

Zinc intake and status in Australian vegetarians

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Vegetarians have a lower incidence of many chronic diseases than omnivores. However, vegetarian diets could potentially result in lower intakes of some minerals, particularly Zn. In a cross-sectional study, dietary Zn intake was measured using 12 d weighed records in ninety-nine vegetarians (ten vegans) aged 18–50 years and forty-nine age- and sex-matched omnivores. In men, the mean daily Zn intake and Zn density values were similar in omnivores, ovolacto-vegetarians and vegans, but in women they were significantly lower in vegetarians (mean intake 6.8 mg *v.* 8.4 mg in omnivores) and few achieved the recommended intake. Significantly more vegetarian than omnivorous women had a daily Zn intake < 6 mg (44% *v.* 13%). Mean serum Zn concentrations were similar in female omnivores and vegetarians, despite the differences in intake. However, omnivorous men had a lower mean serum Zn concentration (0.85 µg/ml *v.* 0.95 µg/ml) and more subjects had levels below the reference range of 0.72–1.44 µg/ml than ovolacto-vegetarians ($P < 0.01$). Overall more women than men had low Zn concentrations; and these women generally had intakes below 6 mg/d. There was a significant correlation between serum Zn concentration and dietary Zn density in vegetarians, especially females ($P < 0.001$), but not in omnivores. Ovolacto-vegetarians did not have a significantly greater risk of low Zn status than omnivores.

Zinc: Vegetarians

Vegetarian diets have become increasingly popular in a number of countries in recent years for health, philosophical, ecological and religious reasons. In the 1995 National Nutrition Survey (McLennan & Podger, 1995) self-reported prevalence of vegetarianism in Australian adults was 4.9% in females and 2.6% in males. The highest prevalence was in females aged 19–24 years (6.2%). Vegetarians appear to have a lower morbidity and mortality from a number of chronic diseases, including cardiovascular disease and some cancers (Dwyer, 1988). These effects may be attributable to diet and to other lifestyle differences. However, there is concern over whether vegetarians, and particularly vegans, have an adequate intake of several minerals, particularly Fe, Zn and Ca (Kadrobova *et al.* 1995; Donovan & Gibson, 1996).

Zn is an essential trace mineral that is a constituent of enzymes involved in most metabolic pathways, and is important for protein metabolism, cell growth and repair, and immune function. The long-term effects of mild deficiency are unclear, but it has been suggested that they include delayed wound healing, suboptimal immune functioning, increased plasma lipid peroxides and perhaps

reduced taste and smell acuity seen in the elderly (Greger, 1989; Fortes *et al.* 1997). Serum Zn, hair Zn and salivary Zn have been used to assess status, although all have drawbacks (O'Dell, 1996). Zn status is subject to strong homeostatic regulation via the gut and liver, and individuals with poor Zn status absorb Zn more efficiently. The lack of sensitive indicators of Zn status and an absence of specific clinical features of mild deficiency, means that setting the recommended dietary intake (RDI) for Zn has proved difficult (O'Dell, 1996; Sandstead & Smith, 1996). In Australia, the RDI has been set in the range of 6–12 mg/d (National Health and Medical Research Council, 1991), with many people using 12 mg.

In the Australian National Nutrition Survey (McLennan & Podger, 1995) diet assessments using 24 h recall and a simple food-frequency questionnaire indicated that the mean intake of Zn from food and beverages was 16.8 mg in adult Victorian males and 9.7 mg in females, and was below the RDI in most female age groups. The composition of the diet affects the bioavailability of dietary Zn (Gibson, 1994). More Zn is generally available from animal sources than plant sources, and protein, insoluble fibre, phytate and

Abbreviation: RDI, recommended dietary intake.

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some minerals, e.g. Fe, Ca and P, can reduce Zn absorption. About two-thirds of the Zn consumed by most people in Australia is provided by animal products, particularly meat. Good plant sources of Zn are grains, nuts and legumes, and eggs, cheese and milk for ovo-lacto-vegetarians (Messina & Burke, 1997).

A few previous studies have investigated Zn intake in vegetarians, and in vegetarians compared with omnivores, with variable findings (Freeland Graves *et al.* 1980; Levin *et al.* 1986; Alexander *et al.* 1994; Donovan & Gibson, 1996). Reasonable-sized studies by Freeland Graves *et al.* (1980) and Levin *et al.* (1986) in both men and women revealed similar intakes, as assessed using 24 h diet recall and a food-frequency questionnaire. Most studies using diet records have measured dietary intake over just 1–4 d rather than the 12–14 d suggested as being required to obtain a more representative estimate of Zn intake (Marr & Heady, 1986). Few studies have included a measure of Zn status such as serum Zn collected simultaneously with the dietary data.

The present study was conducted to measure Zn intake using 12 d weighed food records in Caucasian Australian vegetarian and vegan adults and in age- and sex-matched omnivores with similar lifestyles, and simultaneously to measure serum Zn concentrations.

Experimental methods

Fifty healthy non-pregnant female vegetarians aged 18–45 years and fifty male vegetarians aged 20–50 years were recruited in Victoria from advertisements on notice boards and in newspapers. For the purpose of the present study, a vegetarian was defined as someone who did not consume red meat, consumed fish or chicken less than once weekly and had been following this diet for at least 6 months. Vegetarian subjects were asked to recruit an age- and sex-matched 'friend' of similar BMI and smoking habit, who ate red meat at least three times per week, and half the subjects were able to do this. All subjects gave written informed consent for participation in the study, which was approved by the Ethics Committee of Deakin University.

All subjects completed a questionnaire requesting details about age, occupation, exercise and smoking habits, nutritional supplement use, the type of diet consumed, and the length of time they had followed the diet. Two volunteers who were trained athletes on special diets and supplements were excluded.

Subjects were asked to complete a 12 d weighed diet record, over three blocks of 4 d including three weekend days, and were given detailed instructions on how to complete this. A set of electronic scales (Bonso Mini-Scale) was provided for each subject. Where it was not possible to weigh food, subjects were told to describe the quantities consumed using either household measures or the photographs of representative portion sizes of common foods provided in the back of the diet record (Edington *et al.* 1988). The food and beverage intakes were entered into the dietary analysis program, 'Diet-3' (Xyris, Queensland, Australia), which is based on Australian food composition tables.

Laboratory measurements

Most subjects had a fasting venous blood sample taken at a time when they were not suffering from any infection during the fortnight after completion of the dietary record. The blood sample was collected in a Zn-free tube, left standing at 4° for 60 min and then centrifuged at 3000 rev./min for 15 min. The serum was separated and stored at –20°. Serum Zn was analysed by flame atomic absorption spectroscopy (GBC 901, Melbourne, Australia) (Fell & Lyon, 1994).

Statistical analysis

All statistical analyses were performed using SPSS version 8.0 (SPSS Australasia Pty Ltd, Sydney, Australia). The Mann-Whitney U-test was performed to determine differences between vegetarians and omnivores for nutrient intake and biochemical measurements, with ANOVA or the Kruskal-Wallis test being performed for greater than two groups depending on whether the Shapiro-Wilks test for normality indicated the data to be normally distributed or not. For dietary Zn intake the Shapiro-Wilks test on ovo-lacto-vegetarians gave a *P* value of 0.05; analysis of results was then undertaken by both ANOVA and the Kruskal-Wallis test, and the results were the same. χ^2 tests were performed to establish if the groups were significantly different with respect to numbers of subjects with values above or below cut-off points for nutrient and biochemical values. Spearman rank correlation and multiple regression analysis was performed to assess the relationships between dietary variables and serum Zn concentrations.

Results

Subject characteristics

Females. Forty-eight female ovo-lacto-vegetarians, two vegans and twenty-four omnivorous controls completed the study. The number of vegans was too small to warrant separate analysis so their data were included with those from the other vegetarian subjects. The vegetarians had been following the diet practice for a mean of 5.2 (range 1–17) years. The mean age of vegetarian subjects was 25.2 years, compared with that of omnivores of 25.3 years. Vegetarians had a mean BMI of 22.5 kg/m² and omnivores 22.4 kg/m². These values were not significantly different. Most vegetarian and omnivorous subjects were students (62.5% and 60.0% respectively), and the remainder were employed full-time except for one vegetarian subject who was unemployed. All subjects reported that they were in good health. There was no significant difference in the prevalence of regular cigarette smoking between the omnivores and vegetarians (8% and 16% respectively) or in reported level of physical activity or menstrual blood loss.

Males. Thirty-nine ovo-lacto-vegetarians, ten vegans and twenty-five omnivorous controls completed the study. The ovo-lacto-vegetarians and vegans had been following this dietary practice for a mean of 11 (range 0.5–44) years and 6 (range 1.5–14) years respectively. The omnivores did not differ significantly from either the ovo-lacto-vegetarians or

Table 1. A comparison of daily dietary intakes in male and female omnivores and vegetarians from the present study (Mean values and standard deviations)

Dietary intake	Males						Females			
	Omnivores (n 25)		Ovolacto-vegetarians (n 39)		Vegans (n 10)		Omnivores (n 24)		Vegetarians (n 50)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	11.0	1.9	10.5	2.4	11.6	2.7	6.9	1.4	6.9	1.9
Protein (g)	108	17	80**	16	81**	24	67	16	54**	15
Carbohydrate (g)	291	77	357	92	414*	97	183	42	211	60
Fat (g)	98	32	82	28	88	38	65	17	60	22
Fibre (g)	26.3	7.7	49.8***	15.9	63.8***	14.6	17.3	4.9	24.4***	8.5
Iron (mg)	15.8	4.5	20.4	7.7	22.9	6.2	9.9	2.9	10.7	4.4

Mean values were significantly different from those for omnivores: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Mann Whitney U test).

vegans with respect to age, smoking habit, BMI, waist : hip ratio or blood pressure.

Dietary intake and zinc measurement

Total energy intakes were similar for males and for females in the different diet groups, but vegetarians derived a significantly lower proportion of their energy from protein compared with omnivores. This was compensated for by a higher proportion of energy being derived from carbohydrate in men (Table 1).

The mean Zn intakes in the different dietary groups are shown in Table 2. In men, the Zn intake and Zn density (mg/MJ energy) were not significantly different on the three types of diet, with mean intake at about the upper end of the RDI. Of male omnivores 36 % had a dietary Zn intake below 12 mg/d as did 64 % male ovolactovegetarians and 70 % vegans (Fig. 1(a)). Two ovolactovegetarians had intakes of less than 6 mg/d.

In both the omnivorous group and the vegetarian-vegan group, the females had significantly lower Zn intakes than the males ($P < 0.001$). In women, the mean Zn intake of vegetarians (6.8 mg/d) was significantly lower than that of omnivores (8.4 mg/d; $P < 0.05$), as was the Zn density (mg/MJ energy), ($P < 0.001$). Only one omnivore and one vegetarian had a Zn intake above 12 mg/d. The number of vegetarians with an intake of less than 6 mg/d (22/50) was significantly greater than the number of omnivores (3/23). If

data from females with a reported energy intake less than 1.2 times the calculated BMR were excluded, Zn intake in omnivores (8.9 (SD 1.8) mg) was still significantly higher than in vegetarians (7.9 (SD 2.4) mg) and serum Zn levels in the two groups were not significantly different.

In ovolactovegetarians the predominant food sources of Zn were fruits, cheese, vegetables and cereals, whereas omnivores obtained much of their Zn from animal foods, i.e. meats and dairy products. Only three subjects (one omnivore) regularly took supplements containing Zn and exclusion of their data did not significantly influence the study results.

Nutrient intakes in the dietary groups are shown in Tables 1 and 2. There was a significant positive correlation between dietary Zn and Fe intakes (r 0.68 for vegetarians, and 0.83 for omnivores, $P < 0.001$) and also between dietary Zn and dietary fibre (r 0.57, $P < 0.001$).

The serum Zn concentrations are shown in Table 3. In men the serum Zn concentration was significantly higher in the ovolactovegetarians than in both the omnivores and vegans ($P < 0.01$). No male ovolactovegetarians had serum concentrations below the lower end of the reference range of 0.72 $\mu\text{g/ml}$, which has been proposed as indicating low status (Tietz, 1987), but three of the twenty-one omnivores, and one of the eight vegans did: these had intakes of 10, 12.4 and 14.1 mg, and 7.4 mg respectively. The vegan took Fe and multivitamin supplements.

Serum Zn concentrations were not significantly different

Table 2. Dietary zinc intakes and zinc density of the diets of male and female omnivores and vegetarians in the present study (Median and mean values, standard deviations, ranges and 95% confidence intervals)

	n	Dietary zinc intake (mg/d)					Zinc density (mg/MJ)	
		Median	Mean	SD	Range	95% CI	Mean	SD
Males								
Omnivores	25	12.3	12.7	2.4	9.5–16.9	11.7, 13.7	1.17	0.19
Ovolactovegetarians	39	11.0	11.1	3.8	5.8–26.1	9.8, 12.4	1.13	0.31
Vegans	10	11.0	11.9	3.8	7.4–18.1	9.2, 14.5	1.10	0.21
Females								
Omnivores	24	8.0	8.4	2.1	4.6–13.2	7.5, 9.2	1.21	0.18
Vegetarians	50	5.8	6.8**	2.4	3.1–17.2	6.1, 7.5	1.00**	0.28

Mean value was significantly different from that for female omnivores: ** $P < 0.01$.

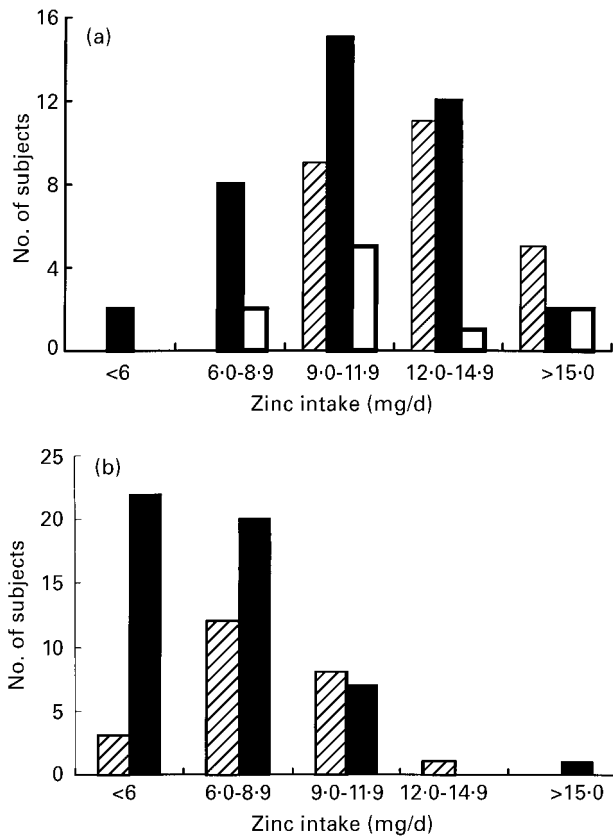


Fig. 1. Frequency distributions of dietary zinc intake in (a) male and (b) female omnivores (▨), ovolactovegetarians (■) and vegans (□) in the present study.

in the female vegetarians and omnivores. Three of the twenty-one omnivores and nine of the thirty-nine vegetarians had concentrations below the reference range (their intakes being 4.8, 5.9 and 7.5 mg in the omnivores and 7.5, 8.6, 5.7, 6.5, 5.7, 5.7, 5.1, 5.1 and 3.1 mg in the vegetarians); thus two and six women respectively had Zn intakes below 6 mg/d.

There was no significant correlation between serum Zn concentration and Zn intake/MJ energy (log values, as not normally distributed) in omnivorous males or females, but the correlation was significant in vegetarians (r 0.48, P < 0.002), particularly female vegetarians. The pattern of the

relationship in female vegetarians is shown in Fig. 2. At lower Zn densities there was no relationship, but at higher values there was a strong positive relationship, with a CV of 0.65 for values above 0.9 mg/MJ. There was no significant effect on serum Zn concentrations of fibre or protein intake.

Discussion

Comprehensive weighed dietary records taken over a period of 12 d have enabled us to obtain data on Zn intake and consumption of other dietary components including fibre, Fe and total energy, and the simultaneous dietary Zn data and serum Zn measurements add to the available data. Energy intakes were low in some subjects but participants were generally lean and trained athletes had been excluded. Although completing diet records may affect consumption or lead to underreporting this should not be different in omnivores and vegetarians.

The Zn intakes were significantly higher in males than females, regardless of the diet. Similar results have been found in other studies comparing Zn intakes of male and female omnivores and vegetarians (Freeland Graves *et al.* 1980; Faber *et al.* 1986; Levin *et al.* 1986). In studies of females only, Zn intakes were generally lower than those found in men in similar populations (Faber *et al.* 1986; Janelle & Barr, 1995; Donovan & Gibson, 1996). In our present study the lower Zn intakes in females were largely related to lower energy intakes, and this is expected to be a factor in other studies, although most do not provide adjusted data and statistical analysis.

The finding of similar dietary Zn intakes in male ovolactovegetarians and omnivores was also obtained in the study by Levin *et al.* (1986) in Israel, but a smaller South African study using 7 d dietary records reported lower intakes in male vegetarians (Faber *et al.* 1986). Serum Zn levels were similar in the former study and were not measured in the latter study.

Our finding of significantly lower Zn intakes in female vegetarians than female omnivores has also been observed in other studies (Freeland Graves *et al.* 1980; Janelle & Barr, 1995), although in some the differences were not statistically significant (Faber *et al.* 1986; Levin *et al.* 1986; Donovan & Gibson, 1996) and the different dietary methodologies make comparison of actual intakes inappropriate. No significant difference in Zn intake was noted in a mixed

Table 3. Serum zinc concentrations in relation to zinc intakes in male and female omnivores and vegetarians in the present study

	n	Zinc intake (mg/d)		Serum zinc (µg/ml)				
		Mean	SD	Mean	SD	Median	Range	95% CI
Males								
Omnivores	21	12.8	2.5	0.85	0.14	0.81	0.66–1.15	0.78, 0.91
Ovolactovegetarians	35	11.4	3.9	0.96**	0.13	0.95	0.72–1.28	0.92, 1.01
Vegans	8	10.9	4.0	0.84	0.15	0.86	0.55–1.02	0.72, 0.97
Females								
Omnivores	21	8.3†	2.1	0.91	0.15	0.92	0.67–1.22	0.84, 0.98
Vegetarians	39	6.8	2.6	0.87	0.18	0.85	0.63–1.31	0.81, 0.93

Mean value was significantly different from those for male omnivores and vegans: ** P < 0.01.
 Mean value was significantly different from that for female vegetarians: † P < 0.02.

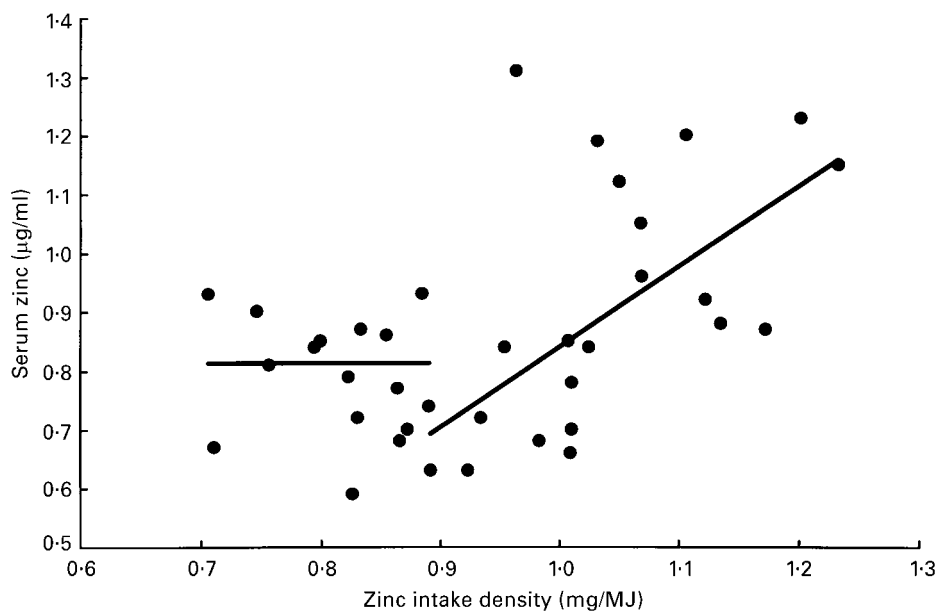


Fig. 2. Relationship between zinc intake density (mg/MJ) and serum zinc concentration in female vegetarians in the present study. Regression lines are shown for zinc intake densities below and above 0.9 mg/MJ.

group of New Zealand males and females, using 12 d weighed records, although the small number of vegans had the lowest mean intake (Alexander *et al.* 1994).

Some low intakes may be due to dietary under-reporting. Several women had low energy intakes, although the mean energy intake expressed as a multiple of the calculated BMR (Schofield *et al.* 1985; Goldberg *et al.* 1991) was the same in omnivores and vegetarians, suggesting similar influences in both groups. Several women reported that they were 'dieting', despite being of normal BMI, but if this was a common feature of their life, they were not excluded. Separate analysis, removing data from women with recorded energy consumption of less than $1.2 \times$ calculated BMR, resulted in an increase in mean Zn intake in both groups, but the intake in the vegetarians was still significantly lower ($P < 0.05$).

In our present study, mean serum Zn concentrations for male and female subjects on all diets were within the laboratory normal 'reference' range for the method (0.72–1.44 $\mu\text{g/ml}$). Physiological stress affects plasma Zn concentration, but in this study (and most with which it is compared) subjects were healthy and blood sampling was not undertaken if they had an infection in order to reduce any influences. Blood specimens were also taken while fasting (between 08.00 and 10.00 hours) to minimize any effect of diurnal variation.

Despite the fact that most female vegetarians had low Zn intakes, serum levels were not significantly lower than in the omnivores. This may reflect compensation with increased absorption of Zn in those with low intakes or status, and/or an adaptation to a long-term vegetarian diet with changes in availability or metabolism. The latter factor might also explain the surprising finding that ovolactovegetarian men had a higher serum Zn concentration than omnivores despite similar intakes, and that more omnivorous men had values

below the reference range. Interestingly, these men had intakes ≥ 10 mg, whereas most of the women omnivores with low serum levels had intakes < 6 mg.

Most of our subjects obtained considerable amounts of their Zn from cheese and eggs, which may be more bioavailable than that from breads, cereals and vegetables. The inhibiting effect of phytate on Zn absorption has been quantified by the ratio phytate:Zn (Gibson, 1994), and foods with high ratios include sorghum, groundnuts, cow-peas, unleavened bread and unprocessed soyabean protein concentrates. These foods may constitute a high proportion of vegetarian diets in some countries and were high in the diet of the Trappist monks who had low serum Zn levels (Harland & Peterson, 1978), but are not common in Australian diets. The vegetarian diets were higher in fibre, but investigators differ in their opinion of whether insoluble cereal and vegetable fibres inhibit Zn bioavailability. In our study there was no relationship between fibre intake and serum Zn concentration, and no relationship was found in Retzlaff's study (Retzlaff *et al.* 1995) in over 300 hypercholesterolaemic men on a low-fat, increased fibre diet. Intakes of Ca and haem Fe, which can also inhibit Zn absorption, were higher in omnivores, although intake from food would probably be insufficient to have a significant effect in the absence of supplement ingestion (Wood & Zheng, 1995).

The pattern of the relationship between Zn density and serum Zn concentration in female vegetarians may indicate that at lower intakes absorbed Zn is rapidly taken into tissues and does not increase the plasma Zn, but at higher intakes it is available to influence the plasma levels. The reason for the strong correlation only in vegetarian women is unclear, although the wider range of values may make this more apparent and perhaps there are differences in binding and metabolism related to the different amino acid profiles

in vegetarians and omnivores. A logarithmic relationship between the daily intake of Zn and the quantity absorbed has been described (World Health Organization Committee, 1996), but our understanding of the mechanisms of Zn homeostasis, metabolism and conservation remains incomplete.

Freeland Graves *et al.* (1980) compared seventy-nine vegetarians (forty-three men) with forty-one non-vegetarians (forty women), but there was no matching by age, BMI etc. and the controls were University students and Faculty, who differed in education status to the subjects. Mean dietary Zn intake (24 h recall) was 8.5 mg in vegetarians, with most subjects having intakes below the recommended dietary allowance (National Research Council, 1989) compared with 10.1 mg in omnivores and only 1.5 mg in the four vegans. Comparisons in men were not possible as only one male omnivore was studied. Serum Zn was not documented separately for men and women, reducing the opportunity for comparison with our study. The Zn content of salivary sediment and hair was, however, noted to be significantly lower in vegetarians, raising the authors' concerns that some women vegetarians had less than satisfactory Zn status.

Anderson *et al.* (1981) studied fifty-six Seventh Day Adventist Canadian women (mean age 53 years) on long-term vegetarian diets. Dietary records (3 d) revealed a mean Zn density of 5.6 mg Zn/4184 kJ (1000 kcal). Mean serum Zn concentration was 0.99 (SD 0.24) $\mu\text{g/ml}$. In these women, who were generally older than those in our present study, both serum levels and Zn intakes were higher with a greater range of values, and the mean Zn density of the diet (mg/MJ) was about 20% greater. No vegetarians had hair Zn levels below 70 mg/kg, suggestive of suboptimal Zn status. In older Seventh Day Adventist women (mean age over 70 years) Nieman *et al.* (1989) found similar mean levels in the twenty-three vegetarians and fourteen non-vegetarians of 6.28 mg and 6.29 mg respectively; energy intake was slightly lower than in the present study, probably because participants were older, but serum Zn was not measured.

In Swedish subjects (Srikumar *et al.* 1992a,b) who changed from a mixed diet to a lacto-vegetarian diet, mean Zn intake remained constant at 12 mg/10 MJ, but energy intake fell by about 10% over 1 year and plasma Zn was 13% lower at 3 months but was then stable.

A small study by King *et al.* (1981) revealed a mean plasma Zn level about 20% lower in pregnant vegetarians than in five non-pregnant vegetarian women, even though the pregnant women consumed more Zn. This is difficult to interpret as proteins change in pregnancy, but as Zn requirement is increased during pregnancy, pregnant vegetarians might be more likely to develop poor Zn status.

Sandstead & Smith (1996) have questioned whether the RDI for Zn might be set too high. Most surveys of subjects with mean intakes below the RDI have shown few with low serum or hair Zn concentrations. A poor correlation between low intake and serum Zn level, as seen in the present study, and a lack of information on what constitutes low status with impaired physiological functioning, compound the problem. The situation is also complicated by other dietary factors, and varying patterns of food intake over time, and lifestyle factors may also be significant in determining Zn requirements (Sandstead & Smith, 1996). The World Health

Organization Committee (1996) suggest the estimation of 'basal' and 'normative' requirements, where basal requirement is the minimal amount needed to replace obligatory Zn loss in persons 'adapted' to low intakes.

There are several studies now indicating that vegetarian diets are more likely to be low in fat and high in fibre and to meet the dietary guidelines of many countries, including Australia, the USA and the UK. It would appear that Zn deficiency is not a specific problem for healthy ovulactovegetarians in Victoria, although it might be in vegans or vegetarians who have additional dietary restrictions, or during pregnancy. Mineral deficiency can, however, occur in both vegetarians and omnivores and it is important that people in high-risk groups, particularly adolescents and premenopausal women and those with low energy intakes, are advised to eat a nutritious balance of mineral-rich foods and that there is the opportunity to assess Zn status in those whose diets and/or lifestyle give cause for concern.

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