Micronutrient status of New Zealand adolescent women consuming vegetarian and non-vegetarian diets

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ABSTRACT

Background and Objectives: Globally, there appears to be an ever-increasing interest in adopting a vegetarian diet. However, there are concerns that avoiding meat may increase the risk of anaemia and micronutrient deficiencies, especially for vulnerable populations, such as adolescent women. The objective of this study was to compare the micronutrient status of vegetarian and non-vegetarian adolescent women in New Zealand. **Methods and Study Design:** Adolescent women aged 15-18 y were recruited from eight locations across New Zealand. Blood samples were analysed for: haemoglobin, serum ferritin, soluble transferrin receptor, zinc, selenium, retinol binding protein, folate, vitamin B-12, vitamin D and parathyroid hormone. **Results:** Of the 182 participants who provided a blood sample, 15% self-identified as vegetarian (n=27). On average, vegetarians had 3.1% (95% CI -5.8 to -0.4, p=0.025) lower haemoglobin, and 8.3% (95%CI -14.1 to -2.1, p=0.004) lower selenium. In contrast, serum folate was 80.5% (95% CI 45.7 to 123.7, p<0.001) higher. The prevalence of zinc and selenium deficiency was higher among vegetarians (50% and 12%, respectively) than non-vegetarians (21%, and 2%, respectively). **Conclusions:** Adolescent vegetarian women may be at increased risk of deficiency of micronutrients commonly found in animal products, including zinc and selenium, and may benefit from following dietary practices that enhance micronutrient intake and absorption.

Key Words: vegetarianism, adolescence, haemoglobin, iron status, zinc

INTRODUCTION

Anecdotally, the popularity of vegetarianism appears to be increasing across the western world.[1-3] However, its prevalence is not well documented. Recent market research data suggest that 12% of Australians,[4] 10% of New Zealanders,[5] 8% of Canadians[6] and 5% of people in the USA,[7] follow a vegetarian diet. Reasons behind the adoption of a vegetarian dietary pattern seem to be centred around animal welfare and environmental concerns,[8] while many religions encourage vegetarianism, or discourage consumption of particular meats, and many people eat less meat than they would like, for economic reasons.

Compared to non-vegetarians, “true” vegetarians can be defined by their avoidance of all meat, poultry, and fish products.[9] Further distinctions can be made based on the inclusion of some animal products such as the inclusion of eggs (ovo-vegetarians) or dairy (lacto-vegetarians). “Vegetarianism”, however, is a term that is often used, particularly by the

general public, to describe an array of dietary patterns that all involve the avoidance of red meat,[10] but may include some fish or poultry.

In general, studies have shown that a vegetarian diet is associated with lower total and low-density lipoprotein cholesterol, body mass index and blood pressure;[3,11,12] as well as lower risk of some non-communicable diseases including ischaemic heart disease,[13] type 2 diabetes mellitus and some cancers.[14,15] Despite these health benefits, eliminating meat, and for vegans, animal products in general, without accounting for the micronutrients they supply may result in inadequate dietary intake and suboptimal micronutrient status. This is particularly important for adolescent women who have increased micronutrient requirements to support growth, sexual maturation, and the onset of menarche.[16] Micronutrients of concern in this population include iron, vitamin B-12 and zinc[17] with rates of suboptimal biochemical status of these micronutrients in adolescent vegetarian women reported to be as high as 58%,[18] 4-10%,[19,20] and 18-24%,[21] respectively.

Most of the studies on the health effects of a vegetarian diet were conducted in adults in the 90s and early 2000s, and a substantial number of them were conducted in Seventh Day Adventist populations.[14,22-24] Much less is known about the current health impacts of vegetarian diets in adolescent women from the general population. It is plausible that changes in the food supply, driven by the increased popularity of vegetarianism, along with increased visibility of sustainable farming and food production methods, may have changed the dietary habits of modern populations. Therefore, the aim of this study was to compare the micronutrient status of vegetarian and non-vegetarian adolescent women in New Zealand.

MATERIALS AND METHODS

Study Design

Data used in this study were collected as part of the SuNDiAL (Survey of Nutrition, Dietary Assessment And Lifestyles) Project, a nationwide cross-sectional survey of adolescent women aged 15 – 18 y. A detailed summary of the methods is presented elsewhere.[25] The study was approved by the University of Otago Human Ethics Committee (Health), H19/004, and is registered with the Australian New Zealand Clinical Trials Registry, ACTRN12619000290190.

Participants

The SuNDiAL project was conducted across eight locations throughout New Zealand (Dunedin, Wanaka, Christchurch, and Nelson in the South Island; and Wellington, New

Plymouth, and Whangarei in the North Island). High schools in these regions were invited via email, and 13 schools participated. Data collectors gave in-person recruitment presentations to either the whole school, individual year groups or individual classrooms, based on the school's preference. Recruitment (and data collection) was originally planned to occur at two timepoints: from February to April 2019, and July to September 2019. Adolescents who self-identified as being a woman, were between 15 and 18 years of age, could speak and understand English, and reported not being pregnant were eligible to participate. Online informed consent was obtained from all participants, and the parent or guardian of participants under 16 years of age. At consent, participants were given the option to indicate if they were prepared to provide a blood sample.

Initially the data collection plan did not involve deliberate targeting of vegetarian participants. However, at the end of the first phase of data collection it was apparent that the prevalence of vegetarians in the recruited sample was lower than expected. Therefore, targeted recruitment of vegetarians within the participating schools was undertaken in the second phase of data collection. In addition, targeted recruitment of vegetarians from the general public (provided they met the inclusion criteria) occurred at a third time point (November 2019 to February 2020). This targeted recruitment was conducted in two cities in the South Island, Dunedin and Christchurch, via word of mouth and social media advertisements. When participants were recruited from the general public data, collection occurred at research clinics instead of schools.

Demographics

Prior to data collection, participants provided online consent. After consent, an online questionnaire was given to participants to complete in their own time. Online consent and questionnaires were administered through REDCap (Research Electronic Data Capture, production server version 9.3.3). This questionnaire included basic demographic and health questions. It also asked, "*Are you vegetarian or vegan?*" but no formal definition of vegetarianism or veganism was provided. If the participant answered yes to this question, they are considered vegetarian in the analysis presented here. It was considered that self-identified vegetarianism was more relevant than strictly defined vegetarianism because this is the group that would be reached by health advice aimed at "vegetarians". Supplement use was also collected in the online questionnaire; participants were also requested to upload photographs of their supplements so that micronutrient content could be confirmed.

Anthropometry

At both the school and clinic data collection session, weight (measured using one of the following scales: Medisana PS420 (Nuess, Germany); Slater 9037 NK3R (Kent, United Kingdom); Seca Alpha 770 (Hamburg, Germany); or Soehnle Style Sense Comfort 400 scales (Backnang, Germany)), and height (measured using a Seca 213 (Hamburg, Germany) or Wedderburn (Sydney, Australia) stadiometer) were measured using standardized procedures in duplicate to the nearest 0.1 kg or cm, respectively, with the participant wearing no shoes and light clothing. A third measurement was taken if the first two measures differed by more than 0.5 units, and the mean of the closest two measurements used as the 'true' value. Body mass index was calculated by weight (in kg), divided by height (in m) squared. Body mass index z-scores for age and height were used to classify participants as healthy weight, overweight or obese using the WHO (World Health Organisation) growth reference data.[26]

Blood collection and analysis

A trained phlebotomist collected non-fasting blood samples during the in-school data collection session from participants who consented to participate in this aspect of the study. Blood was collected, in the morning, from a vein in the antecubital fossa using trace element free vacutainers (BD (Becton, Dickson and Company), Franklin Lakes, New Jersey) and placed on ice immediately after collection before being transferred to a local clinical diagnostic laboratory (Southern Community Laboratories or an affiliated Laboratory) where the coagulated blood sample was centrifuged on arrival. A complete blood count, Serum B-12 and folate concentrations were measured immediately using electrochemiluminescence immunoassay. Remaining serum was aliquoted and frozen before being transferred to the Department of Human Nutrition on dry ice, and then stored at -80°C. An aliquot was sent to Germany (on dry ice) for the measurement of serum ferritin (accuracy 101%; Intraassay coefficient of variation (CV) 2.25%), soluble transferrin receptor (accuracy106%; CV 3.95%), retinol binding protein (accuracy 100%; CV 3.61%), α-1-acid glycoprotein (accuracy 101%; CV 8.09%) and C-reactive protein (accuracy 97%; CV 5.84%) using a combined sandwich ELISA technique in the VitMin Laboratory of Dr J Erhardt.[27] Another aliquot was used for the measurement of serum zinc (accuracy 97%; CV 1.1%) and selenium (accuracy 101%; CV 2.0%), which was performed using inductively coupled plasma mass spectroscopy (Agilent 7500ce ICP-MS; Agilent Technologies) in the Centre of Trace Element Analysis, Department of Chemistry, University of Otago, New Zealand. The final aliquot was used for the measurement of serum vitamin 25-hydroxyvitamin D (accuracy 96%; CV 4.4%) and

parathyroid hormone (PTH) (accuracy 96%, CV 1.9%), which was performed using an electrochemiluminescence binding assay and an electrochemiluminescence immunoassay, respectively (Cobas e 411 Analyzer) in the Department of Human Nutrition Laboratory, University of Otago. New Zealand.

After adjustment for inflammation (see statistical analysis section), abnormal micronutrient status for analysed biomarkers was defined using the following cut points: Anaemia: haemoglobin <120 g/L;[28] iron deficiency anaemia: haemoglobin <120 g/L and a serum ferritin <15 µg/L; iron depletion: serum ferritin <15 µg/L; [29] serum zinc <10.09 µmol/L,[30] serum selenium <0.82 µmol/L,[31] serum folate <6.8 nmol/L,[32] serum B-12 <148 pmol/L,[33] serum 25-hydroxyvitamin D 30-50 nmol (insufficiency), and <30 nmol/L (deficiency).[34] The serum transferrin receptor cut-off derived from the original ELISA method (>8.3 mg/L) was converted to account for the rise of the Tina-quant assay (Roche Diagnostics GmbH) which yields values that are 30% lower; thus, a cut-off of >5.3 mg/L was used.[35] Iron deficiency was also assessed using total body iron (TBI) calculated from the equation of Cook and colleagues.[36] Total body iron concentrations of ≤ 0 mg/kg represent tissue iron deficiency and are linearly related to body iron stores in adults.[37]

Statistical analysis

Three hundred participants were required to give 80% power to detect a difference of 0.5 SD in micronutrient intakes between vegetarians and non-vegetarians to α=0.05. This assumed a 20% prevalence of vegetarianism and a design effect of 1.5.25 As provision of a blood sample was an additional opt-in assessment, participant numbers were likely to be lower. However, even if blood sampling was as low as 50% of the wider sample this would still provide the same power to detect a difference of 0.7 SD.

All statistical analysis was undertaken in Stata 17.0 (StataCorp, Texas). The regression-based BRINDA method,[38] was used to adjust biomarkers for inflammation (identified using both CRP and AGP). Variables were log-transformed as necessary to improve homoskedasticity of residuals, and adjustment was not made if there was no evidence of a relationship with AGP and CRP. Biomarkers that were adjusted for inflammation were: serum ferritin, soluble transferrin receptor, retinol binding protein, zinc, and vitamin B-12.

Unpaired t-tests (for continuous variables) and Fisher's exact tests (for categorical variables) were used to assess differences in demographics, dietary intake, and supplement-use by vegetarian status. Geometric means and 95% CI for all biomarkers (except total body iron, which can be negative in value) were calculated for vegetarians and non-vegetarians.

Mean differences, 95% CI, and *p*-values were determined using linear regression models with the biomarker log-transformed and the mean difference back-transformed to represent the percent difference between the groups. Total body iron was described using mean and SD, and not log-transformed in the regression model, so the mean difference is presented as the absolute difference. The proportions of each group with abnormal micronutrient status were calculated and the Fisher's exact test used to test for differences between the groups. Sensitivity analyses were undertaken that determined the mean difference in biomarkers only in those participants who were not taking a supplement containing a relevant micronutrient.

RESULTS

Demographics and micronutrient intakes

Of the 272 participants who completed at least one component of the SuNDiAL project, 182 provided a blood sample, of whom 14.8% were self-identified vegetarians (n=27) (Table 1). Vegetarians tended to be slightly older than non-vegetarians, but a similar percentage of both groups self-identified as Māori (indigenous people of New Zealand). The mean BMI z-score for self-identified vegetarians was significantly lower than for non-vegetarians (p=0.042). Iron supplements were the micronutrient supplement most consumed in the past month. Vegetarian participants were significantly more likely to use iron (33% versus 14%, p=0.021) and vitamin B-12 (30% versus 12%, p=0.035) supplements in the previous month than non-vegetarians (Table 1).

Micronutrient status of vegetarians and non-vegetarians

On average, vegetarians had 3.1% (95% CI –5.8 to –0.4, p=0.025) lower haemoglobin, 8.3% (95% CI -14.1 to –2.1, p=0.009) lower selenium, and 80.5% (95% CI 45.7 to 123.7, p<0.001) higher serum folate concentrations when compared to non-vegetarians (Table 2). While not statistically significant, this sample of vegetarians also had lower concentrations of serum ferritin, total body iron, zinc, retinol binding protein, serum B-12, and vitamin D. Sensitivity analyses using only data from participants who were not taking supplements containing relevant micronutrients are presented in Supplementary Table 1. This shows that when participants using supplements were removed, differences in biomarkers between vegetarians and non-vegetarians are greater for haemoglobin, serum ferritin (now statistically significant), soluble transferrin receptor (now statistically significant), total body iron (now statistically significant), selenium, and retinol binding protein. Differences for serum folate and serum B-12 were smaller. Vitamin D concentrations varied according to the season of data collection

(irrespective of vegetarian status) with those collected at the end of summer/early autumn (February to April) having higher concentrations (geometric mean: 38.2 nmol/L, 95% CI 35.5 to 41.1 nmol/L) compared to those collected at the end of winter/beginning of spring (July to September; 28 nmol/L 95% CI 26.1 to 30.7; *p*=0.008) and those collected over the late spring/summer (November to February; 23.5 nmol/L, 95% CI 16.6 to 33.; *p*=0.037). Selenium concentrations varied by location (irrespective of vegetarian status), with those living in the North Island having higher concentrations (geometric mean 93.7 µg/L; 95% CI 91.5 to 96.0) compared to those living in the South Island (85.4 µg/L; 95% CI 82.2 to 88.7; *p*=<0.001).

Prevalence of suboptimal micronutrient status

The prevalence of suboptimal micronutrient status is outlined in Table 3. Anaemia was identified in 7% of participants (5% non-vegetarians and 15% of vegetarians, *p*=0.082). Iron depletion was identified in 15% of participants (10% non-vegetarians and 15% vegetarians, *p*=0.105). Vitamin D insufficiency was identified in 54% of participants (55% non-vegetarians and 48% vegetarians), and deficiency was identified in 30% participants (29% non-vegetarians and 44% vegetarians, *p*=0.229). The prevalence of zinc deficiency was higher in vegetarians (50%), compared to non-vegetarians (20%). Sensitivity analyses using only those who did not take relevant micronutrient supplements (Supplementary Table 2) showed that those with suboptimal status tended to be those who were not taking supplements.

DISCUSSION

The results of the present study showed that vegetarian woman participants had a higher prevalence of zinc and selenium deficiency albeit better folate status than their non-vegetarian woman counterparts. Iron and vitamin B-12 status, was also lower, on average, in vegetarians compared to non-vegetarians (in line with well acknowledged concerns about these micronutrients) but these differences were not statistically significant in the current sample. A concerning proportion of both vegetarian and non-vegetarian participants were deemed to be deficient in iron, zinc, and vitamin D.

Iron and vitamin B-12 are notable micronutrients at risk in vegetarian diets, particularly among those who do not consume supplements and omit all foods of animal origin. While the prevalence of anaemia in vegetarian participants (14.8%) was nearly three times greater than non-vegetarian participants (5.2%), the difference was not statistically significant (*p*=0.082). There was also no statistically significant difference between the two groups for the prevalence of iron deficiency anaemia, although the overall prevalence in both groups was

very low (n=5 across the entire sample). Interestingly, one-third of vegetarian participants in the present study were consuming iron containing supplements. The prevalence of iron deficiency, as measured by total body iron, was somewhat concerning ranging from 11% in non-vegetarian participants to 18.5% in vegetarian participants. Total body iron is an indicator of the size of the functional iron deficit that is independent of haemoglobin concentrations.

Similar to iron, concentrations of serum B-12 were also lower in vegetarians, although this difference was not statistically significant. The prevalence of vitamin B-12 deficiency was very low across the entire study population with just three participants being identified as deficient. To our knowledge, the risk of vitamin B-12 deficiency among vegetarian adolescents has been investigated in only two studies, in both of which the participants were classified as vegans, with deficiency ranging from approximately 4% to 10%.[20,39] While vegans, who do not consume any animal source foods, are at higher risk of developing a vitamin B-12 deficiency, the majority of vegetarians in the present study were not classified as such, and many were consuming foods that naturally contain vitamin B-12 such as eggs, milk, yoghurt, and cheese (data not shown, see Peddie et al[40]). In addition, a substantially higher proportion of vegetarian participants (30%) were consuming vitamin B-12-containing supplements compared to non-vegetarian participants (12%). It should also be highlighted that the present study used serum concentrations of B-12 as a biomarker of status, rather than serum holo-transcobalamin or methylmalonic acid, which are said to be more sensitive and specific biomarkers of vitamin B-12 status.[33] Moreover, the use of more than one vitamin B-12 biomarker has been recommended, with further validation work on vitamin B-12 biomarker cut-off values needed to improve identification of deficiency. For example, the cut-off value of serum B-12 (< 148 pmol/L) used in the present study is lower than those used elsewhere (ranging from < 200 to 260 pmol/L)[33] and thus, number of individuals with a vitamin B-12 deficiency in the current sample may have been underestimated.

While the concentration of serum zinc did not differ significantly between the two diet groups in the present study, the prevalence of deficiency was significantly higher in vegetarian participants (50%) compared to non-vegetarian participants (20%). In a similar study conducted among Canadian adolescent women, the prevalence of low serum zinc concentrations tended to be higher in adolescents excluding either red meat, poultry, fish, or red meat alone, compared to their omnivorous counterparts.[21] Similarly, the high prevalence of zinc deficiency reported among both vegetarian and non-vegetarian participants in the present study is also quite concerning, particularly given that zinc requirements peak during adolescence to support pubertal growth.[41] The last national nutrition survey of New Zealand

children aged 5-15 years conducted nearly two decades ago reported a much lower prevalence of zinc deficiency (i.e., 14%).[42,43] Low serum zinc status may be attributable to inadequate intakes of readily available zinc from animal source foods in both dietary groups. Further investigation into the total amount of zinc in the diet, including the intake of enhancers and antagonists of zinc absorption,44 is needed to better understand zinc nutriture in this sample population.

Mean serum selenium concentrations were significantly lower among vegetarian participants, resulting in a significantly higher proportion of vegetarians classified with low selenium status (11%) compared to non-vegetarian participants (2%) using a cut-off said to ensure optimal activity of iodothyronine 5'deiodinase, a selenoenzyme associated with thyroid function.[31] To our knowledge, this is the first time that selenium status has been determined in a sample of adolescent vegetarians. Soil in New Zealand is relatively low in selenium; however poultry and animals are routinely supplemented with selenium through the top-dressing of pastures as well as inorganic supplements, and the use of high selenium meal for poultry feed.[45] Hence, the higher intake of animal source foods among non-vegetarian participants likely contributed to adequate intake and improved status compared to their vegetarian participants. Research in New Zealand has also consistently shown large geographical variation in soil selenium, and consequently differences in selenium intakes and status from the North Island to the South Island of the country. In particular, selenium concentrations in bread are known to differ significantly related to the greater use of higher selenium containing Australian wheat in the North Island in comparison to locally grown wheat in the South Island. Unsurprisingly, we observed a difference in selenium status based on participant location, and hence this factor was considered when comparing the selenium concentrations of vegetarian and non-vegetarians in the final analysis.

The concentrations of serum folate among both diet groups were relatively high compared to cut-off values, although vegetarians had significantly higher status compared to non-vegetarians. Consistent with our findings, higher concentrations of serum folate in vegetarian populations have been previously reported in adolescents[39] and adult women,[46,47] although, one recent study of German adolescents contradicts these findings.[48] The association between a vegetarian dietary pattern and increased serum folate may be explained by higher consumption of folate-rich foods that are synonymous with a vegetarian dietary pattern such as green leafy vegetables and legumes.49 While the amount of dietary folate intake is unknown, consumption of synthetic folic acid via voluntary fortified foods and/or

supplements were also likely contributing to adequate folate status, particularly the higher concentrations of serum folate concentrations observed among vegetarian participants.

Lastly, we also observed that vegetarians had slightly lower concentrations of 25-hydroxyvitamin D when compared to non-vegetarians, although again this difference was not statistically significant. Notably, the prevalence of low concentrations of 25-hydroxyvitamin D was high in both groups. Approximately half of the sample population had serum vitamin D concentrations less than 50 nmol/L. Moreover, we did observe differences in concentrations of 25-hydroxyvitamin D based on seasonal timing of blood collection, with the highest concentrations reported for those recruited late summer and autumn. The inclusion of location in the statistical modelling only slightly attenuated differences in 25-hydroxyvitamin D between vegetarians and non-vegetarians (unadjusted model not shown). Similar to folate, vitamin D fortification of foods is voluntary in New Zealand. Very few food products are fortified with vitamin D in New Zealand and universal supplementation is not recommended for children and adolescents even with low vitamin D intakes.

Several limitations should be considered when evaluating the results. The data in this study were collected from seven locations across New Zealand, however, the results are limited by the fact that the sample is not nationally representative (it is less ethnically diverse and slightly less socioeconomically deprived that the general New Zealand population) The study included a diverse group of self-identified vegetarians with varied dietary patterns. Future studies should examine micronutrient status and risk of insufficiency in vegetarian subgroups such as vegans and lacto-ovo-vegetarians. In addition, the study did not gather data on participants' adherence or duration of their dietary patterns, which could affect the outcomes and conclusions. The study also did not collect data on oral contraceptive use, which can lower serum zinc concentrations.[49] Future studies should consider collecting information about oral contraceptive use in this age group. The sample size was too small to detect, to statistical significance of $p<0.05$, potentially meaningful differences between the groups. However, the dependence on *p*-values for inference is not recommended.[50] Sampling bias may be present due to convenience sampling methods and targeted recruitment of vegetarians. Although data were collected throughout the country the sample is not representative, and this may limit the generalisability of results to the wider population of adolescent women.

In conclusion, this study provides valuable information on the micronutrient status of adolescent women in New Zealand, including those adopting contemporary dietary habits. Results from this study indicate that those identifying as being a vegetarian may be at increased risk of both selenium and zinc deficiency and suboptimal iron status; micronutrients

commonly found in animal foods. Future research should aim to examine the long-term impact of following a vegetarian dietary pattern on health and should consider the possibility that supplements may play a protective role against low micronutrient status in female adolescent vegetarians. However, it remains important that vegetarian adolescent women follow dietary practices that enhance both intake and bioavailability of micronutrients to reduce the risk of micronutrient deficiencies. Potential interventions include dietary diversification to enhance non-haem iron absorption (particularly by including foods containing vitamin C), reduction in intake of inhibitors of iron and zinc absorption (particularly by using preparation methods that reduce phytate in foods), increase in selenium intake (for example Brazil nuts are a particularly useful vegetarian source of selenium),[51] and monitoring at risk micronutrient status of vegetarians to ensure deficiencies are avoided.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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Table 1. Demographic characteristics (n=182)

	Non-vegetarians (n=155)	Self-identified vegetarians (n=27)	*p*-value[†]
Age, mean (SD) years	16.7 (0.9)	17.1 (0.9)	0.031
Māori ethnicity, n (%)[‡]	23 (14.8)	4 (14.8)	>0.999
Household level deprivation, n (%)[§]			0.529
1-3 (Low)	61 (39.6)	14 (51.9)	
4-7	66 (42.9)	9 (33.3)	
8-10 (High)	27 (17.5)	4 (14.8)	
BMI z-score, mean (SD)[¶]	0.77 (0.95)	0.37 (0.80)	0.042
Menarche, n (%)	155 (100)	26 (96.3)	0.148
Micronutrient supplements in the last month, n (%)			
Iron	21 (13.6)	9 (33.3)	0.021
Zinc	19 (12.3)	4 (14.8)	0.754
Seleniun	16 (10.3)	3 (11.1)	>0.999
Vitamin A	13 (8.4)	3 (11.1)	0.711
Folate	19 (12.3)	5 (18.5)	0.363
Vitamin B-12	19 (12.3)	8 (29.6)	0.035
Vitamin D	15 (9.7)	3 (11.1)	0.734

[†]p-value determined by t-test for continuous variables and Fisher's exact test for categorical variables.
[‡]Six participants identified as "Pacific" and seven identified as "Asian" – none of these identified as a vegetarian; the remaining ethnicity category was "New Zealand European and Others".
[§]Household level deprivation determined by NZ Deprivation Index deciles. The NZ Deprivation index is an area-based measure of scioeconomic deprivation in NewZealand based on 9 variables including hoursehold income, employment, house ownership and qualifications.
[¶]BMI z-score determined using WHO growth reference data. Four non-vegetarians were missing BMI data.

Table 2. Differences in biomarkers between self-identified vegetarian and non-vegetarian women adolescents (n=182)

	Geometric mean (95% CI)[†]		Mean percent difference (95% CI)[‡]	p-value
	Non-vegetarian (n=155[§])	Self-identified vegetarian (n=27[§])		
Haemoglobin, g/L	134 (133, 135)	130 (126, 134)	-3.1 (-5.8, -0.4)	0.025
Serum ferritin[¶], µg/L	39.8 (35.6, 44.5)	32.3 (24.0, 43.4)	-18.9 (-39.4, 8.7)	0.159
Soluble transferrin receptor[¶], mg/L	4.59 (4.43, 4.74)	4.94 (4.43, 5.50)	7.7 (-1.8, 18.1)	0.114
Total body iron, mg/kg	3.64 (2.72)	2.81 (3.15)	-0.83 (-1.97, 0.32)	0.157
Zinc[¶], µmol/L	11.0 (10.8, 11.2)	10.7 (10.0, 11.4)	-3.1 (-8.1, 2.1)	0.240
Selenium[††], µmol/L	1.14 (1.12, 1.17)	1.04 (0.93, 1.15)	-8.3 (-14.1, -2.1)	0.009
Retinol binding protein[¶], µmol/L	1.60 (1.51, 1.68)	1.45 (1.26, 1.67)	-9.1 (-21.1, 4.7)	0.183
Serum folate, nmol/L	18.5 (17.1, 20.1)	33.4 (28.8, 38.8)	80.5 (45.7, 123.7)	<0.001
Serum B-12[¶], pmol/L	466 (430, 505)	381 (306, 475)	-18.2 (-33.7, 0.9)	0.060
Serum Vitamin D, nmol/L[‡‡]	34.2 (32.2, 36.3)	27.1 (22.7, 32.3)	-13.8(-26.6, 1.2)	0.070
PTH, pmol/L[‡‡]	24.3 (22.8, 25.9)	31.3 (27.6, 35.5)	14.4 (-4.0, 36.3)	0.132

[†]All presented at geometric means (95% CI), except for total body iron which is presented as mean (SD).
[‡]School cluster, nested within data collection phase included in this model as a random effect to account for location and season of data collection .
[§]n=130 non-vegetarians and n=20 participants had serum folate data; one vegetarian was missing zinc and selenium data
[¶]Adjusted for inflammation using the BRINDA method.
[††]Location (North or South Island) included in the model as a random effect to account for known differences in status based on geography.
[‡‡]School cluster, nested within data collection phase included in this model as a random effect to account for location and season of data collection.

Table 3. Proportion of vegetarians and non-vegetarians with abnormal biomarker status (n=182)

Cut-points for abnormal status	Non-vegetarians (n=155) n (%)	Self-identified vegetarians (n=27) n (%)	p-value[†]
Anaemia	8 (5.2)	4 (14.8)	0.082
Hb < 120 g/L			
Iron depletion			
Ferritin <15 µg/L	15 (9.7)	4 (14.8)	0.492
Iron deficiency anaemia			
Hb < 120 g/L and Ferritin < 15 µg/L	3 (1.9)	2 (7.4)	0.160
Soluble transferrin receptor			
>5.3 mg/L	32 (20.7)	12 (44.4)	0.013
Total Body iron (TBI)			
<0 mg/kg	17 (11.0)	5 (18.5)	0.333
Low zinc status[‡]			
<10.09 µmol/L	31 (20.0)	13 (50.0)	0.002
Low selenium status[‡]			
<0.82 µmol/L	3 (1.9)	3 (11.5)	0.039
Low serum folate[‡]			
< 6.8 nmol/L	1 (0.8)	0	>0.999
Low serum B-12			
< 148 pmol/L	2 (1.3)	1 (3.7)	0.384
Vitamin D			0.229
Insufficient			
30-50 nmol/L	85 (55.2)	13 (48.2)	
Deficient			
<30 nmol/L	44 (28.6)	12 (44.4)	

[†]p-values for difference between self-identified vegetarians and non-vegetarians using a Fisher's exact test.
[‡]n=130 non-vegetarians and n=20 participants had serum folate data; one vegetarian was missing zinc and selenium data.

Supplementary Table 1. Proportion of vegetarians and non-vegetarians with abnormal biomarker status in those not taking micronutrient supplements (n=182)

	Supplement	Number of non-vegetarians not taking supplement	Number of vegetarians not taking supplement
Haemoglobin, g/L	Any micronutrient supplement	131	16
Serum ferritin, µg/L‡	Iron	134	18
Soluble transferrin receptor, mg/L‡	Iron	134	18
Total body iron, mg/kg	Iron	134	18
Zinc, µmol/L‡	Zinc	136	22
Selenium, µg/L	Selenium	139	23
Retinol binding protein, mmol/L‡	Vitamin A	142	24
Serum folate, nmol/L	Folic acid	112	16
Serum B-12, pmol/L‡	Vitamin B-12	136	19
Serum Vitamin D, nmol/L§	Vitamin D	15	3
PTH, pmol/L§	Vitamin D	15	3

	Mean percent difference (95% CI)†	p-value
Haemoglobin, g/L	-4.1 (-7.5, -0.5)	0.025
Serum ferritin, µg/L‡	-31.1 (-52.1, -1.0)	0.044
Soluble transferrin receptor, mg/L‡	13.2 (1.1, 26.6)	0.031
Total body iron, mg/kg	-1.6 (-3.0, -0.2)	0.029
Zinc, µmol/L‡	-3.6 (-8.9, 2.0)	0.202
Selenium, µg/L	-12.4 (-18.1, -6.4)	<0.001
Retinol binding protein, mmol/L‡	-11.5 (-23.4, 2.2)	0.097
Serum folate, nmol/L	77.4 (40.7, 123.7)	<0.001
Serum B-12, pmol/L‡	-4.1 (-7.5, -0.5)	0.025
Serum Vitamin D, nmol/L§	-12.8 (-26.4, 3.4)	0.115
PTH, pmol/L§	14.3 (-5.3, 37.8)	0.163

†Mean differences (95% CI) estimated with a linear regression model using log-transformed values and back-transformed to present the difference in terms of a percent difference for all except for total body iron which is presented as an absolute difference.
‡Adjusted for inflammation using the BRINDA method
§School clusters, which vary by location and season of data collection, were accounted for with a random effect.

Supplementary Table 2. Proportion of vegetarians and non-vegetarians with abnormal biomarker status in those not taking micronutrient supplements (n=182)

Cut-points for abnormal status	Supplement	Non-vegetarians		Self-identified vegetarians		p-value[†]
		Number not taking supplement	Abnormal status n (%)	Number not taking supplement	Abnormal status n (%)	
Anaemia						
Hb < 120 g/L	Any micronutrient supplement	131	7 (5.3)	16	3 (18.8)	0.079
Iron depletion						
Ferritin <15 µg/L	Iron	134	13 (9.7)	18	4 (22.2)	0.121
Iron deficiency anaemia						
Hb < 120 g/L and Ferritin < 15 µg/L	Iron	134	2 (1.5)	18	2 (11.1)	0.069
Soluble transferrin receptor						
<5.3 mg/L	Iron	134	26 (19.4)	18	8 (44.4)	0.031
Low zinc status[‡]						
<10.09 µmol/L	Zinc	136	25 (18.4)	22	11 (50.0)	0.002
Low selenium status[‡]						
< 0.82 µmol/L	Selenium	139	3 (2.2)	23	3 (13.0)	0.038
Low serum folate[‡]						
< 6.8 nmol/L	Folate	112	0	16	0	-
Low serum B-12						
< 148 pmol/L	Vitamin B-12	136	2 (1.5)	19	0	>0.999
Vitamin D						
Insufficiency						
30-50 nmol/L	Vit D	140	75 (54.0)	24	13 (54.2)	
Deficicency						
<30 nmol/L	Vit D	140	42 (30.2)	24	10 (41.7)	

[†]p-values for difference between self-identified vegetarians and to non-vegetarians using a Fisher's exact test.
[‡]n=130 non-vegetarians and n=20 participants had serum folate data; one vegetarian was missing zinc and selenium data.