), but metabolisable energy (ME) is now widely adopted.

Gross energy (GE) represents the total energy concentration in feeds and animal tissues, the main constituents are fat, protein and carbohydrate with approximately 39, 24 and 17.5 MJ GE/kg DM respectively. Gross energy concentrations will therefore vary according to composition, but for most ruminant feedstuffs fall in the range of 18-19 MJ GE/kg DM. Only a proportion of the GE consumed by the animal is available for maintenance or for incorporation into body tissues or animal products, because of the losses encountered during digestion and metabolism. Because energy contained in the faeces represents not only that portion of the dietary GE which has not been digested, but also some energy from metabolic sources (*e.g.* dead micro-organisms, endogenous secretions *etc.* excreted in the faeces), the difference between the GE intake and the faecal energy output is termed the *apparent* digestible energy (DE) intake. Metabolisable energy accounts for the fraction of the DE intake which does not appear in the urine or in eructated and exhaled methane gas (produced during normal rumen fermentation). This forms a relatively constant proportion of the DE intake as ME = 0.81DE.

ME is available to the animal as nutrients absorbed from the digestive tract. During the metabolism of these nutrients, a portion of the ME is converted to heat. Unless the animal is in a particularly cold environment, this heat is of no value and is a further energy loss to the animal. The energy remaining is termed net energy (NE), and is used by the body tissues to meet the requirements of maintenance and production. The efficiency with which the ME is converted to NE for any process (termed a k value) is defined by the relationship:

#### k = NE retention / ME intake

ME requirements can therefore be calculated as:

ME requirement = NE requirement / k

The k value (the partial efficiency of utilisation of ME) varies for the differing processes of maintenance, lactation, pregnancy and liveweight change. In addition, the efficiency of ME utilisation is affected by factors associated with the feed consumed. Energy costs associated with the muscular work involved in propulsion of food through the intestinal tract and heat of fermentation are higher for fibrous feeds of lower ME concentration. The lower value of k for poorer quality feeds is also associated with lower digestibility and higher actetate:propionate ratios in the volatile fatty acids (VFA) absorbed from the rumen. Values of k can be therefore be calculated as a function of the dietary ME concentration (MJ ME/kg DM).

#### **Basal maintenance**

Maintenance is defined as when energy requirements for life processes and replacement of body tissue lost through turnover are met, but there is no net retention of energy in body tissues or products. For growing, pregnant or lactating animals, maintenance is a theoretical concept but, in the factorial approach, is estimated as the base to which the production requirements are added. When animals are fasted, tissues are mobilised to provide energy for support of life processes and this is manifested as fasting heat production (FHP). FHP is therefore a measure of the NE requirements for maintenance and varies with a number of factors including size, age and sex of the animal. Heavier animals generally have higher maintenance ME (ME<sub>m</sub>) requirements than lighter animals, because of the larger tissue mass to be maintained. ME<sub>m</sub> does not

increase in direct proportion to body weight, so it is conventional to use the exponent of liveweight,  $kg^{0.75}$ , as the basis for comparing  $ME_m$ . Young animals and intact males have lower fat:protein ratios in their body tissues, and as protein turns over at a faster rate than fat, their maintenance requirement is increased. The equations for calculating basal maintenance requirements can be found in CSIRO (2007).

#### **Production support**

European studies, with dairy cattle fed grass indoors, found maintenance costs to be 10-27% greater than estimates made some twenty earlier (Agnew and Yan, 2000; Bruinenberg *et al.*, 2002). Improvements in genetic merit over this period have resulted in higher milk yields, and this raises the question as to whether maintenance costs are a function of productivity. In growing and lactating animals, additional ME is required to maintain the productive tissues (udder, muscle and fat cells) and to support extra work by the liver and digestive tract and increased blood flow. CSIRO (2007) estimate this as equivalent to 10% of the ME used directly for production, based on the work of Graham *et al.* (1974) with growing lambs, and these support costs have then been added to the basal maintenance requirements in their calculations (Corbett and Ball, 2002; CSIRO, 2007).

However, it is equally valid to apportion these support costs to production, rather than to maintenance. Nicol and Brookes (2007) have chosen to take this approach, so that maintenance requirements are not dependent on level of production. Hence ME requirements for milk production and liveweight gain are increased by 10%.

#### Grazing activity

Grazing imposes energetic costs associated with harvesting of pasture which can be separated into a) those of walking during grazing and b) harvesting and processing herbage, which is dependent on the amount of herbage consumed. CSIRO (2007) includes these as additional maintenance costs, whereas Nicol and Brookes (2007) include walking in maintenance, but apportion harvesting costs to each individual factor, according to the dry matter (DM) intakes needed to supply the ME requirement for that process. Other activities, *e.g.* walking to and from milking or to water, require additional energy expenditure.

### METABOLISABLE ENERGY REQUIREMENT CALCULATIONS

An example of the two different approaches can be seen in Table 1. This shows calculated daily ME requirements for a 475 kg Jersey x Friesian cow, grazing pasture (11 MJ ME/kg DM), producing 1.0 kg milksolids, gaining 0.1 kg live weight and walking 2 km for milking.

	CSIRO	Nicol & Brookes
Basal Maintenance	51.7	51.7
Tissue Support	7.2	
Grazing	3.6	1.7
Additional Walking	3.5	3.5
Maintenance	66.0	56.9
Milk Production	67.3	67.3
Tissue Support	0.0	6.7
Grazing	0.0	1.8
Lactation	67.3	75.9
Liveweight Gain	4.2	4.2
Tissue Support		0.4
Grazing		0.1
Liveweight Gain	4.2	4.8
Total ME requirement	137.5	137.5

Table 1: Daily ME requirements for a dairy cow (MJ/day), as calculated by CSIRO (2007) and Nicol and Brookes (2007).

The marginal efficiency of ME use for milksolids production and liveweight gain are shown in Table 2.

Table 2: Marginal efficiency of ME (NE/ME) for milksolids production and liveweight gain.

	CSIRO	Nicol & Brookes
NE retained in milk (MJ)	41.7	41.7
ME required (MJ)	67.3	75.9
Marginal Efficiency	0.62	0.55
NE retained in liveweight gain (MJ)	2.5	2.5
ME required (MJ)	4.2	4.8
Marginal Efficiency	0.59	0.52

## DISCUSSION

There is no difference in the total ME requirements calculated by the two methods. However, with Nicol and Brookes (2007), maintenance costs are not affected by level of production, and tissue support and appropriate grazing costs are included in the production requirements. In calculating the production response in grazing livestock to additional feed inputs, it is conventional to use the marginal efficiency of ME above maintenance (Holmes *et al.*, 2002). Assuming 50% of the additional ME consumed is partitioned towards milk production, the response to increasing feed intake by 1 kg DM, containing 11 MJ ME/kg DM, can be calculated as:

CSIRO (2007): 82 g milksolids/kg DM Nicol and Brookes (2007): 73 g milksolids/kg DM This results in an apparent increased response of approximately 13% using the CSIRO (2007) approach. However, this has not taken into account the 16% increase in maintenance costs, which have to be met from the extra feed consumed.

#### CONCLUSION

It is our contention that the Nicol and Brookes (2007) approach has advantages over that of CSIRO (2007), in that the calculation of maintenance requirements is not dependent on animal productivity and that tissue support costs ought really be considered as a function of production, rather than maintenance. This should therefore provide more realistic estimates of responses in practice to changes in ME intake, because there is no need to adjust the maintenance requirement when assessing the likely effects of changes in feeding level on animal production.

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# The effect of heat processing on selenium balance in cats fed dietary inorganic and organic selenium

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## ABSTRACT

The bioavailability of a nutrient is an important factor to consider when formulating petfoods. Commercial petfoods are subject to several methods of heat treatment and these processes decrease the nutritive value of the diet. The aim of this study was to investigate the influence of heat treatment on selenium (Se) availability in cats. Twenty cats were fed a commercial canned cat food containing 0.5  $\mu$ g Se/g DM (control) or the control diet supplemented with inorganic or organic Se before or after heat treatment to give total Se concentrations of 3  $\mu$ g Se/g DM. Diets were fed for 11 days and faecal and urine samples were collected daily, pooled for the five day collection period (last five days) and used for the analysis of total Se concentrations and subsequent estimations of apparent absorption and retention. Blood samples were obtained before and after the 11 day treatment period. Heat processing decreased the apparent availability of inorganic Se and the utilisation of organic Se, therefore it was concluded that heat processing effected absorption and utilisation of Se in cats fed commercial petfoods.

#### **INTRODUCTION**

Formulation of a well-balanced petfood requires knowledge of the nutrient requirements of the animal, the composition of ingredients and the bioavailability of nutrients within those ingredients (Dzanis, 1994). As an essential trace element and key antioxidant, an adequate intake of dietary selenium (Se) is important for the maintenance of optimum health. In petfoods, Se originates from the ingredients used to formulate the food, such as grains, cereals, animal tissues, plant and animal by-products (Mumma *et al.*, 1986), or can be added as a supplement. There is large variation in the Se content of petfoods (Simcock *et al.*, 2005), which may be attributed to the variety and source of the Se content of dietary ingredients. In addition, as there are no regulations governing the inclusion of Se in petfoods, supplementation occurs at the discretion of the manufacturer. Historically, supplementation in animal feeds has been in the form of inorganic sodium selenite (Sunde, 1997), however organic forms of Se, such as selenised yeasts, appear to be more beneficial due to their increased bioavailability, decreased toxicological risk, ability to increase production in animals and improve Se status in both animals and humans (Power, 2005).

In order to preserve shelf life, increase palatability, and attain a certain physical form, unprocessed petfood is subjected to heat treatment during extrusion, baking, pasteurisation, canning or sterilisation (Hendriks *et al.*, 1999). Commercial petfoods are highly processed, and heat treatment is thought to decrease the nutritive value of the diet (Hendriks *et al.*, 1999; National Research Council, 2006). Antioxidant losses of up to 50% have been reported in extruded petfoods (Tucker, 2004), and many Se compounds

are unstable and volatile (Higgs *et al.*, 1972), therefore it is possible that heat treatment has an effect on Se availability in the diet.

The aim of this study was to assess the effect of heat treatment on the apparent absorption and retention of inorganic and organic Se in cats.

#### **MATERIALS AND METHODS**

Twenty domestic cats (ten male, ten female) aged between 1 and 8 years and weighing between 2.61 and 6.09 kg were used in the study. One month prior to commencement of the trial all animals were fed the control diet, a commercial moist feline diet with a Se concentration of  $0.5\mu g/g$  DM, to standardise Se exposure. Four cats were allocated to each of five nutritional treatment groups and fed the control diet, or the control diet supplemented with inorganic (Inorg; sodium selenite – Nutritech International Ltd, Auckland, NZ) or organic (Org; SelPlex<sup>TM</sup> - Alltech Inc, Kentucky, USA) Se before (-) or after (+) heat treatment to give total Se concentrations of 3  $\mu$ g Se/g DM. Target and actual Se concentrations are shown in Table 1.

Table 1: Selenium concentrations in feline diets supplemented with inorganic and organic Se sources.

Group	Target level	Actual level*		
	µg Se/g DM)			
Control	0.5	0.48		
Inorg+	3.0	2.39		
Inorg-	3.0	2.65		
Org+	3.0	2.76		
Inorg-	3.0	3.50		

\*Actual levels obtained from mean of quadruplicate samples pooled over trial

Diets were fed for 11 days which included a six day adaptation period and a five day collection period. Blood samples were obtained before and after the 11 day feeding period. Faeces, urine and sub-samples of each diet were collected daily, pooled for the collection period and used for the analysis of total Se concentrations and subsequent estimations of apparent absorption and retention. Due to discrepancies between dietary Se intakes of cats fed the treatment groups, data was standardised by applying a correction factor to Se concentrations of faeces, urine and the second plasma sample. Data were analysed by one way ANOVA and multiple comparisons determined using Duncan's test (SAS, v8.02).

## RESULTS

In the initial plasma sample, Se concentrations in control animals were lower than those in the treatment groups (p<0.05), but there were no differences in Se concentrations between groups of cats fed the different treatment diets (Table 2). After the 11 day treatment period, plasma Se concentrations were highly variable between groups, and with the exception of cats fed Org-, concentrations increased numerically in treated animals between days 0 and 11, this increase was only significant in cats fed the Org+ diet (p<0.05; Table 2).

		Plasma Se (µmol/L)	P > F			
Time	Diet	Mean $\pm$ SEM	Diet	Time	Diet x Time	
Day 0	Control	$4.5\pm0.2^{\mathrm{a}}$	0.0003	0.0168	0.3309	
	Inorg+	$5.3\pm0.7^{ m ab}$				
	Inorg-	$5.9\pm0.3^{\mathrm{b}}$				
	Org+	$6.1 \pm 0.2^{b}$				
	Org-	$5.8\pm0.2^{\mathrm{b}}$				
Day 11	Control	$4.5\pm0.4^{\mathrm{a}}$				
·	Inorg+	$8.6\pm1.7^{\rm b}$				
	Inorg-	$7.0 \pm 1.0^{\mathrm{b}}$				
	Org+	$9.6\pm2.2^{\mathrm{b}}$				
	Org-	$5.8 \pm 1.0^{\mathrm{ab}}$				

Table 2: Plasma Se concentrations in cats before and after being fed control (0.5  $\mu$ g Se/g DM) or test diets (3.0 $\mu$ g Se/g DM)

Differences between treatments are indicated by different letters (p<0.05)

Table 3 shows faecal and urinary excretion, apparent absorption and retention of total Se (controls), and supplemented Se (treatments) as a percentage of dietary intake. Se excretion in faeces of cats fed the Org+, Org- and Inorg+ diets were half that of cats fed the control diet, and cats fed the Inorg- diet excreted the least amount of Se in their faeces (p<0.0001). Up to four times as much Se was absorbed by cats fed the treatment diets, with over six times more absorbed by those fed Inorg- (p<0.0001). More than twice as much Se was excreted in the urine of cats fed supplementary Se compared to those fed the control diet, and urinary excretion was highest in cats fed the Inorg- diet (p<0.0001). Retention of Se as a percentage of dietary intake was highly variable and with the exception of cats fed both the Org diets, there were no significant differences.

Table 3: Se absorbed, excreted or retained by cats fed a control diet, or diet supplemented with inorganic (Inorg) or organic (Org) Se before (-) or after (+) heat processing

Preterio				
	Se excreted in faeces <sup>1</sup> (%)	Se absorbed <sup>1,2</sup> (%)	Se excreted in urine <sup>1</sup> (%)	Se retained <sup>1,3</sup> (%)
Controls:	$86.5\pm0.8^{\rm a}$	$13.6\pm1.0^{\text{a}}$	$19.1\pm3.2^{\rm a}$	$-4.5 \pm 3.7$
Treatments <sup>4</sup>				
Inorg+	$49.8\pm6.2^{b}$	$50.2\pm6.2^{\rm b}$	$50.4\pm4.3^{\rm b}$	$4.3 \pm 4.2$
Inorg-	$19.8\pm4.2^{\rm c}$	$80.2 \pm 4.2^{\circ}$	$87.7\pm8.0^{\rm c}$	$-7.5 \pm 11.5$
Org+	$41.3\pm5.5^{\mathrm{b}}$	$58.7\pm5.5^{\rm b}$	$67.8\pm2.4^{\rm d}$	$-12.5 \pm 7.1^{a}$
Org-	$46.3 \pm 4.5^{b}$	$53.7\pm4.5^{\rm b}$	$38.1 \pm 2.3^{b}$	$15.8\pm6.5^{\mathrm{b}}$
a 1 11	11.00	11.00	0.05	

Columns with different superscripts are different (p<0.05)

<sup>1</sup> calculated as a percentage of dietary intake

<sup>2</sup> calculated from the difference between dietary intake and faecal excretion

<sup>3</sup> calculated from difference between dietary intake, faecal and urinary excretion

<sup>4</sup> values represent absorption, excretion or retention of supplemented Se only (calculated by difference from the amount of Se in the control diet)

#### DISCUSSION

In this trial difficulties were encountered obtaining similar dietary Se concentrations across all treatment diets due to various factors in the commercial operation used to produce them. However some differences were observed that indicated a potential effect of heat processing on supplemented Se.

Although plasma Se concentrations varied between cats in the different groups on day 0, concentrations of Se in control animals were unchanged after 11 days and there were no significant differences in Se concentrations in cats fed the different treatment diets at this time. Plasma Se levels are thought to reflect dietary Se intakes (Reilly, 1993), and Se concentrations increased in cats fed the treatment diets at the end of the 11 day feeding period, although they were highly variable and only significantly higher in those fed the Org+ diet. It is possible that the feeding period was not long enough to increase plasma Se concentrations in some of the groups or that the difference in dietary Se concentrations between control and treatment diets was not sufficiently large to elicit a significant increase.

The results show increased Se absorption in cats fed the diets supplemented with Se compared to the control diet, suggesting absorption is affected by the level of dietary Se intake. Both inorganic and organic forms of Se are reported to be well absorbed in humans, with approximately 60% of inorganic Se and 20-30% of organic Se secreted in urine (Robinson *et al.*, 1997). The increased apparent absorption of cats fed the inorganic Se added after processing (Inorg-) indicated that a greater amount of Se was available to cats following ingestion compared to Se subjected to heat treatment (Inorg+). If the high level of Se absorbed from the diet and subsequently excreted in the urine of cats fed the Inorg- diet is considered in relation to the way in which inorganic Se is typically utilised and metabolised (Combs and Combs, 1986; Patterson *et al.*, 1989; Daniels, 1996), this may reflect increased availability. It would seem that the majority of Se from high dietary levels absorbed by cats fed Inorg- was not required for selenoprotein synthesis, and was therefore methylated and excreted in urine. It was concluded that heat processing decreased feline absorption of inorganic Se.

In contrast, cats fed the diet with organic Se added after processing (Org-) showed a similar level of absorption to the diets in which supplemented Se was exposed to heat treatment (Inorg+, Org+). However the amount of Se excreted in the urine by these cats was considerably lower than in the other groups (38% compared to 50% and 68% respectively), and consequently, a significant amount of this Se was retained in the body. The differences between the Org- and Inorg+ groups can be explained by the different ways in which these forms of Se are metabolised and utilised (Combs and Combs, 1986; Daniels, 1996; Wolffram, 1999), and may indicate that heat processing affected the ability of organic Se to be incorporated into body proteins.

Heat processing therefore affects the way in which Se is absorbed and utilised by cats fed commercial diets, and this should be considered when formulating petfoods.

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## Broiler performance is adversely affected by higher pelleting temperatures

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## ABSTRACT

A trial was conducted to determine the effects of high pelleting temperatures on broiler performance, apparent metabolisable energy (AME) and pellet quality. Two basal diets (maize and wheat) were pelleted after conditioning at 60, 75 and 90 °C for 30 seconds and each of the six dietary treatments was fed to six replicate cages of broiler chickens (eight per cage) from day 1 to 21 post-hatch. Weight gain was influenced (p<0.001) by grain type and pelleting temperature. Increasing the pelleting temperature decreased weight gain in both wheat- and maize-based diets. Increasing the pelleting temperatures, however, increased (p < 0.001) feed per gain of broilers, with birds fed low pelleting temperature diet having a lower feed per gain than those fed diets pelleted at moderate and high temperatures. AME was influenced (p < 0.001) by grain type, but not (p>0.05) by pelleting temperatures. Pellet durability index (PDI) was influenced (p<0.01) by pelleting temperature, with moderate and high pelleting temperature diets having a higher PDI than low pelleting temperature diet. There was an interaction (p<0.001) between grain type with pelleting temperature for PDI. Increasing the pelleting temperature improved PDI in wheat-based diet, but no effect on PDI in maizebased diet. Overall, the present data demonstrate that increasing the pelleting temperature from 60 to 90 °C has adverse effects on the performance of broiler starters fed wheat- and maize-based diets.

## **INTRODUCTION**

Feed processing includes the physical, chemical and thermal processing of a feed prior to consumption by animals (Maier and Bakker-Arkema, 1992). The most common processing operations in feed manufacturing plants are receiving the raw materials, grinding (particle size reduction), mixing, heating (thermal treatment), packing and loading. Nearly every one of these operations can have either a negative or positive influence on subsequent animal performance and can certainly influence the profitability of a poultry production company (Behnke and Beyer, 2002). Among these operations, thermal treatment is very important as the most chemical and physical changes happen during this process. The processing technology of poultry feeds involves a wide range of thermal treatments including extrusion, expansion, conditioning and pelleting. But when the cost-benefit is considered, pelleting is the widely used thermal processing method. Pelleting plays a central role in feed manufacturing processes, especially in the broiler industry. Pelleting agglomerates smaller feed particles with the help of mechanical pressure, moisture and heat, and is known to positively influence the weight gain, feed intake and feed efficiency of broilers, which can be attributed to less feed wastage, higher nutrient density, no selective feeding, improved palatability, improved starch digestibility, increased nutrient intake, changes in physical form and decreased energy spent for eating (Peisker,

2006). Optimal conditioning temperature is a debatable pelleting variable. Heat applied during conditioning may aid in the destruction of pathogens (i.e. Salmonella), and antinutritive factors found in some ingredients (i.e. trypsin inhibitor in soybean meal). High temperatures are needed for proper agglomeration of nutrients and are essential for achieving high pellet quality (Thomas and van Der Poel, 1996). Pelleting, however, can also negatively affect feed quality and result in poorer broiler performance, if appropriate temperatures are not used. Bedford *et al.* (2003) reported that temperatures above 65 °C have adverse effects on broiler performance fed wheat-based diets. The aim of the present experiment was to test the effect of conditioning temperature on feed quality, energy utilisation and performance of broilers fed maize- and wheat-based diets.

## MATERIALS AND METHODS

Two broiler starter diets, each based on one of the grains (maize or wheat) were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Table 1). Both diets were isocaloric and isonitrogenous. Each formulated diet was then divided to three equal batches and was pelleted using a pellet mill (Richard Size Limited Engineers, Orbit 15, Kingston-upon-Hull, England) capable of manufacturing 180 kg of feed/h and equipped with a die ring (3-mm holes and 35-mm thickness). The mash diets were conditioned pre-pelleting to three different temperatures (60, 75 and 90 °C). Conditioning temperature was measured at the outlet of the conditioner. Each of the 6 dietary treatments was fed to six pens, each housing eight broiler chickens, from day 1 to 21 post-hatch. Feed and water were offered *ad libitum* throughout the trial. Body weights and feed intake by cage were recorded at weekly intervals throughout the trial. Mortality was recorded daily. Feed per gain values were corrected for the body weights of any birds that died during the course of the experiment.

A classical total excreta collection assay was conducted during the last 4 days of the trial for the determination of AME values. Pellet durability index (PDI) was determined in a Holmen Pellet Tester (New Holmen Pellet Tester, TekPro Ltd., Norsolk, UK) using the method described by Svihus *et al.* (2004). The data were subjected to two-way analysis of variance and differences were considered to be significant at p<0.05 and significant differences between means were separated by the Least Significant Difference test.

Ingredient	Maize-based diet	Wheat-based diet
Wheat	-	647.2
Maize	617.5	-
Soybean meal	278.6	228.4
Meat and bone meal	80	80
Soybean oil	8.7	27.6
Limestone	2.3	2.1
Salt	1.6	1.5
Sodium bicarbonate	0.9	0.1
Lysine HCl	2.3	3.7
DL-methionine	2	2.6
L-threonine	0.1	0.8
Trace mineral-	3	3
Titanium oxide	3	3
Calculated analysis		
Metabolisable	3010	3010
Crude protein	230	230
Lysine	13.8	13.8
Methionine	5.5	5.6
Methionine +	9.2	9.2
Threonine	8.5	8.5
Calcium	10	10
Available	5.2	5.2

Table 1: Composition and calculated analysis (g/kg as fed) of the basal diets

#### RESULTS

The influence of dietary treatments on the performance of broilers, AME and pellet durability index of the diets is presented in Table 2. Weight gain was influenced (p<0.001) by grain type, with weight gain of birds fed the maize-based diet being greater than those fed the wheat-based diet. The main effect of conditioning temperature on weight gain was significant (p<0.001), but there was an interaction (p<0.01) between grain type with conditioning temperature. Increasing the conditioning temperature decreased weight gain in wheat-based diets, but birds fed high conditioning temperature maize-based diet had a similar weight gain to those fed the low conditioning temperature maize-based diet.

Grain type had no effect (p>0.05) on feed intake. Feed intake was influenced (p<0.001) by conditioning temperature, with the intake of the low conditioning temperature diet being greater than those of the moderate and high conditioning temperature diets. A significant (p<0.05) interaction was observed between grain type and conditioning temperature; for maize-based diets, birds fed the high conditioning temperature diet had a higher feed intake than those fed the moderate conditioning temperature diet. However, increasing the conditioning temperature decreased feed intake in wheat-based diets.

Birds fed the maize-based diet had a lower (p<0.001) feed per gain compared to those fed the wheat-based diet. Increasing the conditioning temperature increased (p<0.001) feed per gain of broilers, birds fed the low conditioning temperature diet had a lower feed per gain than those fed moderate and high conditioning temperature diets.

There was, however, a tendency (p=0.06) for a grain type x conditioning temperature interaction, with low conditioning temperature having the lowest feed per gain in birds fed maize- and wheat-based diets.

There was no main effect (p>0.05) of conditioning temperature and interaction (p>0.05) between grain type with conditioning temperature on the AME of diets. However, the main effect of grain type was significant (p<0.001) for AME, with greater AME values in the maize-based diets. The PDI of pellets was not influenced (p>0.05) by grain type. A significant (p<0.001) interaction was observed between grain type and conditioning temperature; increasing the conditioning temperature improved PDI in the wheat-based diet, but had no effect on PDI in the maize-based diet.

## DISCUSSION

The present results indicate that feeding broiler chickens with maize-based diets increased weight gain and improved feed per gain ratio compared to birds maintained on wheat-based diets. These improvements can be attributed to higher apparent metabolizable energy (AME) and better nutrient digestibility. Regardless of the grain type, increasing the conditioning temperature resulted in lower body weight gain and feed intake of birds and also impaired the feed per gain ratios. Similar findings have been reported in a number of studies (Silversides and Bedford, 1999; Bedford *et al.*, 2003; Cowieson *et al.*, 2005; Creswell and Bedford, 2006; Kirkpinar and Basmacioglu, 2006).

It is noteworthy that in spite of an improvement in PDI with increasing conditioning temperature in wheat-based diets, all performance parameters were negatively affected. Therefore, despite the fact that high pellet quality (low levels of fines) is reported to improve performance of pelleted feeds (Jensen, 2000; Kenny, 2008); it seems that high pellet quality does not appear to overcome the negative effects of high temperature conditioning.

Grain type	Conditioning	Weight gain	Feed intake (g)	Feed per gain	AME	PDI <sup>3</sup> (%)
	temperature <sup>2</sup>	(g)		(g/g)		
Maize	Low	1040 <sup>a</sup>	1272 <sup>ab</sup>	1.228	14.16	81.4 <sup>ab</sup>
	Moderate	960 <sup>b</sup>	1203 <sup>c</sup>	1.265	14.19	81.6 <sup>ab</sup>
	High	1015 <sup>a</sup>	1279 <sup>a</sup>	1.261	14.27	79.3 <sup>ad</sup>
Wheat	Low	1021 <sup>a</sup>	1338 <sup>d</sup>	1.315	13.08	76.7 <sup>d</sup>
	Moderate	925°	1235 <sup>bc</sup>	1.344	13.18	82.7 <sup>bc</sup>
	High	908 <sup>c</sup>	1255 <sup>ab</sup>	1.383	13.10	85.1 <sup>c</sup>
$SEM^4$	C	11.6	14.9	0.009	0.071	0.938
Main						
Grain						
Maize		1005	1251	1.251 <sup>a</sup>	14.21 <sup>a</sup>	80.7
Wheat		951	1276	1.347 <sup>b</sup>	13.12 <sup>b</sup>	81.5
Conditioni	ng					
Low	-	1030	1305	$1.271^{a}$	13.62	79.0
Moderate		942	1219	1.304 <sup>b</sup>	13.68	82.1
High		961	1267	1.322 <sup>b</sup>	13.69	82.2
Probabiliti	es, p≤					
Grain type	· •	***	NS	***	***	NS
Conditioni	ng	***	***	***	NS	**
Grain type	x C.	**	*	0.06	NS	***

Table 2: Influence of grain type and conditioning temperature on the weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broilers (1-21 days posthach)<sup>1</sup>, AME (MJ/kg dry matter) and pellet durability index (%)

<sup>a,b,c,d</sup> Means in a column not sharing a common superscript are significantly different (p<0.05) NS, not significant; \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001. <sup>1</sup> Each value represents the mean of six replicates (8 birds per replicate). <sup>2</sup> Low (60 °C); Moderate (75 °C); High (90 <sup>3</sup> Each value represents the mean of six replicates. <sup>4</sup> Pooled standard error of mean.

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### Satiety – why we feel full

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The prevalence of obesity has increased rapidly in recent years. Obesity is associated with increased risk of the development of type 2 diabetes, cardiovascular disease, hypertension and stroke. While hunger and appetite represent the physiological and psychological need and desire to eat, respectively, satiety is commonly referred to as the physiological and psychological feeling of fullness. Protein, and its relationship with satiety, is gaining more interest with the re-emergence of the popular "high protein" diets. It is widely believed that protein is more satiating than either carbohydrate or fat, which may help facilitate weight loss over the longer term. However, within proteins, the effect on satiety appears to be dependent on the source of protein. There is some evidence that dairy whey protein produces a stronger effect on satiety versus casein, egg albumin and carbohydrate. The development of high-satiety foods offers an opportunity for the food industry to assist individuals with weight reduction and management.

### **Effects of Probiotic on weaner pig performances**

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An experiment was conducted to investigate the effect of different probiotics on the growth performance, blood parameters and faecal scores of piglets in the 3-week post-weaning period. A total of 120 piglets from four farms, differing in their health status, were fed one of 4 different diets (control, and control + probiotics 1, 2, or 3) *ad libitum* for 3-weeks post-weaning. Over the three-week experimental period no difference in mean daily feed intake (413 ± 68 g/d SD), gain (308 ± 65 g/d) or feed conversion ratio ( $1.36 \pm 0.19$  g/g) were observed between dietary treatment groups.

Over the entire experimental period, the farm of origin had a significant effect on all white blood cell types except for the number of Basophils. The number of white blood cells increased from day 0 to day 14, but was identical on days 14 and 21. During the experiment the numbers of white blood cells were significantly higher for piglets fed the probiotic 1, due to the increased number of monocytes for this group. No effect of probiotic on the incidence of post-weaning diarrhoea was found, as indicated by the similar faecal scores across treatment groups. It is concluded that the probiotics used in this study did not have any significant effect on the piglets post-weaning growth performances, blood parameters or diarrhoea.

## Estimation of genetic potential for maximum protein deposition rate and energy partitioning in growing pigs.

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When computer pig models are used commercially to simulate a pig response to management and feeding strategies, it is important that the genetic performance potential of the pig is characterized correctly. The genetic performance potential is defined as the 'operational maximum protein deposition potential' (PdMax), which is the maximum protein deposition rate (g/d) that can be achieved for a certain type of pigs under specific conditions. Another intrinsic pig parameter is the minimum lipid to protein ratio (MinLP), which represent the energy partitioning between lipid and protein deposition when energy intake but not protein intake is limiting.

In a trial, with males and females pigs from four genotypes, values for MinLP and PdMax were estimated for 64 individual pigs. The average live weight of the pigs was 31 kg at the start of the trial, and the pigs were slaughtered at an end weight of approximately 70 kg.

In order to find a value for MinLP, the pigs were fed initially a diet for a period of four weeks, which was restricted in energy, but not limiting in protein and/or amino acids. After four weeks, a new diet was offered, which was not limiting for both energy and protein, in order to allow expression of PdMax. This diet was fed until the pigs reached the end weight. Pigs were fed ad libitum. Body weight and feed intake were recorded weekly, carcass weight and P2 backfat were measured at slaughter.

The feed intake and body weight data were used to compare two methods of on-farm estimation of PdMax and MinLP: one method which uses live weight and P2 measurements to derive body composition at the end of the growing period, and one which uses the growth model itself to find the best prediction of the individual growth curves. Significant differences were found between genotypes and sexes for values of PdMax, but not for MinLP

# Immune enhancing potential of a canola oil-based supplement in the cat

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**Background:** Interest in the use of functional foods as a non-invasive way to modulate and optimise the human immune system is beginning to transfer into the companion animal industry with pet owners seeking a more natural way of maintaining their animals' health. To date, dietary supplementation trials have been carried out primarily in humans and mice. These trials have demonstrated the ability of a variety of ingredients such as probiotics and specific ratios of  $\omega$ -3 and  $\omega$ -6 fatty acids to modulate a range of immune functions. However, little data exists for companion animals especially cats.

**Objective:** To assess potential immune enhancing benefits of dietary supplementation with a NZ made canola oil based dietary supplement, in the cat.

**Design:** Sixteen adult, domestic cats: 8 per group were fed *ad libitum* a commercial moist control diet with or without oil supplementation (18.95ml/kg diet ; MyBeau ®, VitaPower Ltd, Wanganui, NZ) for 28 days. Blood samples (day 0, 14, 28) were used to assess immune responses: lymphocyte proliferation, cell surface markers, phagocyte function.

**Outcomes & Conclusion:** Dietary supplementation resulted in significant enhancement of lymphocyte proliferative responses to PHA (p=0.026). This indicates specific groups of T-cells can be up-regulated by consumption of the oil-based supplement, priming them to proliferate in response to an appropriate antigenic challenge (e.g. bacterial infection). A time dependant increase (p=0.0003 at 4 weeks) in phagocytic activity indicates a greater ability to fight infection and disease in the cats fed the MyBeau  $\circledast$ -supplemented diet compared to control fed animals.

## Omega-3 PUFA status from farmed salmon compared to salmon oil capsules

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**Background**: Salmon and fish oil are good sources of long chain (LC)  $\omega$ -3 PUFA which are well recognized for their health benefits. It may be better to consume these fatty acids from fish than from supplements. The  $\omega$ -3 index is a marker of risk for sudden cardiac death.

**Objectives**: To investigate the most effective method of increasing red blood cell (RBC) LC  $\omega$ -3 status from consumption of farmed salmon compared to salmon oil capsules.

**Design**: Healthy volunteers (n=44) were randomly assigned to one of 4 groups consuming 2x120g servings of farmed salmon/week or 2, 4 or 6 capsules of salmon oil/day, for 8 weeks. Salmon (after cooking) and capsules were analysed for their fatty acid content. RBC fatty acid levels were analysed and  $\omega$ -3 index was calculated. Using linear regression analysis predictive models were fitted to the capsule data to predict changes in RBC LC  $\omega$ -3 levels and  $\omega$ -3 index with intakes of LC  $\omega$ -3 from capsules in amounts equivalent to that consumed from salmon.

**Outcomes**: LC  $\omega$ -3 intakes from salmon and 2, 4 and 6 capsules were 0.82, 0.24, 0.47 and 0.68 g/day. Results from the predictive models (R<sup>2</sup> = 0.32 for RBC EPA (p=0.001); 0.15 for RBC DHA (p=0.03); 0.31 for  $\omega$ -3 index (p=0.001)) showed that increases in RBC LC  $\omega$ -3 levels and  $\omega$ -3 index were similar with intakes of 0.82 g LC  $\omega$ -3 from salmon and capsules (salmon vs capsule, RBC EPA: 0.80 vs. 0.94%; RBC DHA: 0.93 vs. 1.11%;  $\omega$ -3 index: 1.74 vs. 2.10%).

**Conclusion**: Consumption of similar amounts of LC  $\omega$ -3 PUFA either from salmon (2 weekly servings) or salmon oil (daily dosage) was equally effective in increasing RBC LC  $\omega$ -3 status. The  $\omega$ -3 status can be improved by either method according to consumer preference.

# The effect of probiotics (Yakult®) on breath hydrogen patterns after lactulose and symptoms in IBS patients with an early breath hydrogen rise – a proof of concept study

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**Background:** Distal small intestinal bacterial overgrowth (DSIBO) is common in irritable bowel syndrome (IBS) and is directly related to the genesis of symptoms. Reduction of intestinal bacterial load with antibiotics is efficacious, but the effect of probiotics remains untested.

**Objectives:** To examine whether Yakult® can reverse DSIBO and reduce IBS symptoms.

**Design:** Eighteen patients with IBS (Rome II criteria) and DSIBO (early rise in breath hydrogen after lactulose (ERBHAL),  $\leq 60$  min) were studied. After a one-to-two week run in period, 56 ml (one bottle) of *Lactobacillus casei* Shirota strain (Yakult®) was consumed daily for six weeks. Lactulose breath testing was repeated at the end of the treatment period and symptom severity was rated weekly using a 10 cm visual analogue scale (VAS).

**Outcomes:** Of 14 patients completing the study, nine (64%) had reversal of ERBHAL, with the median time of first rise increasing from 45 to 75 minutes (p=0.03). There was no significant improvement in symptoms, except for wind (p=0.04). Patients with moderate/severe baseline symptoms who no longer had ERBHAL after treatment improved overall symptoms (55% reduction; n=6) compared to those with persistent ERBHAL (12% reduction; n=5; p=0.18).

**Conclusion:** These findings suggest there may be a role for Yakult® in the treatment of IBS with probable DSIBO. A double blind, randomised, controlled trial of this probiotic is indicated.

# Effect of ω-3 and ω-6 fatty acids on cytokine levels in feline whole blood cell cultures

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**Background:** Human studies indicate that the varying levels of cytokines are responsible for the regulation and biological function of immune cells. The effects of different  $\omega$ -3 and  $\omega$ -6 concentrations on the production of the anti-inflammatory cytokines IL-4 and IL-10 and pro-inflammatory IFN- $\gamma$  by feline cells are not well defined.

**Objective:** To investigate the effect of  $\omega$ -3 and  $\omega$ -6 fatty acids on IL-4, IL-10 and IFN- $\gamma$  levels in feline whole blood cell cultures *in vitro*.

**Design:** Whole blood from 10 healthy adult cats was diluted 1:4 in complete RPMI-1640 medium and cultured for 72 hours at 37°C in a 5% CO<sub>2</sub> atmosphere, in the presence or absence of different concentrations (0 (control); 0.001; 0.01 and 0.1 %) of  $\omega$ -3 (DHA) or  $\omega$ -6 (AA) fatty acids. Culture supernatants were frozen prior to cytokine analysis by ELISA.

**Outcomes:** Incubation with DHA or AA at a concentration of 0.1% significantly (p<0.05) increased IL-10 levels. Incubation with AA or DHA had no significant effect on IL-4 levels in feline whole blood cell cultures. IFN- $\gamma$  levels in culture supernatants showed a very small increase (p=0.051) when incubated with the highest concentration of AA (0.1%). When incubated with DHA, IFN- $\gamma$  levels did not change in the blood cell culture supernatants.

**Conclusion:** This data suggests that both  $\omega$ -3 and  $\omega$ -6 fatty acids may play a role in regulating IL-10 production in the cat and further study is needed to confirm this result *in vivo* and to investigate possible mechanisms of action.

## Are dietary blackcurrant and green tea polyphenols available for metabolism in the brain?

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**Background:** Berry fruit and green tea are each considered to be healthy foods due to their polyphenol contents. Their health benefit comes from the ability of polyphenols to reduce oxidative damage. Flavanoids are the most common polyphenols in the human diet and are found in fruit, vegetables, grains and beverages. These compounds show high antioxidant activity *in vitro*. Blackcurrants are rich in anthocyanins while green tea is rich in catechins. The processes of digestion and absorption in the gastrointestinal tract and interactions with other food compounds may result in inhibition or enhancement of their digestion, absorption and metabolism and their subsequent activity *in vivo*. A number of studies have found that flavonoids accumulate in tissues but there little work on the effects of dietary fibre on their digestion, absorption, activity and metabolism.

**Objective:** To determine if dietary antioxidants from green tea and blackcurrant were digested and absorbed from the gastrointestinal tract and subsequently available for metabolism within the brain and other tissues, and to determine if the presence of different levels of fermentable dietary fibre affected these measures of bioavailability using the laboratory rat as the animal model.

**Design:** Forty male rats were fed blackcurrant and green tea extracts in diets containing pectin (0, 4 and 8%) for 28 days. At the end of the study, plasma, urine, faecal and brain samples were collected and analysed for flavonoid metabolites by LC-MS.

**Outcomes:** Anthocyanins, catechins and flavonols were excreted in the faeces as parent compounds. In the urine and plasma, however, it was mostly the metabolites of the dietary anthocyanins, catechins and flavonols that were present. No polyphenols were detected in brain. Higher pectin levels may be responsible for increased absorption of catechins as indicated by the differences in metabolites excreted in the urine.

**Conclusion:** The flavonoids from green tea and blackcurrant extracts were available to the body *in vivo* but were not found in the brain tissues. Dietary soluble fibre addition appears to increase the availability of catechins based on the higher metabolite levels in the urine.

#### Nutrition screening in older adults with fall related fractures

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**Background:** In New Zealand hip fractures are the most common injury in older people admitted to hospital. Under-nutrition is an independent risk factor and is associated with longer hospital stays, higher co morbidities and more infections than persons who are well nourished. Nutrition screening after admission can identify patients at risk. These patients can then be referred to a dietitian for nutrition assessment and support. Regardless of age malnourished patients who receive nutrition support have a more successful recovery.

**Objective:** To identify nutrition risk in older patients admitted to Waikato Hospital with fractures due to falls using the validated 'Malnutrition Universal Screening Tool' (MUST).

**Design:** A descriptive survey to screen patients over 65 years with fractures due to falls for nutrition risk within 24 hours of admission. The survey was undertaken over a four week period. Screening included the determination of BMI (height and weight measures included MUAC, ulna length or recall), unplanned weight loss in past 3-6 months and an acute disease effect (no nutritional intake for >5 days). For each of these determinants scores were assigned for 0=Low risk, 1=Medium risk, 2=High risk. Scores were added to provide an overall score for risk of malnutrition. Patients at high risk were referred to a dietitian. Dietetic referrals were assessed at baseline and as a result of screening.

**Outcomes:** Thirty patients were screened used MUST. Most were women (22), NZ European (29) with mean age of  $80.10 \pm 10.72$  years. Nearly half of the patients had a history of falls (13) and 6 a history of fracture. There was an average unplanned weight loss for all patients of 2.5kg over the previous 3 to 6 months. Using MUST; patients were classified at low (43%), medium (30%) and high (27%) risk of malnutrition. Repeat screening was undertaken after one week in patients at low or medium risk. This indicated an increased prevalence of patients at high risk (37%) but no change in low or medium risk. As a result of nutrition screening dietetic referrals increased by two-fold during the four week period.

**Conclusions:** MUST is used as a nutrition screening tool because of its ease of use, simplicity, reliability and validity. In the four weeks MUST was trialed at Waikato hospital it was shown to be a practical method to identify older patients at nutrition risk. For maximum patient benefit it is important that the entire care pathway (screening, assessment, treatment and monitoring) be followed. The implications of implementing nutrition screening on dietetic workloads are unknown. Further investigations are needed to assess the benefits among larger subject groups.

#### Iron status and cognitive function in female university students

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**Background:** Recent research from the United States suggests that non-anaemic iron deficiency (ID) may affect cognitive function in adults. There are currently no New Zealand data.

**Objective**: To determine any association between iron status and cognitive function as part of the larger WISE Study investigating iron status of female university students aged 18-44yrs.

**Design:** Serum ferritin (SF) and haemoglobin (Hb) were analysed on fasted venipuncture blood samples. Women with non-anaemic iron deficiency (SF<20 $\mu$ g/L, Hb $\geq$ 120g/L) were compared with women with normal iron status (SF $\geq$ 20 $\mu$ g/L, Hb $\geq$ 120g/L). Three cognitive tests were completed online in English: a letter number sequencing task - working memory (max score 21); a finding words task - processing speed (max score 200); and a word recall task - long-term memory (max score 25). Socio-demographic and other data were also collected.

**Outcomes:** Non-anaemic iron deficiency was not associated with differences in cognitive function in students whose first language was English (n=150). In students for whom English was not their first language (n=52) those with ID (n=10) scored significantly lower on working memory (mean [95% CI]: 9.7 [6.8, 12.5] *vs.* 13.3 [12.1, 14.5], p=0.02) and processing speed (34.9 [26.9, 42.9] *vs.* 50.5 [46.8, 54.3], p=0.001) compared to those with normal iron stores (n=42) even after controlling for age.

**Conclusions:** Students whose first language is not English may experience a reduction in processing and working memory skills when working in English if they have poor iron status. This may affect academic studies.

## Osteoporosis knowledge and health beliefs among a sample of South Asian women in Auckland, New Zealand

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**Background**: Prevalence of osteoporosis is expected to increase due to the ageing population. Osteoporosis knowledge and health beliefs are important factors for the prevention of osteoporosis among South Asian women, the second largest Asian ethnic group in New Zealand.

**Objective**: To determine the level of osteoporosis knowledge and health beliefs in a sample of South Asian women living in Auckland, New Zealand.

**Design**: 102 women aged >20 yr of South Asian origin completed the validated Osteoporosis Knowledge Test (OKT) and the Osteoporosis Health Belief Scale (OHBS) online. The maximum possible scores that could be achieved were 26 for the OKT.

**Outcomes:** Mean OKT score was  $15.1 \pm 4.14$  indicating a low level of osteoporosis knowledge. Half of these women thought osteoporosis was a serious disease but only 29% agreed that there is a good chance they would get osteoporosis. Participants perceived many benefits and few barriers to regular physical activity and dietary calcium intake for the prevention of osteoporosis. These women reported a high health motivation with 95% agreeing that keeping healthy was very important.

**Conclusion**: Osteoporosis education interventions are required among this population group. Future research is required to determine whether osteoporosis knowledge and health beliefs relates to osteoporosis preventive behaviours among South Asian women.

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Intending authors are recommended to submit their papers to a Nutrition Society member or a colleague prior to submission to the editor to confirm the paper is in the recommended format of the Proceedings of the Nutrition Society. **One copy of each paper** should be posted to the Editor **within one month of the conference**.

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Smith, A.B. (1980). Fat digestion in Teal. In: *Birds of New Zealand*. (Ed. E.F. Jones). Pp. 21-29. Palmerston North, ADB Press.

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Smith, A.B. and Brown, C.D. (1982). A multienzyme technique for estimating protein digestibility. *New Zealand Journal of Science* 5, 123-130.

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