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An update on selenium status and requirements in New Zealand

CHRISTINE D. THOMSON
Department of Human Nutrition, University of Otago, Dunedin, New Zealand

ABSTRACT

New Zealand has long been known as a country with a low selenium environment and all early research in human subjects indicated that our selenium status was amongst the lowest in the world. However, we have documented a steady and continuing increase in selenium status of New Zealanders during the past decade. The reasons for this change include an increase in the selenium content of our animal foods, changes in dietary patterns to more varied diets including an increase in consumption of imported foods, in addition to importation of Australian wheat. This review outlines the consequences of New Zealand's low selenium status in terms of overt selenium deficiency, consequences of suboptimal levels of selenoproteins or 'marginal selenium deficiency', and protection against chronic disease and maintenance of optimal health. The paper also discusses requirements and recommendations for selenium based on prevention of deficiency, intakes for maximal levels of selenoproteins and on requirements for optimal health. In New Zealand, we are left with the paradox of a community with low selenium status living in a naturally selenium-deficient environment for animals, yet with apparently healthy human residents, albeit with relatively high incidence of cardiovascular disease and some cancers. As we learn more about selenium deficiency and function, the role of selenium in health and disease is becoming clearer, but there are many questions remaining.

INTRODUCTION

Muriel Bell 1898-1974

Dr Muriel Bell was elected an Honorary Member of the Nutrition Society of New Zealand at the first Annual General Meeting in February 1966, "in recognition of her sterling services to the science of Nutrition." After her death in 1974, the Council of the Society established the Muriel Bell Memorial Lectureship to commemorate her excellence as a nutritionist, and as a humane, inquisitive and dedicated person.

Dr Bell had a lifelong interest in the importance of trace elements in animal and human nutrition. This included her early interest in iodine and goitre as well as in other trace elements. She introduced

Marion Harrison to trace element research in their work on fluorine that led to the fluoridation of water supplies in New Zealand. Marion Robinson (nee Harrison) later became my supervisor and mentor when she introduced me to the frustrating yet fascinating topic of selenium in human nutrition.

Marion Robinson presented the first Muriel Bell Memorial Lecture in 1975 (Robinson, 1975). This was most appropriate given her association with Dr Bell and because of her own significant accomplishments. The title of her talk was "The Moonstone: More About Selenium". Three decades later, we know even more about selenium, but there are still many questions to answer. In that talk she described some of our early research on selenium metabolism, selenium balance and selenium status and response to selenium supplements, which has provided the basis of much research around the world.

Introduction

New Zealand has long been known as a country with a selenium environment and all early research in human subjects indicated that our selenium status was amongst the lowest in the world (Robinson, 1989). But the implications of low selenium status in terms of human health were not clear. In 1988 Ray Burk described the situation as "selenium deficiency in search of a disease" (Burk, 1988), reflecting the uncertainties at that time. There were many 'selenophiles' who believed that selenium was a cure for all ills, including cancer, cardiovascular disease (CVD), rheumatoid arthritis, male infertility and many others, while other researchers were more cautious because of the lack of clear evidence for these associations. We now know much more about selenium deficiency and function, which helps to clarify the role of selenium in health and disease, but there are many questions remaining (Thomson, 2006).

Selenium exerts its functions through the selenoproteins, which contain selenocysteine at their active sites (Kryukov *et al.*, 2003). There are 25 mammalian selenoproteins, not all of which we know the function. The most well known of these include several glutathione peroxidases and three thioredoxin reductases involved in antioxidant and redox functions, three iodothyronine deiodinases involved in thyroid metabolism, and selenoprotein P (SelP) which appears to function in selenium homeostasis and oxidant defence (Burk & Hill, 2005; Gromer *et al.*, 2005), but there are many others the functions of which are not clear. When selenium intake is limited, there is a clear priority for selenium supply, to both specific tissues and to specific selenoproteins, resulting in a "hierarchy" of importance of selenoproteins (Behne *et al.*, 1988). Many of the effects of selenium deficiency or health effects can be attributed to these proteins, but some actions of selenium, such as proposed anti-cancer properties, may operate independently of the selenoproteins.

Update on selenium status in New Zealand

New Zealand with its unusually low iodine, fluorine and selenium environment has provided a natural laboratory for the study of these anionic trace elements. Jim Oldfield, a pioneer in the field of selenium nutrition in farm animals in Oregon, once commented at a trace element workshop in Dunedin that "….really all of New Zealand is a trace element workshop (Oldfield, 1981).

From the time we first began studying selenium in New Zealand, all our studies indicated that our selenium status was lower than most other countries studied at that time (Robinson, 1989). The low status resulted in low activities of the selenoenzyme glutathione peroxidase (GPx) in erythrocytes and plasma, both of which responded to supplementation with selenium in various forms (Thomson, 2004b), a response not observed in countries with higher selenium status.

During the past decade, however, we have documented a steady and continuing increase in selenium status of New Zealanders without intentional intervention (Thomson & Robinson, 1996; Thomson, 2004b). This has been monitored through baseline plasma selenium concentrations in many intervention trials during this period. The increase does not seem to be abating, with even higher plasma selenium in children in 2002 (Thomson *et al.*, 2007), in adults in 2004 (Thomson *et al.*, 2006) and in elderly Otago residents in 2005/2006 (Campbell *et al.*, 2006)

The reasons for the increase in selenium status are several. Clearly the major influence on plasma selenium is dietary intake, and in New Zealand this is closely aligned to geographical location or origin of foods, but there are a number of other factors such as dietary patterns, ethnicity, and geographical distribution of ethnic groups in the country (Thomson, 2004b). A major influence is the importation of Australian wheat into some parts of the country. This is graphically illustrated in the Children's Nutrition Survey, which shows a decline in serum selenium concentrations in children from the North of the North Island to the South Island (Thomson *et al.*, 2007). But there were other factors influencing this variation, such as the interaction between dietary patterns of different ethnic groups and the geographical distribution of these groups. For example, Pacific Island people, who reside mainly in the North Island, have a high intake of fish, also a major contributor to selenium status.

The increase in selenium status is not just due to Australian wheat, as importation has been occurring for some decades (Watkinson, 1981). Another factor is an increase in the selenium content of our animal foods, perhaps due to more efficient supplementation of our sheep and cattle. Although bio fortification of food crops for human consumption has not been used as in Finland (MTT Agrifood Research Finland, 2005), food selenium content has increased indirectly as a result of protecting our animal population. Other reasons might be the consumption of more varied diets that include nuts and legumes, many of which are imported from countries with higher selenium soils. Brazil nuts are a particularly good source of selenium and a recent study has shown that selenium in these nuts is at least equally bioavailable as selenomethionine for increasing plasma selenium and raising GPx activities to maximal levels (Thomson *et al.*, 2006).

Consequences of low selenium status in New Zealand

Selenium deficiency

Our interest in selenium was sparked in the late 1960s, when Hickey (Hickey, 1968) reported that many Southland farmers were dosing themselves with 'Selovet' – a veterinary preparation for the treatment and prevention of white muscle disease, ill-thrift and other selenium responsive diseases in sheep and cattle – for relief from a mysterious fibromyalgia widespread in Southland. This was one of the first indications of a possible role for selenium in human health in New Zealand (Thomson & Robinson, 1980). The late Dr Peter Snow, a Tapanui GP, referred to Southland residents, who

seemed to be prone to an epidemic form of muscular rheumatism (personal communication) and sought help from their veterinary surgeons who advised the use of selenium. Selenium appeared to help the condition and so we undertook a double blind trial to determine the effectiveness of selenium supplementation in preventing these muscular symptoms (Robinson *et al.*, 1981). Symptoms improved in approximately half the subjects in both placebo and supplemented groups, suggesting that beneficial effects ascribed to selenium may have been due to a placebo effect. The usual dose taken by farmers was 5 mg selenium as sodium selenate, nearly 100 times the current recommended intake of 60-70 µg/day (NHMRC, 2006), and there was concern about the effects of such large intakes on the human population.

This muscular syndrome was mirrored in a surgical patient in New Zealand, who had been on total parenteral nutrition (TPN) for some time, and presented with a severe muscular syndrome that prevented her from walking (van Rij *et al.*, 1979). At that time alimentation fluids did not contain trace elements and selenium deficiency was suspected. Supplementation dramatically reversed the symptoms (van Rij *et al.*, 1981). Selenium deficiency in TPN was subsequently reported in other parts of the world with patients presenting with varying symptoms of cardiomyopathy and muscle syndromes including muscle pain, fatigue and proximal weakness (Chariot & Bignani, 2003). Inclusion of trace elements in fluids has now eliminated this problem. The mechanism behind this muscular involvement is unknown. It is tempting to speculate that selenoprotein W, which is abundant in skeletal and cardiac muscle, but is eliminated from tissues in selenium deficiency, or selenoprotein N may be involved (Rederstorff *et al.*, 2006).

The only known natural selenium deficiency syndromes, Keshan Disease, an endemic cardiomyopathy that occurs during preadolescent and adolescent years and Kaschin Beck disease, an endemic osteoarthritis, both of which occur in low selenium areas of China (Yang *et al.*, 1988), were not seen in New Zealand as selenium intakes were much higher than those of around 7µg/day in endemic areas. More recently selenium deficiency has been observed in HIV-positive patients where poor selenium status appears to be associated with disease progression and early mortality in HIV-positive patients (Rayman, 2002), however, this has not been reported in New Zealand.

Consequences of sub-optimal selenoprotein levels

There is a gap between the intake of selenium associated with overt deficiency such as Keshan disease and the amount to maximize activities of the selenoenzyme GPx, on which requirements and recommendations are based (Thomson, 2004a). This gap may be referred to as marginal deficiency. The relevance of long lasting GPx depression due to marginal selenium deficiency to the development of chronic disease is a matter of ongoing debate. Because of our low soil selenium and low selenium status, New Zealanders have so far existed in this gap. The question frequently asked is how could the New Zealand population be so healthy and active with such low selenium status? We could not find any adverse effects on generally healthy New Zealanders (Robinson, 1989). This is perhaps partly due to lack of appropriate biomarkers for sub-clinical effects of selenium deficiency. There has been some progress in recent years, and there is increasing evidence that selenium intakes in the range between overt deficiency and requirements for selenoproteins are associated with increased risk from a number of medical conditions including cancer, CVD, altered immune function,

viral infection and /or replication, male infertility, inflammatory disorders and auto-immune thyroid disease (Rayman, 2002).

Few of these conditions have been studied in New Zealand, though asthma and thyroid disorders are of particular interest. The questions being asked are: To what extent can the effects of selenium deficiency or health effects be attributed to selenoproteins? Are some actions of selenium eg proposed cancer-preventive properties operate independently of selenoproteins? How do these effects relate to requirements and recommendations?

Asthma: New Zealand has a high incidence of asthma and there is some evidence that habitual low selenium status and the associated low activities of GPx may exacerbate or increase the risk of asthma in New Zealand children (Flatt *et al.*, 1990; Shaw *et al.*, 1994). However, further research is required to determine the nature of the relationship between selenium and asthma and the mechanisms.

Thyroid disorders: Myxoedemetous cretinism, a disorder of skeletal growth, neurological development, hypothyroidism and thyroid atrophy has been linked to severe deficiency of both selenium and iodine. This contrasts with neurological cretinism, which occurs in areas of very severe iodine deficiency and deficient or adequate selenium (Vanderpas *et al.*, 1993). These conditions have never occurred in New Zealand, in spite of a history of iodine deficiency goitre prior to salt iodization in the 1930s (Hercus *et al.*, 1925). There is some evidence of associations between low selenium status and elevated thyroid volume, and alterations in thyroid hormone concentrations in individuals with and without low iodine status, but at present there is no direct evidence for selenium-dependent alteration of deiodinase expression or activities in humans in vivo (Kohrle *et al.*, 2005). Our research indicates that the marginal selenium deficiency in New Zealand does not greatly affect expression of deiodinases, probably because of their high position in the hierarchy of selenoproteins and the high potential of the thyroid gland for adaptation to altered challenges (Thomson *et al.*, 2005).

Possible beneficial effects 'supranutritional intakes: requirements for optimal health

In recent reviews of nutrient recommendations (Food and Nutrition Board of the Institute of Medicine, 2000; NHMRC, 2006), there has been a move to consider another criterion when making recommendations, the intake that allows for optimal nutrition and optimal health. This has renewed interest in the question of possible beneficial health effects of nutrients, such as antioxidants, in larger than recommended intakes, often termed 'supranutritional' intakes. These beneficial effects might include maintenance of good health and the reduction of other disease not caused by nutritional deficiencies, such as cancer and CVD. Selenium is one of these nutrients, as evidence grows for a protective effect against some forms of cancer, and enhancement of immune function (Rayman, 2002). Only a few clinical trials have been conducted to determine whether selenium intakes above the level to maximize GPx activity affect disease outcome favourably.

Selenium and cancer: The evidence for beneficial effects of supranutritional intakes of nutrients, particularly for selenium, is often conflicting and controversial. Selenium was once known as a carcinogen, but there is now increasing evidence for a role in cancer prevention and in protection against the toxic effects of chemotherapy (Rayman, 2005). Evidence comes from *in vitro* and animal

studies, case-controlled prospective studies with human subjects and a limited number of randomized controlled trials (RCT) of selenium supplementation in humans. The strongest support comes from the Nutritional Prevention of Cancer (NPC) trial, in which subjects with a high risk of skin cancer were supplemented with 200µg Se/day or a placebo for several years, with the effect on skin cancer as the primary outcome (Clark *et al.*, 1996). There was no effect on skin cancer, but a dramatic reduction in the risk of other cancers, notably prostate cancer. This has been supported by a number of case-controlled studies of selenium and prostate cancer (Whanger, 2004; Rayman, 2005). However, some have viewed these results of the NPC trial with caution, because they were not primary outcomes of the trial, and observations have yet to be confirmed. Furthermore, new evidence from the trial showed that, although other cancer risk reductions were achieved, squamous cell carcinoma and total nonmelanoma skin cancers increased by 25% and 17% respectively in patients receiving selenium (Duffield-Lillico *et al.*, 2003). Further trials are necessary to clarify the role of selenium in cancer prevention.

The incidence of certain cancers in New Zealand is high, in particular prostate and colon cancers, but it is difficult to correlate this with selenium status. Ferguson *et al.* (2004) have reported an inverse correlation between regional differences in soil selenium levels and regional differences in incidence of colon cancer across the country. However, it is likely that these regional differences are related to importation of high selenium Australian wheat rather than soil levels, as bread and wheat products are the only major food sources of selenium that show geographical variation (Vannoort & Thomson, 2005; Thomson *et al.*, 2007). Fruit and vegetables contribute little to total selenium intake, and animal foods, which provide most of our dietary selenium, do not show much variation in selenium content because livestock in low selenium areas are all supplemented.

The mechanism often quoted as being the cancer-protective effect is selenium's role as an antioxidant. There is some evidence for a role for selenoproteins in cancer prevention through antioxidant roles of GPx enzymes, redox regulation roles of thioredoxin reductase and hormonal regulation of deiodinases (Diwadkar-Navsariwala & Diamond, 2004; Rayman, 2005). However, the known metabolic functions of the selenoproteins do not fully explain the chemo protective effects. In most studies preventive effects were observed at selenium dosages that far exceed those needed for maximal expression of GPx enzymes. Thus, should the cancer protective effect of selenium be confirmed, evidence suggests that intakes required are higher than recommendations based on those necessary to prevent nutritional deficiency or for optimization of functional proteins (Thomson, 2004a). Low molecular selenium compounds have also been implicated. There is evidence for anti-carcinogenic activities of several intermediary metabolites of selenium including seleno-diglutathione, hydrogen selenide, methyl selenol and methylated metabolites of selenide, which are directly anti-carcinogenic (Rayman, 2005). In fact Drake (2006) provides convincing arguments that pro-oxidative rather than antioxidant properties of selenium compounds best account for their observed anti-cancer effects. This supports the hypothesis that supranutritional exposures of selenium can reduce cancer risk, but it is likely that selenium can function as a cancer preventive agent through both nutritional and supranutritional mechanisms.

Nutrigenomics, the study of how individual genetic differences can affect the way we respond to nutrients in foods we eat, associates gene variants (single nucleotide polymorphism, SNP) with

differential responses to nutrients and then relates this to disease states. Several SNPs in selenoproteins have been identified (Diwadkar-Navsariwala & Diamond, 2004). Research suggests that for some individuals who are genetically predisposed to cancer, requirements for selenium differ from those in the normal population. For example, individuals carrying a nucleotide polymorphism at codon 593 of human GPx1, which changes cytosine (C) to thymine (T), appear to have an increased risk of lung, breast and prostate cancers (Diwadkar-Navsariwala & Diamond, 2004). This risk may be associated with increased susceptibility to DNA damage and to differences in response to selenium supplementation.

Selenium and cardiovascular disease: Although early epidemiological studies suggested that the risk of CVD is higher in people with low selenium intake, evidence from more recent clinical studies is controversial and inconclusive (Huttunen, 1997; Rayman, 2002). The only large RCT investigating the efficacy of selenium supplementation alone in the prevention of CVD, indicated no overall benefit of supplementation (Stranges *et al.*, 2006). Nevertheless, because of the antioxidant potential of several selenoproteins, a number of potential links between selenium status and mechanisms leading to atherosclerosis have been studied, including LDL oxidation, platelet aggregation and endothelial dysfunction (Birringer *et al.*, 2002; Rayman, 2002).

Requirements and recommendations for selenium

Prior to the 1980s there was little information on selenium in humans and so a 'guestimate' of requirements (50-200µg/day) was extrapolated from animal studies, some of which were carried out in New Zealand. Later, the traditional method of determining requirements from metabolic balance studies was not helpful as New Zealanders could be in metabolic balance on a diet of 20 µg/day, while US citizens needed around 80 µg and Chinese men residing in selenium deficient areas of China were in balance at even lower intakes of less than 9 µg/day (Thomson, 1989). This demonstrated that we can adjust our selenium homeostatic mechanisms to keep in balance over a wide range of dietary intakes by adaptation.

The minimum requirement for prevention of deficiency disease can be determined by comparing the selenium intakes in areas in China where the natural selenium deficiency Keshan Disease was endemic (~ 7 µg/day), with those in areas where it was not (~ 17-20 µg/day) (Yang *et al.*, 1987). Thus we may conclude that the minimum requirement to prevent deficiency symptoms in these areas is around 17 µg/day (Thomson, 2004a).

More recently, the assessment of physiological requirement of a certain nutrients is determined from the intake needed for full expression of, or to maximize the activity or concentration of, an enzyme or some other biochemically functional protein. For selenium, this concept becomes rather complex, as there are, in fact, 25 functional selenoproteins in the human body. Furthermore, the level of selenium necessary to maximize the activity of each of these proteins is not the same because of the hierarchy of selenoproteins (Behne *et al.*, 1988). For example, in selenium deficiency, expression of the selenoproteins iodothyronine deiodinase 1 and phospholipid GPx (GPx4) takes priority, while the activity of cellular GPx (GPx1) is reduced dramatically. Selenoproteins most essential for life have higher positions in the hierarchy of selenoproteins than do those that are less essential. Thus the ideal biomarker by which to assess full expression of all selenoproteins would be the selenoprotein with

the lowest position on the hierarchy. Only a few selenoproteins are easily accessible for measurement in humans; plasma or serum contains two, GPx3 and SelP.

When we started investigating selenium requirements in humans, the only selenoprotein for which there were sufficient data was cellular GPx, which had been identified as a selenoenzyme in 1973. This is now known as GPx1 and is only one of a family of six GPx enzymes. Our group was the first to show a linear relationship between blood selenium concentrations and GPx activities in individuals with low selenium status (Rea *et al.*, 1979). When we supplemented New Zealanders with selenium, their GPx activity increased, indicating that their selenium status was inadequate for optimal function of this selenoenzyme (Thomson *et al.*, 1988; Thomson *et al.*, 1993). Such an increase, however, was not observed in US residents who had naturally higher selenium status. Fortunately, GPx is low in the hierarchy of selenoproteins; otherwise we might not have seen a response to selenium supplementation. Current recommendations are based on intakes required for maximal levels of plasma GPx3. Recent research by Xia and Burk and colleagues suggests that SelP may be even lower in the hierarchy, and they have concluded that SelP may be a better biomarker for whole body protein expression than plasma GPx (Xia *et al.*, 2005). On the other hand, our work showed that similar intakes are required for maximal levels of both plasma GPx and plasma SelP in Otago residents (Duffield *et al.*, 1999). Clearly more data are required to clarify this, but when adequate data on optimization of SelP are available, the current recommendation may need to be revised upward. The recently released Australian/New Zealand Nutrient Reference Values (NRVs) have used the criterion of maximal plasma GPx, but in establishing these values, we did in fact accommodate this new data on SelP, and chose the upper end of the range or rounding up rather than rounding down in our calculations (NHMRC, 2006).

Other selenoproteins have been considered as criteria for estimating requirements. The selenoenzyme, iodothyronine 5' deiodinase, is required for the synthesis of thyroid hormones, converting the inactive thyroxine (T_4) to the active tri-iodothyronine (T_3), and therefore the ratio of T_4 to T_3 may be used to assess adequacy of selenium supply. We have studied the interrelationship between selenium and iodine by investigating the effects of selenium status and of selenium supplementation on thyroid hormone metabolism in New Zealand residents. These studies have indicated that our intake of selenium here in New Zealand, is not sufficient for maximal activity of GPx. It does, however, seem to be marginally adequate for the optimal functioning of the deiodinases (Thomson, 2004a; Thomson *et al.*, 2005). The deiodinase enzymes are higher on the hierarchy of selenoproteins decreasing only in severe selenium deficiency, and therefore require lower intakes for optimal activity.

In the review of the US/Canadian Dietary Reference Intakes (Food and Nutrition Board of the Institute of Medicine, 2000) and the Australian/New Zealand NRVs (NHMRC, 2006), the intake of nutrients that allows for optimal nutrition and optimal health was also considered, including the possible protective effects of selenium against cancer and CVD. Evidence from the NPC trial and other studies suggested that the intakes required are likely to be somewhat higher than recommendations based on those necessary to prevent nutritional deficiency or for optimization of functional proteins (Thomson, 2004a). It was considered that there were insufficient data to reliably estimate a specific intake. However, it is possible to estimate an 'unofficial' intake range for the

cancer protective effect using results from case-controlled studies and one intervention trial, of around 75 to 125 µg/day (Thomson, 2004a). Further dose-control trials are needed to strengthen such proposals and to determine more precisely the minimum intakes for a protective effect.

It is clear, then, that when discussing requirements for selenium we need to consider the question – requirement for what? This is a difficult question for the expert committees charged with setting recommended intakes of nutrients, because the evidence for beneficial effects of supranutritional intakes of nutrients is often conflicting and controversial. If we put this information together with other information we have produced on physiological requirements for optimization of GPx, SelP and the deiodinases, we can see (Table 1) that there is a range of intakes necessary to meet requirements depending on the criterion used (Thomson, 2004a).

Table 1 Estimates of requirements for selenium

	µg Se/day
Minimum requirement for prevention of Keshan disease	20
Physiological requirement (EAR) for maximal expression of	
glutathione peroxidase	45-55
Selenoprotein P	~60-70
Requirement for optimal function of iodothyronine 5' deiodinases	35-45
Possible protection against some cancers	~120

Do New Zealanders meet these requirements? Intakes in New Zealand are well above the minimum requirements for prevention of Keshan disease. However, results of the Children's Nutrition Survey clearly show that in 2002, intakes did not reach these recommendations. Intakes are probably sufficient for optimal thyroid function as long as iodine intake is sufficient, but not for maximal expression of GPx (Thomson *et al.*, 2005). Results of supplementation trials, showing an increase in GPx confirms this. However, as our selenium status creeps upwards, the increase in GPx following supplementation has not been as pronounced as in previous years (Thomson *et al.*, 2006).

The future

There is an increasing tendency towards supplementation and fortification of foods with some nutrients. For selenium it has never been clear whether this is necessary or not – even less so now our selenium status seems to be increasing (Thomson & Robinson, 1996; Thomson, 2004b). The evidence available has not been strong enough to justify it. The definitive studies which focus on possible cancer or cardiovascular disease protective effects have yet to be completed. In spite of this there are frequent calls for increasing selenium intakes by supplementation or by other means. For example, the wheat industry has proposed that growers be required to apply selenium as part of their fertiliser programme to wheat for human consumption in low selenium areas of the South Island, not just for animal feeds. Whether this move would impact on human health is debatable as a similar move in Finland in 1985 has increased selenium status, but has not resulted in obvious reductions in the incidence of chronic diseases, or any other health benefits that can be attributed to selenium in that country (Pietinen *et al.*, 1996).

Perhaps nutrigenomics will provide the missing link in identifying the relationship between individual requirements for nutrients and the susceptibility to disease. But will the approach change the concept of recommended nutrient intakes? Data used to set recommended dietary

intakes and safe upper limits are established from a wide population, and therefore recommendations should accommodate most genetic variations that exist (Stover, 2006). On the other hand, with the re-evaluation of the criteria used to determine recommended intakes to include optimal health, the effect of genetic variations on requirements and susceptibility to disease may allow dietary recommendations to be individualized according to genotype, with the aim of ultimately reducing our risk of degenerative diseases.

CONCLUSION

In less than 40 years selenium has gone from being a feared toxin to an essential nutrient and potential anti-carcinogenic agent. Recommendations for selenium as outlined in the new Australia/New Zealand NRVs are based on physiological requirements for optimal levels of selenoproteins such as GPx (NHMRC, 2006), but there is a move towards recommending higher intakes of selenium for prevention of cancer and other chronic diseases. It is clear that there may be different levels of requirements depending on criteria used (Thomson, 2004a). In New Zealand, we are still are left with the paradox of a community with low selenium status living in a naturally selenium-deficient environment for animals, yet with apparently healthy human residents, albeit with relatively high incidence of CVD and some cancers. We were often criticised for not recommending intervention on a national scale, but in fact there has not been sufficient good evidence available to justify it, particularly in this era of evidence-based medicine and public health. Now we are seeing an improvement in the selenium status of the New Zealand's human population that is secondary to the improvement of the selenium status of livestock, but we have yet to see any beneficial effects of that in terms of disease outcome. Furthermore, we need to be aware of the risk from high intakes of a trace element such as selenium, which has a long early history of toxicity effects. In 1970 Krehl described the situation: "Selenium is perverse, contradictory and elusive, and yet all the while intriguing". He called it the Maddening Mineral (Krehl, 1970).

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Total phenolics content and antioxidant activities from commercial coffee drinks

AED BEKHIT[1], K WANG[2], BO SUN[3], R SEDCOLE[1], SL MASON[1]

[1]*Agriculture and Life Sciences Division, Lincoln University, New Zealand*

[2]*Hunan Engineering & Technology Center for Natural Products, Hunan Agricultural University, Changsha 410128, People's Republic of China*

[3]*Ningxia University, People's Republic of China*

ABSTRACT

The aim of this study was to determine the phenolics concentrations of six commercial coffee drinks (espresso, long black, filtered, flat white, latte and cappuccino) from 4 different coffee retailers (A, B, C and D), and to investigate their antioxidant activities. Total phenolics concentration was determined using the Folin–Ciocalteu method and antioxidant activities of coffee were estimated using the DPPH and the superoxide anion (O_2^{-}) scavenging activity (SASA) methods. On the basis of total phenolic content per gram of freeze dried coffee, retailers varied ($P < 0.001$) widely with black coffee (espresso, long black, and filtered) having 2.5 to 3 fold variation compared with 1.5 to 2 fold variation in white coffee (flat white, latte and cappuccino). Black coffee was higher ($P < 0.001$) in total phenolics content (as Gallic acid equivalent; GAE)/g freeze dried coffee compared with white coffee. Based on coffee serve, apart from long black, total phenolics content GAE/ cup from different retailers was not different ($P > 0.05$). White coffee had higher ($P < 0.001$) total phenolics content GAE/ cup compared with black coffee. Superoxide anion scavenging ability per gram of freeze-dried coffee was higher ($P < 0.001$) in black coffee than white coffee (53.10 -54.09 % SASA; 2mg freeze-dried coffee/ml for black coffee compared with 9.54- 9.66 % SASA; 2mg/ml for white coffee). However, because of the higher freeze-dried yield in white coffee, the overall %SASA per serve was higher ($P < 0.001$) in white coffee than in black coffee. Total antioxidant activity as determined with DPPH had the same trend as in SASA assay. Regressions between total phenolics content and antioxidants activities as determined by SASA and DPPH assays were significant ($P < 0.001$) with $R^2 = 0.66$ and $R^2 = 0.34$ for SASA and DPPH, respectively. The data show that coffee is a good source for antioxidants, but variation between retailers and within retailers can affect the daily intake of total phenolics from coffee.

INTRODUCTION

Coffee is one of the most widely consumed beverages throughout the world for its physiological effects as well as its pleasant taste and aroma. Coffee is known to possess antioxidant properties (del Castillo et al., 2002; 2005; Yanagimoto et al., 2002; 2004) and provides a significant source of phenolic compounds in the diet (Kilmartin and Hsu, 2003). The antioxidative activity of coffee and its free radical scavenging capability have attracted much attention of recent years. Current research revealed relatively high level of antioxidant properties in coffee compared with other popular beverages, such as tea, cocoa, wine and juice, known to contain antioxidants (Richelle et al., 2001; Yamaguchi et al., 1998). These properties have a role in the prevention of diabetes, arteriosclerosis, neurodegenerative diseases and cancer (Higdon and Frei, 2006; van Dam, 2006), which will depend on the oxidant-antioxidant balance in the body (Meyer et al., 1998). Phenolic compounds (e.g. chlorogenic acids, caffeic, ferulic, vanillic and cumaric acid) are the main source for the antioxidant activity in coffee (Daglia et al., 2000; del Castillo et al., 2002). However, little is known on the level of phenolics and the antioxidant capacity in commercial coffee drinks. Therefore, the objectives of

the present study is to determine the phenolics content of six commercial coffee drinks (espresso, long black, filtered, flat white, latte and cappuccino) from 4 different coffee retailers, and to investigate their antioxidant activities.

MATERIALS AND METHODS

All the chemicals and reagents were HPLC grade or highest grade available from Sigma. Six commercial coffee drinks (espresso, long black, filtered, flat white, latte and cappuccino) were purchased from 4 different coffee retailers (A, B, C and D) on three non-consecutive days (n = 3). Samples were freeze-dried (FD) and stored at -20°C. Total phenolics concentration was determined using the Folin–Ciocalteu method (Spanos and Wrolstad, 1990) and antioxidant activities of coffee were estimated using the DPPH (Brand-Williams et al., 1995) and the superoxide anion ($O_2^{\cdot-}$) scavenging activity (SASA) (Siddhuraju and Becker, 2007) methods. All analyzes were carried out in triplicates and the mean values were used in the statistical analysis.

Statistical analysis

The data was analyzed using the general linear model protocol in MINITAB (Release 14.1). The significance of the difference between means was determined by Tukey's multiple comparison test ($P < 0.05$). Values reported are the mean of three samples ± SD.

RESULTS AND DISCUSSION

The volume of coffees and weight of FD samples varied significantly ($P < 0.001$) among coffee drinks but not among retailers (Table 1). Total phenolic content expressed as GAE/g of FD coffee was different among retailers ($P < 0.001$) with black coffees (espresso, long black, and filtered) having 2.5 to 3 fold variation compared with 1.5 to 2 fold variation in white coffees, flat white, latte and cappuccino (Figure 1). Black coffee samples were higher ($P < 0.001$) in total phenolics content GAE/g FD extract compared with white coffee. However based on a coffee serving, total phenolic content GAE/ cup from different retailers was not different ($P > 0.05$). White coffee had higher ($P < 0.001$) total phenolics content GAE/ cup compared with black coffee. Direct comparison with total phenolic values reported in the literature was not possible because of the wide range of coffee drinks used in the present study (compared with brewed or espresso/Turkish types reported in literature) or because the basis for total phenolic calculations were different. In the present study 3 retailers (A, B and C) had higher total phenolic content in espresso coffee (mean 6.97, 3.85 and 7.63 mg GAE/ ml of espresso, respectively) compared with the average total phenolic content in Turkish coffee (2.4 mg GAE/ml of Turkish coffee) reported by Gunduc and El (2003). Retailer D had same amount of total phenolic in espresso (2.41 mg GAE/ml of espresso).

Superoxide anion scavenging ability per g of freeze-dried coffee was higher ($P < 0.001$) in black coffee than white coffee (53.10 -54.09 % SASA at 2mg freeze-dried extract/ml for black coffee compared with 9.54- 9.66 % SASA; 2mg/ml for white coffee). However, because of the higher freeze-dried yield in white coffee, the overall %SASA per serve was higher ($P < 0.001$) in white coffee compared with black coffee (Fig. 2). Total antioxidant activity as determined with DPPH had a similar trend as in SASA assay. Studies on the effect of direct addition of milk to coffee generated mixed results with some studies showning a decrease in antioxidant activity when milk is added

(Kilmartin and Hsu, 2003; Sanchez-Gonzalez et al., 2005) while other studies found no effect (Dupas et al., 2006; Richelle et al., 2001). In the present study, the higher antioxidant capacity in white coffee seems to be the result of different preparation techniques used in coffee making. For example, the extraction time with white coffee is generally higher than black coffee since there is no restriction on the serving volume as white coffee normally has larger volume (Table 1). Support for this contention comes from studies that demonstrated the increase of coffee extracts with the increase of brewing time (Lee et al., 1992) and that only 33% and 65% of total phenolics that was available in ground coffee were extracted in espresso and brewed coffees, respectively (Sanchez-Gonzalez et al., 2005).

Regressions between total phenolics content and antioxidants activities as determined by SASA and DPPH assays were significant ($P < 0.001$) with $R^2 = 0.66$ and $R^2 = 0.34$ for SASA and DPPH, respectively. This may reflect the chemical constituents in coffee that contributed to the differing inhibition mechanisms in both assays.

Conclusions- The data shows that coffee is a good source for antioxidants, but, variation between retailers and within retailers as well as the choice of coffee type can affect the daily intake of total phenolics and antioxidant capacity from coffee. The reported worked highlighted the *in vitro* antioxidant activities of commercial coffee drinks, which serve as indication for their potential antioxidant activity. *In vivo* and metabolic studies will be required for full understanding of the nutritional contribution of such beverages to human well being.