

Are changes in food consumption patterns associated with lower biochemical zinc status among women from Dunedin, New Zealand?

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Reductions in red meat and increases in cereals in the diet may compromise the intake and bioavailability of Zn. In this cross-sectional study of 330 premenopausal New Zealand women aged 18–40 years, we have assessed the inter-relationships among dietary intakes (via computer-administered food-frequency questionnaire), biochemical Zn status, and anthropometric indices, and compared our results with earlier data. Fasting serum (12.00 (SD 1.36) $\mu\text{mol/l}$) and hair Zn (2.71 (SD 0.36) $\mu\text{mol/g}$) were lower than those for young Dunedin, New Zealand, women in 1973 (non-fasting serum Zn 18.6 (SD 4.6) $\mu\text{mol/l}$, hair Zn 2.99 (SD 0.35) $\mu\text{mol/g}$). Further, our mean serum Zn was at the 25th percentile of the US National Health and Nutrition Examination Survey (NHANES) (1976–1980) reference sample for women aged 20–44 years. Meat–poultry–fish contributed only 28 % total Zn in the present study, a level comparable with that from cereals–nuts–legumes (27 %), compared to about 40 % in 1989. Significant negative correlations existed between serum Zn and dietary [phytate]:[Zn] molar ratios ($r -0.163$, $P < 0.01$); 35 % had diets with [phytate]:[Zn] >15 , a level said to compromise Zn status. Mean serum Zn of a subgroup of non-oral contraceptive users free of infection was higher in the red-meat eaters ($n 149$) compared with non-red-meat eaters ($n 48$) (12.2 v. 11.8 $\mu\text{mol/g}$, $P < 0.05$). In contrast, serum Zn was lower in those with dietary [phytate]:[Zn] ratios >15 v. <15 (i.e. 11.9 v. 12.3 $\mu\text{mol/l}$, $P = 0.04$). We postulate that the lower biochemical Zn status of these New Zealand women may be associated in part with changes in food selection patterns, which have led to a reduction in the bioavailability of dietary Zn.

Zinc: Food: Diet: Bioavailability: Indices

Inadequate intakes of dietary Zn are not uncommon among premenopausal women in industrialized countries. Several national food consumption surveys have reported average intakes of Zn below dietary recommendations for this age group (Life in New Zealand Survey, 1992; McDowell *et al.* 1994; McLennan & Podger, 1997). Such inadequacies have often been associated with low energy intakes, arising from concerns about body weight, coupled with a sedentary lifestyle (Moser-Veillon, 1990; Houston *et al.* 1997). Temporal changes in food selection patterns, specifically reductions in the consumption of red meat, may also be implicated. Certainly, a decline in red-meat consumption has been reported in New Zealand (Laugesen & Swinburn, 2000) as well as the UK (Whitehead, 1995), USA (Popkin *et al.* 1989) and Canada (Zafiriou, 1985), concomitant with an increase in intakes of unrefined cereals, nuts and legumes.

Among young women, these trends appear to be related to perceived health benefits, as well as ethical, ecological and economic concerns (Richardson, 1994). Such changes in food selection patterns have the potential to compromise Zn bioavailability and thus further exacerbate dietary Zn inadequacies. Red meat is a very rich source of readily available Zn, whereas cereals contain high levels of phytic acid (myo-inositol hexaphosphate), a potent inhibitor of Zn absorption (Oberleas & Harland, 1981). To date, very few studies have examined the potential impact of such changes in food selection patterns on the biochemical Zn status of premenopausal women. This is unfortunate, because for this age group, sufficient Zn is critical for replenishing tissue Zn pools depleted by the increased demands for Zn during the pubertal growth spurt as well as for pregnancy (World Health Organization, 1996). Adequate Zn nutrition is critical for optimal growth and sexual development,

Abbreviation: OCA, oral contraceptive agent.

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skeletal maturation and mineralization, pregnancy outcome, and immune and cognitive function (King, 1996; Prasad, 1996).

In the present study, we have assessed the biochemical Zn status of a self-selected sample of premenopausal women living in Dunedin, New Zealand, using a combination of biochemical Zn indices. We selected serum Zn because it can be used to assess the Zn status of groups of individuals, provided the blood samples are taken under standardized conditions (Pilch & Senti, 1984; English & Hambidge, 1988; Tamura *et al.* 1994; Brown, 1998). Hair Zn concentrations were also measured, as an index of chronic suboptimal Zn status (Hambidge *et al.* 1972; Walravens *et al.* 1983; Gibson *et al.* 1989), and to examine relationships between hair Zn and anthropometric indices of body composition observed in an earlier study (Gibson *et al.* 2000). Finally, we chose to measure the activity of alkaline phosphatase in serum, a Zn-metalloenzyme which has also been used to assess Zn status (Nanji & Anderson, 1983; Baer *et al.* 1985). In the present study, we also evaluated the impact of certain non-dietary and dietary factors on these three biochemical Zn indices, in an effort to identify possible aetiological factors associated with suboptimal Zn status among these premenopausal Dunedin women.

Experimental methods

Participants

Details of the participants and data collection for this study have been described earlier (Heath *et al.* 2000b). Briefly, premenopausal women aged 18–40 years (mean age 26.4 (SD 6.7) years) were recruited mainly via publicity in the media, university, and community groups in Dunedin and surrounding areas from March 1996 to May 1998. The inclusion criteria were the absence of chronic disease, pregnancy and lactation, and consumption of a Western-type diet. Informed, written consent was secured from each participant after the nature of the study had been fully explained to them. The study protocol was approved by the Human Ethics Committee, Southern Regional Health Authority, Otago, New Zealand.

Data collection, dietary and anthropometric assessment

Participants attended morning clinics held either at the Southern Community Laboratories' Hanover Street Clinic or the University of Otago. Morning peripheral venepuncture blood samples were taken after an overnight fast with participants in the sitting position using trace-element-free evacuated tubes (Becton Dickinson, Rutherford, NJ, USA). Hair samples were also obtained at this time from the occipital portion of the scalp with stainless-steel scissors. Participants were then requested to complete a validated computer-administered food-frequency questionnaire designed to assess habitual intakes and dietary modifiers of Fe and Zn absorption over the last month (Heath *et al.* 2000a). A validation study against 11 d weighed diet records (Heath *et al.* 2000a) demonstrated that the Food-Frequency questionnaire was able to correctly classify the

protein, Ca, Fe, Zn, meat–fish–poultry, dietary fibre and phytate intakes of 73–88 % of participants to within one quartile of the record (35–51 % correctly classified), and grossly misclassified only 0–6 % (0–3 of forty-nine participants to opposite quartiles). Because the Food-Frequency questionnaire was not designed to assess fat and carbohydrate intakes, a 24 h recall was also used to determine the average energy intakes for the group. Energy intakes were calculated using the Diet Entry and Storage program (NutriComp, Dunedin, New Zealand) and the New Zealand food composition database (Burlingame *et al.* 1995).

Pretested questionnaires, designed to obtain information on health, socio-economic and demographic status, lifestyle, exercise and activity levels, mood and appetite, medical and menstrual history, habitual food consumption patterns of the participants, and their use of alcohol, medications, cigarettes, oral contraceptive agents (OCA), and vitamin and mineral supplements, were also administered by trained interviewers. Data on menstrual blood loss (via a validated menstrual blood loss questionnaire) (Heath *et al.* 1998), number and timing of blood donations and nosebleeds during the last year, were also collected as described previously (Heath *et al.* 2000b).

Dietary intakes of selected nutrients, dietary fibre (as NSP), phytic acid, and [phytate]:[Zn] molar ratios were calculated from the computerized food-frequency questionnaire using the New Zealand computerized nutrient database derived from the FOODFILES of the New Zealand food composition database (Burlingame *et al.* 1995). Phytate values were added to the nutrient database using selected analysed and literature values, as described in Donovan & Gibson (1995). For composite dishes, the phytate, meat, fish, and poultry content were calculated from recipes published in the New Zealand nutrient database, from Holland *et al.* (1988, 1991, 1992) or from the Edmonds Cookery Book (1987). Foods were categorized into seventeen food groups to determine the major food sources of Zn. Probability analysis, which takes into account the variation in requirement between individuals (National Research Council, 1986), was used to calculate the proportion of individuals likely to have Zn intakes below their own individual requirements. The average requirement estimates from the UK dietary reference values (Department of Health, 1991; National Research Council, 1986) were used because the Australian recommended dietary intakes (Truswell *et al.* 1990), used by New Zealand, are currently being revised, and do not stipulate the mean requirement estimates for Zn. Further, the dietary pattern of the UK is known to be similar to that of New Zealand.

The height, weight, biceps, triceps and subscapular skinfold thicknesses, and mid-upper arm circumference of participants were recorded in duplicate in the morning clinics with participants in the fasting state, using standardized procedures and calibrated equipment (Lohman *et al.* 1988). A portable anthropometer (Central Workshop North, University of Otago, Dunedin, New Zealand) was used to measure height to the nearest 0.1 cm, with participants standing erect, shoeless or with bare feet. Body weight was recorded to the nearest 0.1 kg using

digital scales (SECA-770, Vogel & Halke GmbH & Co., Hamburg), with participants wearing light clothing and no shoes. Quetelet's index (BMI) as weight (kg)/height (m²), sum of skinfold thicknesses, arm fat area and arm muscle area were then calculated using standardized equations (Frisancho, 1990).

Biochemical assessment

Trace-element-free techniques were used during the handling and analysis of all the blood samples. The latter were refrigerated immediately after collection (Tamura *et al.* 1994). Serum was separated within 2 h, and aliquots frozen in trace-element-free polyethylene vials at -20°C for later analysis (English & Hambidge 1988). Serum Zn was analysed by flame atomic absorption spectrophotometry (Perkin Elmer 2690, Ebos Group Ltd, Auckland, New Zealand), using a standardized procedure (Smith *et al.* 1979). Serial replication of aliquots from a pooled serum sample and quality control sera were used to check the precision and accuracy of the analytical methods. The CV for Zn in a pooled serum sample was 4.3 % (*n* 20). Values for the quality control sera (Bovine Serum Reference Material no. 1598; National Institute of Standards and Technology, Gaithersburg, MD, USA) were 13.6 (SD 0.9) µmol/l (CV 1.7 %, *n* 8) compared with the certified value of 13.9 (SD 1.5) µmol/l. Serum alkaline phosphatase activity was determined via a colorimetric procedure using *p*-nitrophenol phosphate as the substrate and commercial kits (Sigma Chemical Co., St Louis, MO, USA). The mean Zn concentration of a quality control serum (Sigma Enzyme Control 2-N; Sigma Chemical Co.) was 70.3 (SEM 1.08) U/l (CV 4.8 %), within the expected range of 65.13–78.49 U/l. The mean value for seven replicates of pooled serum was 19.4 (SEM 0.60) U/l (CV 8.2 %). The presence of infection was assessed by serum C-reactive protein ≥10 mg/l using a nephelometer (Behring BNA; Dade Behring, Auckland, New Zealand). The C-reactive protein assay had a CV 2.4 %. Haemoglobin and serum ferritin were also assayed by methods described in Heath *et al.* (2000b).

All hair samples were collected from the occipital portion of the scalp, washed by a modification of the non-ionic detergent procedure of Harrison *et al.* (1969), acid digested with ultrapure HNO₃ (700 g/l) (AristaR; BDH Laboratory Supplies, Palmserston North, New Zealand), then analysed for Zn by flame atomic absorption spectrophotometry. The CV for Zn in aliquots of a pooled powdered hair sample was 2.5 % (*n* 7) by atomic absorption spectrophotometry. Aliquots of a certified reference material for human hair (Commission Bureau of Reference, Reference Material no. 125, Geel, Belgium) were analysed: the mean value was 3.05 (SD 0.11) µmol/g (CV 3.5 %, *n* 24), compared with the certified value of 3.04 (SD 0.08) µmol/g.

Statistical analysis

All data were analysed using the Statistical Package for Social Sciences (SPSS for windows version 7.5.1; SPSS, Chicago, IL, USA). Checks for normality using the

Kolmogorov–Smirnov or Shapiro–Wilk test showed that the data conformed to the statistical tests used. Pearson product moment correlation coefficients were calculated to assess relationships among age, anthropometric, biochemical and dietary variables, where appropriate. Differences in frequency distributions of dietary variables of those who included and those who excluded red meat were examined using Fisher's exact test. Avoidance of red meat was determined by responses to the questionnaire on habitual food consumption patterns. ANOVA using the General Linear Model procedure was used to investigate the explanatory effects of: (1) OCA use, infection, age, blood loss (via menstruation, nosebleeds, and blood donation) on the biochemical Zn indices; (2) selected dietary variables on biochemical Zn indices; (3) selected anthropometric indices on biochemical Zn indices. All tests were considered significant at *P* < 0.05; correlation tests with *P* > 0.05 but *P* < 0.10 were considered for incorporation into the ANOVA because of potential associations.

Results

Participant characteristics

The present analysis included women (*n* 330) for whom blood, hair, anthropometric, and dietary measurements were obtained. Characteristics of the participants have been described earlier (Heath *et al.* 2000b). Briefly, the premenopausal women were mainly Caucasian (95 %), with New Zealand Maori or Pacific Island, (2 %) and other (3 %) and had a mean socio-economic status (using the six class New Zealand socio-economic index scale (Davis *et al.* 1997)) equivalent to class 3, technical workers. The majority of the women were nulliparous (80 %). The mean value of parity amongst those women who had children was 2.1. Of the participants, 15 % smoked, with 10 % smoking a mean value of five cigarettes/d, and 5 % smoking fifteen cigarettes/d; 14 % had consumed alcohol in the past month. OCA were used by 39 % of the women. Of the 38 % of women taking a multi-vitamin and/or mineral supplement, only eight regularly consumed a special Zn supplement. The amount of supplemental Fe being taken ranged from 0.06 mg/d (for an infrequently

Table 1. Intakes of energy, selected nutrients and antinutrients* (Median values and 1st and 3rd quartiles for 330 premenopausal women)

	Median	Quartiles	
		1st	3rd
Energy (kJ)	7969	6282	9775
Protein (g)	72.9	59.4	88.4
Ca (mg)	717	515	909
Fe (mg)	10.7	8.6	13.0
Zn (mg)	9.9	7.9	12.3
Zn density (mg/MJ)	1.3	1.0	1.6
Meat, poultry and fish (g)	102	59	146
Dietary fibre as NSP (g)	20.9	16.2	26.6
Phytate (mg)	1165	679	1713
[Phytate]:[Zn] molar ratio	12	7	17

* For details of procedures, see p. 72.

Table 2. Contribution of selected food groups to dietary Zn intake (%)

Food group	Present study*	Comparable studies (age of subjects)			
		15–24 years†	25–44 years†	15–24 years‡	25–44 years‡
Meat, poultry, and fish	27.9	40	39	31	32
Cereal products, nuts and legumes	26.7	25	26	34	31
Dairy products	18.4	–	–	14	17
Vegetables	7.6	–	–	7	5
Other	19.4	–	–	14	15

* Premenopausal women (18–40-years-old; *n* 330) using a Food-Frequency questionnaire. For details of procedures, see p. 72.

† 1989 Life in New Zealand Survey data; LINZ Research Unit, University of Otago, (personal communication).

‡ 1997 New Zealand National Nutrition Survey data; LINZ Research Unit, University of Otago (personal communication).

|| Excludes legumes.

taken multi-vitamin–mineral supplement) to 105 mg/d (for an Fe-only supplement).

Dietary results

Table 1 shows the median values (1st and 3rd quartiles) for the daily intakes of selected nutrients, antinutrients, and [phytate]:[Zn] molar ratios for the 330 participants. In this group, 35 % of the women had diets with [phytate]:[Zn] molar ratios >15. The median daily intake of dietary Zn was well above the UK reference nutrient intake of 7.0 mg/d (Department of Health, 1991). Probability analysis suggested that only 6 % of the women were at risk of inadequate intakes of dietary Zn. Of the participants, 22 % (*n* 74) excluded red meat in their diets; the remainder consumed omnivorous diets. As expected, more of the women who did not eat red meat had dietary [phytate]:[Zn] molar ratios >15 compared with red-meat eaters (i.e. 72 v. 24 %, *P* < 0.0001).

The contributions of Zn from selected food groups (%) for the premenopausal Dunedin women is shown in Table 2, and compared with data from the 1989 Life in New Zealand Survey (1992) and the 1997 National Nutrition Survey (NNS97 data, LINZ Research Unit, University of Otago; personal communication) for women aged 15–24 and 25–45 years (European and others excluding Maori and Pacific Island people). Note that the contribution of dietary Zn provided by cereals, legumes and nuts was very similar to that of flesh foods for both the women from Dunedin and those in the New Zealand 1997 National Nutrition Survey. Milk and milk products were also an important secondary source of Zn; only a very small proportion of Zn was provided by vegetables.

Anthropometric results

Mean values for height, weight, BMI, triceps, biceps and subscapular skinfold thicknesses, sum of skinfold thicknesses, mid-arm circumference, arm fat area and arm muscle area are shown in Table 3. Overall, on average the women of the present study were taller, lighter, with smaller triceps and subscapular skinfold thicknesses, and a lower BMI than the women of a similar age (European and others excluding Maori and Pacific Island people) in the 1997 New Zealand National Nutrition Survey (Russell *et al.*

1999). Of the 330 participants, 61.5 % had a BMI within the designated healthy range for New Zealand (i.e. 20–25 kg/m²); corresponding proportions with BMI <20 and >25 were 13.6 % and 24.9 % respectively, of whom 5.8 % were >30, the cut-off for BMI considered indicative of obesity in New Zealand (Nutrition Task Force, 1991).

Biochemical Zn indices

Table 4 compares the mean values for serum and hair Zn concentrations for the women of this study and for a group studied in Dunedin in 1973 (JM McKenzie, unpublished results). Mean values for alkaline phosphatase activity, haemoglobin, and serum ferritin for the women studied here are also given. Of the 330 women, 17 % (*n* 56) had serum Zn values below the cut-off of 10.71 µmol/l suggested to be indicative of mild Zn deficiency for fasting morning samples (Pilch & Senti, 1984). Of the non-OCA users (*n* 197) without infection, 12 % (*n* 23) had low serum Zn values compared with less than 1 % in the 1973 Dunedin study (JM McKenzie, unpublished results) and 2.4 % of US non-OCA users aged 20–44 years in the National Health and Nutrition Examination Survey II survey (Pilch & Senti, 1984). Our mean hair Zn value was also lower than that reported for the 1973 Dunedin study, despite using the same analytical method (i.e., atomic absorption spectrophotometry). Unfortunately, serum alkaline

Table 3. Selected anthropometric variables*

(Mean values and standard deviations for 330 premenopausal women)

	Mean	SD
Height (cm)	166.2	6.2
Weight (kg)	64.6	11.1
BMI (kg/m ²)	23.3	3.7
Biceps skinfold thickness (mm)	7.6	3.7
Subscapular skinfold thickness (mm)	13.0	5.8
Triceps skinfold thicknesses (mm)	18.9	5.2
Sum of skinfold thicknesses (mm)	39.5	13.5
Mid-upper arm circumference (mm)	296	35
Arm muscle area (mm ²)	4489	980
Arm fat area (mm ²)	2558	927

* For details of procedures, see p. 72.

Table 4. Selected biochemical variables for women of the present study (*n* 330) and Dunedin women in 1973 (*n* 49)*†

	Present study		1973 Dunedin study*‡	
	Mean	SD	Mean	SD
Serum Zn ($\mu\text{mol/l}$)	12.00	1.36	18.6	4.6
Hair Zn ($\mu\text{mol/g}$)	2.71	0.36	2.99	0.35
Serum alkaline phosphatase (U/l)	21.5	7.1	–	–
Haemoglobin (g/l)	131.5	8.2	–	–
Serum ferritin ($\mu\text{g/l}$)	42.0	36.3	–	–

* JM McKenzie, unpublished results.

† For details of procedures, see p. 72.

‡ a.m. non-fasting sample, non-oral-contraceptive-agent users, mean age 20.1 (SD 0.3) years.

phosphatase activity in this earlier Dunedin study was not available for comparison. Currently, specific criteria for interpreting hair Zn concentrations for premenopausal women have not been established. Of the 330 participants, 21 % had a hair Zn concentration below a cut-off value of 2.44 $\mu\text{mol/g}$ established earlier in a study of 11-year-old New Zealand children (Gibson *et al.* 2000).

Associations among biological, dietary, anthropometric, and biochemical Zn variables

Several significant relationships between both non-dietary and dietary factors and biochemical indices of Zn status were observed. They are now discussed in turn.

Age. As expected, most variables of body fatness (triceps skinfold thickness, sum of skinfold thickness, arm fat area, BMI) as well as mid-upper arm circumference correlated positively with age ($P < 0.05$). Selected dietary variables, including intakes of protein, Fe, Zn (per d), and meat–fish–poultry (g/d), also correlated positively with age because the omnivores were significantly older as a group than those (*n* 74) who excluded red meat (26.9 *v.* 25.0 years, $P = 0.03$).

Oral contraceptive agents. The use of OCA had a marked negative effect on serum Zn as well as on alkaline phosphatase activity, the decrease being significant for serum Zn ($P < 0.05$), and alkaline phosphatase ($P < 0.01$) (after removing the confounding effect of age); OCA use did not appear to impact significantly on hair Zn concentrations (Table 5).

Infection. Of the non-OCA users, the few participants with serum C-reactive protein >10 mg/l (*n* 5) (Hobbs *et al.* 1996) had a significantly lower concentration of Zn in serum, but not hair; levels of serum alkaline phosphatase activity were similar (Table 5). Of the 197 non-OCA users with C-reactive protein <10 mg/l (mean age 27.8 years), 12 % (*n* 23) had low serum Zn values (i.e. <10.71 $\mu\text{mol/l}$), whereas 18 % had hair Zn concentrations below the cut-off value of 2.44 $\mu\text{mol/g}$. Of the 12 % (*n* 23) with low serum Zn values, five had hair Zn concentrations below the cut-off value of 2.44 $\mu\text{mol/g}$. In these same 197 participants, serum Zn concentrations were not correlated with hair Zn concentrations or alkaline phosphatase activities. Serum Zn concentrations were modestly, but significantly, positively correlated with serum ferritin concentrations (r 0.19, $P = 0.01$) and haemoglobin (r 0.23, $P = 0.001$).

Anthropometry. Several relationships existed between selected anthropometric and biochemical Zn variables (hair Zn and serum alkaline phosphatase) (Table 6), but these were only apparent when the study group was divided into two groups: younger participants aged 18–20 years (*n* 86), and an older group comprising the remainder of the participants (*n* 244). These two age groups were selected because for some of the younger participants, linear growth and skeletal maturation may have only recently ceased (Lindsay *et al.* 1993). After classifying the participants in this way, several significant correlations were observed between variables of body fatness and hair Zn concentrations in the younger group whereas in the older group, such associations were only significant for serum alkaline phosphatase activities. There were no significant correlations with serum Zn concentrations for any of the anthropometric variables for either age group.

Other non-dietary factors. Other non-dietary factors

Table 5. Biological factors impacting on biochemical zinc indices*

	<i>n</i>	Mean serum Zn ($\mu\text{mol/l}$)	Mean hair Zn ($\mu\text{mol/g}$)	Mean serum alkaline phosphatase (U/l)
All participants:				
Oral-contraceptive users	128	11.81	2.68	19.5
Non-users	202	12.11	2.72	22.8
Statistical significance of effect: <i>P</i>		0.05	NS	0.01†
Excluding oral contraceptive users:				
Serum C-reactive protein <10 mg/l	197	12.15	2.72	22.7
Serum C-reactive protein >10 mg/l	5	10.50	2.84	23.4
Statistical significance of effect: <i>P</i>		0.05	NS	NS
Excluding oral contraceptive users and participants with serum C-reactive protein >10 mg/l				
Age ≤ 20 years	50	12.12	2.65	24.7
Age >20 years	147	12.16	2.74	22.1
Statistical significance of effect: <i>P</i>		NS	NS	0.04

* For details of subjects and procedures, see p. 72.

† After first correcting for age.

Table 6. Relationship between anthropometric measures and biochemical indices of Zn status for two age groups†
(Pearson correlation coefficients)

	Age ≤20 years (<i>n</i> 86)		Age >20 years (<i>n</i> 244)	
	Hair Zn	Serum alkaline phosphatase	Hair Zn	Serum alkaline phosphatase
Height	0.098	-0.036	0.002	0.012
Weight	-0.228*	-0.024	-0.023	0.234**
BMI	-0.304**	-0.004	-0.026	0.250**
Biceps skinfold thickness	-0.137	0.101	0.074	0.265**
Subscapular skinfold thickness	-0.193	0.000	0.068	0.225**
Triceps skinfold thicknesses	-0.228*	0.116	0.080	0.228**
Sum of skinfold thicknesses	-0.218*	0.075	0.080	0.257**
Mid-upper arm circumference	-0.229*	0.029	0.008	0.242**
Arm muscle area	-0.179	-0.037	-0.036	0.189**
Arm fat area	-0.229*	0.097	0.058	0.239**

P* < 0.05 (2-tailed), *P* < 0.01 (2-tailed).

† For details of subjects and procedures, see p. 72.

examined included socio-economic status, smoking, consumption of alcohol, stage of the menstrual cycle, and blood losses associated with menstruation (both extent and duration of menstrual bleeding), nosebleeds, and time of any recent blood donation. Neither socio-economic status, alcohol consumption, or stage of the menstrual cycle had any discernible impact on the biochemical indices of Zn status examined. Approximately 15 % of the subjects smoked, which was associated with a non-significant but noticeable increase in alkaline phosphatase activity, but no change in serum and hair Zn concentrations. Blood losses from menstruation, nosebleeds, and recent blood donations did not impact on any of the biochemical Zn indices, although these three sources of blood loss were all identified as significant risk factors for mild Fe deficiency (i.e. serum ferritin <20 µg/l, haemoglobin >120 g/l) in these same young women (Heath *et al.* 2000b).

Use of Fe supplements. Of the participants who did not use an OCA and with no evidence of infection (*n* 197), 17 % regularly took daily supplements containing Fe only, most of which contained less than 60 mg Fe/d. These participants had a mean serum Zn level that was slightly lower than their counterparts not taking any Fe-only supplements (i.e. 11.8 *v.* 12.2 µmol/g, NS). A very small number of these same participants (*n* 8) regularly consumed a daily Fe supplement containing 105 mg elemental Fe. The participants taking this high-Fe dose daily had a significantly lower mean hair Zn concentration than the rest of the group (i.e. 2.43 *v.* 2.73 µmol/g, *P* = 0.01), although their mean serum Zn concentration and alkaline phosphatase activity were similar.

Dietary patterns. In the non-OCA users with serum C-reactive protein levels <10.0 mg/l, the mean serum Zn value was higher in the red-meat eaters (*n* 149) compared with those who excluded red meat (*n* 48) (12.2 *v.* 11.8 µmol/g, *P* < 0.05); no comparable differences existed for mean hair Zn or alkaline phosphatase activity. Further, 19 % of the young women who avoided eating red meat, compared with only 9 % who ate red meat, had serum Zn values below a cut-off of 10.71 µmol/l. In the group as a whole (i.e. *n* 197), significant negative correlations existed between serum Zn concentrations and intakes of dietary fibre (as NSP) and phytate, as well as the [phytate]:[Zn]

molar ratios of the diet; details are shown in Table 7. There were no significant correlations with hair Zn concentrations or serum alkaline phosphatase activity for any of the dietary variables.

Discussion

The most striking finding of the present study was the apparent lower biochemical Zn status of the premenopausal women of this study compared with earlier data for women of this age group in New Zealand (McKenzie, 1979) as well as other Western countries (Pilch & Senti, 1984; Health and Welfare, Canada, 1981; Nishi *et al.* 1981). For example, in non-OCA users aged 20 years (*n* 55, range 18–25 years) studied in Dunedin in 1973, both serum and hair Zn concentrations were markedly higher than levels reported here (i.e. serum Zn 18.5 µmol/l, hair Zn 2.98 µmol/g) (McKenzie, 1979). Such a difference is especially noteworthy because non-fasting morning samples, which were collected in the earlier Dunedin study (JM McKenzie, unpublished results), are known to yield lower serum Zn values than those for the fasting early morning blood samples reported here (Pilch & Senti, 1984).

Several factors were observed to impact negatively on one or more of the biochemical Zn indices examined in these young women, and thus had the potential to confound

Table 7. Relationship between selected dietary variables and serum zinc for 197 non-oral-contraceptive-agent users free from infection†
(Pearson correlation coefficients)

Dietary variable	Serum Zn
Energy (kJ)	-0.007
Protein (g)	-0.057
Ca (mg)	-0.111
Fe (mg)	-0.116
Zn (mg)	-0.098
Zn density (mg/MJ)	-0.019
Meat, poultry and fish (g)	0.103
Dietary fibre (g NSP)	-0.148*
Phytate (mg)	-0.239**
[Phytate]:[Zn] molar ratio	-0.163*

P* < 0.05 (2-tailed), *P* < 0.01 (2-tailed).

† For details of subjects and procedures, see p. 72.

a relationship between Zn status and diet. Of these, use of OCA was the biological factor with the most marked negative effect on both serum Zn and alkaline phosphatase activities (Table 4), independent of age, consistent with earlier findings (Prasad *et al.* 1975; Schiele *et al.* 1983; Pilch & Senti, 1984; Loke *et al.* 1992; Donovan & Gibson, 1995), although not always taken into account (Ball & Ackland, 2000). The reduction in serum Zn levels associated with OCA use appears to arise from alterations in the postabsorptive utilization of Zn induced by oestrogens rather than reflecting an increased requirement of dietary Zn (King, 1986). Infection also played a role in decreasing serum Zn concentrations (Table 4), as noted by others (Zavaleta *et al.* 1995). This effect has been attributed to leucocyte endogenous mediator–interleukin 1 produced by phagocytic cells during the acute-phase response (Beisel, 1977). By contrast, infection did not influence hair Zn or alkaline phosphatase activity, although the latter was significantly reduced by smoking, an effect also observed in pregnant Canadian adolescents (Wolfe *et al.* 1994).

In view of the confounding effects of OCA use and infection on serum Zn concentrations, we compared the mean serum Zn concentration of a subgroup of our women who were non-OCA users free from infection (i.e. C-reactive protein >10 mg/l; Hobbs *et al.* 1996) with that for non-pregnant and non-lactating women aged 20–44 years in the US National Health and Nutrition Examination Survey II 'AM Fasting Sample' reference population (excluding all OCA-users and women with infection) (Pilch & Senti, 1984). The mean serum Zn value for our subgroup was identical to the 25th percentile value for this US reference sample, confirming that the serum Zn concentrations of our New Zealand women were indeed lower than corresponding values for US women studied in 1976–1980.

We also examined the impact of blood loss as a risk factor for suboptimal Zn status in our group. Three types of blood loss were examined: menstrual blood loss (both extent and duration of menstrual bleeding), nosebleeds, and the time of any recent blood donation, none of which had any impact on the Zn status of the women, despite their aetiological role in the development of mild Fe deficiency (i.e. serum ferritin <20 µg/l and haemoglobin >120 g/l) in these same women (Heath *et al.* 2000b).

Lower intakes of dietary Zn *per se* were probably not responsible for the lower biochemical Zn status of the Dunedin women studied here. The median dietary Zn intakes for the group as a whole (i.e. 9.9 mg/d, *n* 330) as well as for our selected subgroup of the non-OCA users without infection (i.e. 10.1 mg/d, *n* 197) were both higher than levels reported in earlier studies of premenopausal women in New Zealand (Guthrie & Robinson, 1977; Life in New Zealand Survey, 1992) and other Western countries (Gregory *et al.* 1990; Moser-Veillon, 1990). We recognize that such comparisons are difficult because of possible biases arising from the selection of the subjects, dietary methods used, and dietary under-reporting (Black *et al.* 1991). Nevertheless, our median dietary Zn intake for the entire group was very close to that of the median values (adjusted for intra-individual variation) for New Zealand

premenopausal women in the recent 1997 National Nutrition Survey in New Zealand (i.e. European and others: 15–24 years 9.8 mg/d, 25–44 years 10.1 mg/d; Russell *et al.* 1999).

Consumption of flesh foods, specifically beef and sheep meat, has declined in New Zealand from an estimated 194 g/capita per d in 1975 to 147 g/capita per d in 1995, based on food balance sheet data from Statistics New Zealand (Laugesen & Swinburn, 2000), a trend consistent with that of several other industrialized countries (Zafiriou, 1985; Popkin *et al.* 1989; Whitehead, 1995). In addition in some young women in New Zealand (Horwath, 1991), and elsewhere (Slattery & Randall, 1988), there have been concomitant increases in the intakes of grain products. As a result, we postulate that differences in food selection patterns, resulting in dietary Zn intakes with a reduced bioavailability, may have accounted for the lower biochemical Zn status noted in these New Zealand women. In earlier studies of premenopausal women in New Zealand (Guthrie & Robinson, 1977) and other Western countries (Gibson & Scythes, 1982; Mares-Perlman *et al.* 1995), flesh foods were said to be the major food source of dietary Zn. Indeed, their contribution was approximately 40 % for women aged 15–44 years in the 1989 Life in New Zealand survey (Table 2) (LINZ Research Unit, University of Otago, personal communication). By contrast, in our present study, the contribution of meat, poultry and fish to dietary Zn intake was markedly lower (i.e. 28 %) and comparable with that for cereal products, nuts and legumes (i.e. 27 %); dairy products were the next major food source of Zn (18 %). DL Alexander (unpublished results) also found cereals to be a major food source of dietary Zn, even for young omnivorous women in Dunedin. In the New Zealand 1997 National Nutrition Survey (NNS97 data; LINZ Research Unit, University of Otago, personal communication) cereals provided 32 % and 30 % dietary Zn for women (European and others excluding Maori and Pacific Island people) aged 15–24 and 25–44 years respectively. Corresponding contributions from flesh foods were 31 and 32 %, suggesting that the shift in the major food sources of Zn reported for the women studied here paralleled a national trend.

Such changes in the major food sources of dietary Zn could potentially have a major impact on the amount of Zn available for absorption. Flesh foods, especially red meat, are rich sources of readily available Zn. Further, their presence is said to facilitate Zn absorption via releasing L-amino acids and cysteine-containing peptides during digestion which form soluble ligands with Zn (Sandström & Cederblad, 1980). Certainly, our present results confirm that the women who included red meat in their diet had a superior biochemical Zn status (based on serum Zn) than those who avoided eating red meat. Indeed, of our selected subgroup (*n* 195), 19 % who excluded red meat, compared with only 9 % who ate red meat, had serum Zn values below the cut-off (10.71 µmol/l) associated with functional consequences of mild Zn deficiency (Pilch & Senti, 1984). Differences in the prevalence of low serum Zn values for young adolescent women consuming lacto-ovo vegetarian, semi-vegetarian, and omnivorous diets have been reported previously (Donovan & Gibson, 1995).

Grain products, nuts and legumes, one of the major food-group sources of dietary Zn for these premenopausal women, contain high levels of phytic acid, a potent inhibitor of Zn absorption which forms insoluble Zn–phytic acid complexes in the intestine, (Sandström & Lönnerdal, 1989). To date, very few dietary surveys have reported intakes of phytic acid. Phytate (and dietary fibre) intakes for these premenopausal New Zealand women were higher than those reported for premenopausal women in the USA (Murphy & Calloway, 1986) and Canadian adolescents (Donovan & Gibson, 1995). The negative effect of phytic acid on Zn absorption can be predicted by the [phytate]:[Zn] molar ratio of the diet. Values >15 have been associated with suboptimal Zn deficiency in human subjects (Harland & Peterson, 1978; Oberleas & Harland, 1981; Turnland *et al.* 1984; Bindra *et al.* 1986; Donovan & Gibson, 1995). Of the women in our study, 35 % had diets with [phytate]:[Zn] molar ratios >15. We also observed a significant inverse relationship between serum Zn concentrations of our subgroup (n 195) and [phytate]:[Zn] molar ratios in the diet (Table 7), consistent with our earlier findings in Canadian vegetarian and omnivorous adolescents (Donovan & Gibson, 1995). Moreover, the women of this group with dietary [phytate]:[Zn] molar ratios >15 had a significantly lower mean serum Zn value than their counterparts with diets with [phytate]:[Zn] molar ratios <15 (i.e. 11.9 v. 12.3 $\mu\text{mol/l}$, $P = 0.04$). Nevertheless, we detected no adverse consequences related to marginal Zn deficiency in these women such as disturbances in appetite, mood, physical growth and body composition, or an increased susceptibility to infection (Hambidge, 2000).

An additional diet-related factor known to have an antagonistic interaction with Zn, and hence with the potential to lower the biochemical Zn status of these women, is the consumption of high doses of Fe supplements (Solomons, 1986). In our present study, serum Zn concentrations for the subgroup were slightly, but not significantly, lower for those women taking Fe supplements. Moreover, for the small group (n 8) consuming a very high daily dose of Fe-only supplements (i.e. 105 mg/d), their mean hair (but not serum) Zn value was significantly lower ($P = 0.01$) compared with those not taking any Fe-only supplements. A similar negative effect on Zn nutriture has been reported for pregnant women taking prenatal Fe supplements both in New Zealand (McKenzie-Parnell *et al.* 1987) and elsewhere (Bloxam *et al.* 1989; Dawson *et al.* 1989).

It is noteworthy that although a low BMI placed these premenopausal women at increased risk for mild Fe deficiency (Heath *et al.* 2000b), BMI was not associated with the biochemical Zn status of the group as a whole. Nevertheless, among the younger (18–20-year-old) women, BMI as well as several other indices of body fatness and body weight (Table 7) had a negative correlation with hair Zn concentrations, a finding noted in earlier studies of New Zealand children (Gibson *et al.* 2000) and New Zealand adolescents (I Jones, personal communication). In these studies, subjects with low hair Zn concentrations were heavier, fatter and/or had higher BMI values than their peers with higher hair Zn values. Inverse

relationships between Zn status and anthropometric indices of body fatness and BMI have also been reported in some adult studies (Chen *et al.* 1988), although in the present study we did not find these effects amongst its' adult (>20-year-old) cohort. Instead, in this older cohort, we noted several significant correlations between anthropometric indices of body fatness and serum alkaline phosphatase activity. Such findings probably arise from inter-relationships noted between total bone mineral density and fat mass and total body mineral density and serum alkaline phosphatase activity. Indeed, total body fat is said to be an important determinant of whole-body bone density in premenopausal sedentary women (Reid *et al.* 1992).

Animal studies investigating the mechanism for the relationship between biochemical Zn status and body composition suggest that the changes may be mediated by an interaction of Zn with insulin activity and thyroid hormone conversion (Begin-Heick *et al.* 1985; Kennedy *et al.* 1986; Chen *et al.* 1991).

In summary, the lower biochemical Zn nutriture of the New Zealand premenopausal women studied may be associated in part with changes in food selection patterns, which have led to a reduction in intakes of red meat, and may be cause for concern. If these premenopausal women enter pregnancy with a compromised Zn status, and continue to receive intakes of bioavailable Zn that do not meet their increased needs, the resultant poor maternal Zn status could have adverse consequences on pregnancy outcome. Certainly, infants born to otherwise healthy Afro-American women with below-average plasma Zn levels at prenatal enrolment had significantly lower birth weights and head circumferences than those infants born to corresponding Zn-supplemented mothers (Goldenberg *et al.* 1995).

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