Dietary factors play a critical role in human health. The aim of this cross-sectional study was to examine micronutrient intake and status of subjects who were habitual meat eaters eating different quantities of meat with those who were habitual vegetarians or vegans. One hundred and thirty-nine healthy male subjects (vegan, n = 18; ovolacto-vegetarian, n = 46; moderate meat-eater, n = 65; and high meat-eater, n = 18) aged 20–55 years were recruited in metropolitan Melbourne. Each volunteer completed a semiquantitative Food Frequency Questionnaire (FFQ) and gave a fasting venous blood sample. Dietary sodium/potassium ratio was significantly lower and vitamin C, fibre and iron intakes were higher in vegetarians than in meat-eaters. High meat-eaters had a significantly higher calcium, retinol and zinc intake than did the other three dietary groups; moderate meat-eaters had the lowest mean intake of fibre, vitamin C and β-carotene. Vegans had a significantly higher β-carotene intake than did the other groups. Serum ferritin and vitamin B12 levels, and haemoglobin concentration were significantly lower in vegetarians than in meat-eaters. Vegans had a significantly higher serum folate concentration than did ovolacto-vegetarian and moderate meat-eater groups. There was no significant difference in serum α-tocopherol concentration. There are differences between the four diet groups that have potential to affect the subjects’ health and susceptibility to chronic diseases including cardiovascular disease and cancer. Based on the present data, high meat-eaters may particularly benefit from altering their dietary pattern to reduce their sodium and saturated fat intake, and moderate meat-eaters from increasing their fibre and antioxidant consumption. Vegetarians, especially vegans, may need to increase their vitamin B12 and zinc intakes.

Key words: meat, Melbourne, minerals, trace elements, vegetarian diet, vitamins.

Introduction
Dietary factors, together with other lifestyle factors such as cigarette smoking, exercise and alcohol consumption, play an important role in determining long-term health. Meat intake has decreased in many Western countries over the last 10 years, and vegetarian diets have become increasingly popular for health, philosophical, ecological and religious reasons. In Australia, in the 1995/6 National Nutrition Survey, the self-reported prevalence of vegetarianism was just under 3% in men, but a number of men continue to have high meat intakes.1 Compared with omnivores, vegetarians have been reported to have a lower incidence of a number of chronic diseases, such as coronary heart disease (CHD), diabetes, osteoporosis, arthritis, kidney stones and some cancers, and generally have lower blood pressure and body mass index (BMI), as reviewed by Dwyer et al.2 As vegetarians tend to differ from omnivores in other behavioural patterns as well as diet, namely, fewer smoke and more participate in exercise, it is difficult to delineate which aspects of the vegetarian diet and lifestyle are protective.3 However, the dietary differences seen in several studies of vegetarians indicate that on average they have a lower fat and sodium intake and a higher fibre and antioxidant intake, all of which may reduce the risk of a range of chronic diseases, including cardiovascular disease and cancer.4 The influence of the meat intake per se is less clear, and many studies do not document the level of intake.

A large epidemiological study found that fibre intake, independent of fat intake, is an important dietary component for the prevention of coronary disease.5 Dietary fibre intake has been reported to be protective against breast cancer and colon cancer.6,7 Antioxidant vitamins play a critical role in the protection from free radical damage in the body, which, among other effects, may cause oxidation of low density lipoprotein cholesterol thought to be involved in the mechanism of atherosclerosis.8 Plasma antioxidant vitamin concentration is a reflection of antioxidant status and is associated with cardiovascular disease in international comparisons and in population groups.9 A low concentration of vitamin E has been associated with an increased risk of CHD, with plasma vitamin E (α-tocopherol) being the strongest predictor of the...
vitamins assessed.\textsuperscript{10–12} Although epidemiological study results are not entirely consistent, numerous studies reported an inverse relationship between antioxidant vitamin intake and several types of cancer, as reviewed by Patterson \textit{et al.}\textsuperscript{13}

Although the vegetarian diet may provide some benefits, this does not necessarily mean it provides the optimum balanced dietary intake, and there has been concern whether vegetarians, and particularly vegans, might have an inadequate intake of a number of micronutrients.\textsuperscript{2} The aim of this cross-sectional study was to examine nutrient intake and status of subjects who were habitual meat eaters with either a high or modest intake and subjects who were habitual vegetarians; this study also specifically included a group of vegans, who are not included or differentiated in most other studies. A focus was on micronutrients likely to be relevant in influencing cardiovascular and cancer risk, with examination of nutrient status by blood analysis for nutrients where it is difficult to assess dietary intake because of the lack of Australian food analysis data, namely, folate, B\textsubscript{12} and α-tocopherol.

**Methods**

**Subjects**

This project was approved by the Human Research Ethics Committee of RMIT University, and all subjects gave written informed consent. A total of 147 healthy, male non-smokers aged between 20 and 50 years were recruited through advertisements in University newsletters and local newspapers. The exclusion criteria for this study were evidence of CVD, hypertension, renal disease, hyperlipidaemia, haematological disorders, diabetes, family history of CVD, excess alcohol intake and drug therapy. According to their habitual dietary intake (based on the semiquantitative Food Frequency Questionnaire (FFQ)), the subjects were divided into vegan (\(n = 18\)), ovolacto-vegetarian (OV) (\(n = 46\)), moderate meat-eater (\(n = 65\)) and high meat-eater (\(n = 18\)) groups. A vegan was defined as someone who ate no meat and eggs, and dairy products less than six times per year. An OV was defined as someone who ate meat less than six times per year but consumed eggs and dairy products. A high meat-eater was defined as someone who consumed \(\geq 285\) g meat/day and a moderate meat-eater as someone who consumed < \(285\) g meat/day (raw weight). For the subjects to be classified into these categories, they had to have been practising their diets for at least 6 months prior to the study.

The dietary intake data of each subject was assessed using a semiquantitative FFQ. It was administered by a nutritionist for at least 6 months prior to the study. One hundred and forty-seven healthy men completed the study, although eight were excluded from the final analysis due to hypertension, dietary supplement use, reported energy intakes below the calculated basal metabolic rate and evidence of family history of CVD. Thus, 139 subjects (18 high meat-eaters, 60 moderate meat-eaters, 43 OV and 18 vegans) were included in the final results.

**Laboratory analyses**

A full blood count was performed on a Coulter STKR (Coulter Electronics Inc, Hialeah, USA).

Serum alpha-tocopherol and retinol concentration was measured by high performance liquid chromatography (HPLC). Serum (300 μL) was deproteinized with 300 μL ethanol containing 0.2 g/L of all-rac-α-tocopherol acetate as the internal standard. After extraction with 300 μL of petroleum ether and evaporation of the solvent, the residue was reconstituted in 60 μL of acetonitrile : dichloromethane : methanol (7 : 2 : 1). Concentration of α-tocopherol was resolved by the reverse phase HPLC (Model LC-10 AD, Shimadzu, Kyoto, Japan) and quantified by spectrofluorometric detection as published by Catignani and Bieri.\textsuperscript{15}

Serum ferritin was measured by a two-site chemiluminescent method on a Ciba Corning Chemiluminescence Systems Analyser (ACS:180, Australian Diagnostics Corporation Ltd, Scoresby, VIC, Australia) using a commercially available kit (Ciba Corning Diagnostics Corporation, Scoresby, Victoria, Australia).

Serum vitamin B\textsubscript{12} and folate were determined by immunochemiluminometric assay (ICMA) method on the ACS 180 using commercially available kits (Ciba Corning Diagnostics Corp., Medfield, MA, USA) as described elsewhere.\textsuperscript{16}

**Statistical analyses**

The data analyses were performed using a StatView software program (Abacus Concepts Inc., Berkeley, CA, USA). Descriptive statistics were initially performed. Analysis of variance was used to establish if differences existed between the dietary periods for each parameter. If a significant difference was found, a further multiple comparison test was performed using Fisher’s PLSD post-hoc tests to determine differences between each pair of dietary groups. The values are reported as mean ± SD in all the result tables unless otherwise specified. \(P\)-values were two-tailed and \(P < 0.05\) was considered as significant. Comparison of the number of subjects in each group with values below the Australian Recommended Daily Intakes (RDI)\textsuperscript{17} were made using multiple \(\chi^2\) tests or Fisher’s exact test.

**Results**

One hundred and forty-seven healthy men completed the study, although eight were excluded from the final analysis due to hypertension, dietary supplement use, reported energy intakes below the calculated basal metabolic rate and evidence of family history of CVD. Thus, 139 subjects (18 high meat-eaters, 60 moderate meat-eaters, 43 OV and 18 vegans) were included in the final results.

**Physiological characteristics**

Table 1 shows the characteristics of the four dietary groups. The median ages (range) were 34.5 years (21–50 years) for the high meat-eater group, 38 years (21–55 years) for the moderate meat-eater group, 31 years (22–54 years) for the OV group and 31 years (22–50 years) for the vegan group. The mean BMI was significantly greater in the high and moderate meat-eater groups than in both the OV and vegan groups. There was a decreasing trend in waist : hip ratio and
diastolic blood pressure from both high and moderate meat-eater groups to OV and to vegans.

**Dietary intake**

High meat-eaters had a significantly higher energy intake than did the other three dietary groups (Table 2). However, the energy sources were quite different in the four dietary groups. Protein, total fat, saturated and monounsaturated fat (MUFA), as percentage of energy, showed a significant decreasing trend from high meat-eaters to moderate meat-eaters to OV and vegans. However, polyunsaturated fat (PUFA) and carbohydrate intake showed the opposite trend.

In vegans the fat was derived mostly from seeds, nuts, avocado and vegetable oils. Fibre and vitamin C intakes showed a significant increasing trend from the meat-eating groups to the OV to the vegans. Retinol and sodium intake showed a significant decreasing trend from the high meat-eaters to moderate meat-eaters to OV and vegans. Dietary iron and potassium intake were significantly lower in the moderate meat-eater group than in both the OV and vegan groups. Vitamin C and β-carotene were substantially higher in the vegan group than in the other three dietary groups.

**Haematological and biochemical parameters**

(Table 4): Both the high and moderate meat-eating groups had significantly higher haemoglobin (Hgb), serum vitamin B12, ferritin and retinol concentrations compared with both OV and vegan groups. The mean serum ferritin concentrations of OV and vegan groups were only 30% of the high meat-eater group and 45% of the moderate meat-eater group. No OV or vegan had a serum ferritin > 200 ng/mL compared with five and 12 in the high and moderate meat-eating groups, respectively ($\chi^2 = 15.1, P < 0.002$). There was a significant trend for the concentration of serum retinol to decrease from both the high and the moderate meat-eater groups to the OV to the vegan groups. The vegans had a significantly higher serum

<table>
<thead>
<tr>
<th>Table 1. Characteristics of subjects in the dietary groups (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High meat</strong> (n = 18)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
</tr>
<tr>
<td><strong>Waist/hip</strong></td>
</tr>
<tr>
<td><strong>Systolic BP</strong></td>
</tr>
<tr>
<td><strong>Diastolic BP</strong></td>
</tr>
</tbody>
</table>

**Table 2. Daily nutrient intakes of the four dietary groups (mean ± SD)**

<table>
<thead>
<tr>
<th><strong>Energy (MJ)</strong></th>
<th><strong>High meat</strong> (n = 18)</th>
<th><strong>Moderate meat</strong> (n = 60)</th>
<th><strong>Ovolacto-vegetarian</strong> (n = 43)</th>
<th><strong>Vegan</strong> (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%E)</td>
<td>19.4 ± 1.3</td>
<td>17.9 ± 2.2 bmb</td>
<td>14.7 ± 2.2 boc, moc</td>
<td>14.1 ± 2.4 bovc, mvc</td>
</tr>
<tr>
<td>Carbohydrate (% E)</td>
<td>40.3 ± 4.4</td>
<td>45.7 ± 6.8 bmb</td>
<td>50.9 ± 6.4 bovc, moc</td>
<td>57.4 ± 5.4 bovc, mvc</td>
</tr>
<tr>
<td>Total fat (%E)</td>
<td>37.8 ± 4.2</td>
<td>32.8 ± 6.1 bmb</td>
<td>32.7 ± 6.2 bohc</td>
<td>28.2 ± 4.3 bovc, mvc, obv</td>
</tr>
<tr>
<td>SFA (%E)</td>
<td>17.4 ± 2.7</td>
<td>14.3 ± 3.2 bmc</td>
<td>11.9 ± 4.0 bohc, moc</td>
<td>6.6 ± 1.6 bovc, mvc</td>
</tr>
<tr>
<td>MUFA (%E)</td>
<td>14.9 ± 1.4</td>
<td>13.0 ± 2.8 bma</td>
<td>12.9 ± 3.2 bohc</td>
<td>11.7 ± 2.7 bovc</td>
</tr>
<tr>
<td>PUFA (%E)</td>
<td>5.6 ± 1.4</td>
<td>5.6 ± 2.3</td>
<td>7.9 ± 2.5 bohc, moc</td>
<td>9.8 ± 3.1 bovc, mvc, obv</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>42.7 ± 12.2</td>
<td>33.0 ± 10.4 bma</td>
<td>54.2 ± 17.5 bohc, moc</td>
<td>76.8 ± 23.2 bovc, mvc, obv</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>251 ± 108</td>
<td>208 ± 146</td>
<td>276 ± 140 bohc, moc</td>
<td>441 ± 186 bovc, mvc, obv</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>5850 ± 1710</td>
<td>3623 ± 951 bmb</td>
<td>3138 ± 1080 bohc, moc</td>
<td>2721 ± 1225 bovc, obv</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>5855 ± 1529</td>
<td>4126 ± 1038 bmb</td>
<td>5014 ± 1411 bohc, moc</td>
<td>5959 ± 1580 bovc, mvc</td>
</tr>
<tr>
<td>Na/K</td>
<td>0.78 ± 0.20</td>
<td>0.77 ± 0.23</td>
<td>0.66 ± 0.25 bohc, moc</td>
<td>0.46 ± 0.17 bovc, mvc, obv</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>24.4 ± 5.3</td>
<td>16.8 ± 3.9 bmc</td>
<td>20.5 ± 5.6 bohc, moc</td>
<td>25.7 ± 9.5 bovc, mvc, obv</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1577 ± 558</td>
<td>1201 ± 360 bmb</td>
<td>1229 ± 459 bohc</td>
<td>977 ± 362 bovc, mvc</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>25.0 ± 5.3</td>
<td>14.6 ± 3.0 bmc</td>
<td>12.3 ± 3.1 bohc, moc</td>
<td>13.4 ± 4.0 bovc</td>
</tr>
<tr>
<td>Retinol (µg)</td>
<td>1640 ± 2210</td>
<td>761 ± 776 bmb</td>
<td>438 ± 216 bohc</td>
<td>218 ± 144 bovc, mva</td>
</tr>
<tr>
<td>β-carotene (µg)</td>
<td>7268 ± 3360</td>
<td>4599 ± 2405</td>
<td>6559 ± 4400</td>
<td>12590 ± 1582 bova, mvc, obv</td>
</tr>
</tbody>
</table>

%E, % of energy; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. aP < 0.05; bP < 0.01; cP < 0.001.
folate concentration than did the moderate meat-eater and the OV groups. Serum α-tocopherol levels were similar in the four dietary groups.

**Discussion**

While a number of studies have compared nutrient intakes and selected plasma/serum constituents in omnivores and vegetarians, this study is unique in that we have examined four groups (high and low meat-eaters, ovolacto-vegetarians and vegans) with widely varying habitual diets and have included more nutrients and plasma/serum variables than other studies. The use of four groups emphasizes the marked difference in nutrient intakes between the extremes of dietary behaviour (i.e. high meat vs vegan).

The validation of the semiquantitative FFQ has been investigated by many studies. All current methods for assessing dietary intake involve some systematic error and limitations. The 10-day weighed diet record does not fully represent the individual’s habitual intake. Prolonging the record period can make the accuracy of the records less reliable and induce subjects to make subconscious changes to their dietary habits. Multiple diet records, namely, four 1-week weighed food records at approximately 3-month intervals, have generally been considered the ‘gold standard’. However, this method is very time consuming, expensive and has a high withdrawal rate.

The FFQ is rapid with a low respondent burden and high response rate. The FFQ relies totally on memory and the ability to estimate serving sizes; however, it may be difficult to assess dietary intake accurately in elderly subjects due to declining memory. Despite some limitations to this method, the FFQ has become the preferred method in nutritional epidemiological studies. Fidanza et al. have developed a semiquantitative FFQ which contains photographs of different food portion sizes, and suggest that the semiquantitative FFQ can be considered to be a useful method for evaluating food intake and ranking subjects according to habitual intake of certain nutrients. Therefore, the semiquantitative FFQ was chosen in the present study.

In this study population, the fibre intake in OV and vegans was significantly higher than the mean intake of the high meat-eaters. The moderate meat-eaters had the lowest intake with the mean below the recommendation for Australians of 35 g per day. α-Tocopherol and retinol status were assessed by measuring plasma concentration because vitamin E data are not currently available for many Australian foods and vitamin A precursors and retinols in food are absorbed and metabolized differently so serum levels provide a better assessment of status. Vitamin C and β-carotene intakes were assessed by FFQ. There was no significant difference between the four dietary groups in plasma α-tocopherol concentration. However, a relatively higher proportion of moderate meat-eaters (65%) had plasma α-tocopherol concentration below the recommended values of above 5.0 µg/mL compared with 50% below this value for the other three dietary groups. Most other studies on vegetarians have not measured plasma levels to allow comparison. That of Krajcovicova-Kudlackova et al. in Slovakia showed significantly higher levels of plasma vitamin C, E and β-carotene in vegetarians than in omnivores. In the present study, all of the subjects had plasma retinol concentrations above the reference range of 0.2–0.6 µg/mL. Dietary vitamin C intake was well above the Australian RDI of 40 mg/d, as found in other studies. However, optimum intake for health benefit may exceed this RDI and the higher intake in the vegetarians and vegans may be beneficial.
higher β-carotene intake from vegetables and fruit in vegans could also provide benefits.

The significantly higher zinc intake in high meat-eaters is likely to be due to the fact that meat is a major source of zinc, and meat contributed 50 and 37% of the dietary zinc intake of high and moderate meat-eater groups, respectively. Cereals and dairy produce also contain zinc but it may be less bioavailable.28

All high meat-eaters had calcium intakes above the Australian RDI (800 mg/day), but a relatively high proportion of vegan (33%) subjects had intakes below the RDI compared with the moderate meat-eaters (13%) and OV (16%), with the difference between meat-eaters and vegetarians being significant (Fisher’s exact test, \( P < 0.04 \)). This result is consistent with the result reported by Alexander et al.22 This is because vegans do not eat dairy products, which are usually the main source of calcium in the Western diet. However, vegans may remain in calcium balance despite lower intake compared with meat-eaters, as lack of animal derived-protein and low phosphorus should reduce their urinary calcium losses.29

The INTERSALT study indicated a positive correlation between the dietary Na/K ratio and systolic and diastolic blood pressure, while cross-population data show a significant positive association between systolic blood pressure and Na/K ratio but not diastolic blood pressure.30 Law et al. in their meta-analysis found a reduction in both systolic and diastolic blood pressure with salt reduction.31 The higher diastolic blood pressure in the meat-eaters may be due to their higher sodium and Na/K intakes, although measures of discretionary Na intake may not be very reliable. However, it may also result from the fact that the meat-eaters are slightly older and heavier, as these characteristics are also known to correlate with elevated blood pressure.

All subjects had iron intakes well above the Australian RDI of 7 mg/day for men. However, 21% of OV and 11% of vegans had serum ferritin concentration below 12 µg/L, often considered as indicative of low iron stores, compared with meat-eaters, as lack of animal derived-protein and low phosphorus should reduce their urinary calcium losses.29

High dietary fibre intakes may also contribute to lower serum vitamin B12 levels in vegetarians. Fibre may block intrinsic factor and/or R proteins which are involved in vitamin B12 absorption or inhibit enterohepatic circulation of vitamin B12 by binding with it to form an unabsorbable complex in the intestine.34 Lower Hgb levels in both OV and vegan groups may be caused by lower dietary intakes of vitamin B12 and/or iron (haem iron). All subjects had a serum folate concentration in the reference range (2–14 ng/mL).35

The present results are in general agreement with previous studies but provide a greater description of meat/animal produce intake to reveal trends. They show that the dietary profiles of both OV and vegan groups are higher in fibre, antioxidants and potassium, and lower in sodium compared with both high and moderate meat-eater groups, even after adjustment for meat intake. They also reveal some differences between those with high and moderate meat intakes. Vegetarianism may provide some protective effect against CVD and some cancers. However, vegetarians, and especially vegans, may be at risk from low calcium, zinc and vitamin B12 intakes and should seek nutritional advice to optimize their intake of these nutrients.

References


