

Blood and urine acid–base status of premenopausal omnivorous and vegetarian women

BY D. BALL* AND R. J. MAUGHAN

Department of Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen AB25 2ZD

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The effect of long-term differences in diet composition on whole-body acid–base status was examined in thirty-three young healthy females. The volunteers were recruited from two separate groups matched approximately for age, height and weight; one group regularly ate meat (omnivores; n 20) and one group did not (vegetarians; n 13). All subjects completed a 7 d weighed intake of food, and from their dietary records, total energy, carbohydrate (CHO), fat and protein content were estimated using computer-based food composition tables. During this week they reported to the laboratory on two occasions, following an overnight fast and separated by at least 48 h. Arterialized venous blood samples were obtained on each visit and these were analysed for blood acid–base status. Haemoglobin and packed cell volume, serum total cholesterol and HDL-cholesterol, serum albumin and total protein were also determined. Two 24 h urine collections were completed; the volume was recorded and samples were analysed for pH, titratable acid and Mg and Ca concentration. Total energy intake of the omnivores was greater ($P = 0.0003$) than that of the vegetarian group. Dietary intake of CHO ($P = 0.024$), fat ($P = 0.0054$) and protein ($P = 0.0002$) were higher in the omnivorous group than in the vegetarians. There were no differences between the two groups with respect to blood CO_2 partial pressure, plasma HCO_3^- and blood base excess, but blood pH was slightly higher in the omnivores ($P = 0.064$). Measures of urine acid–base status suggested a lower pH in the omnivore group, but this difference was not statistically significant; a greater titratable acid output was observed with the omnivorous group compared with the vegetarians (48.9 (SE 20.3) v. 35.3 (SE 23.3) mEq/24 h; $P = 0.018$). Although the dietary intake of Ca was not different between the two groups, urinary Ca excretion of the omnivores was significantly higher (3.87 (SD 1.34) v. 3.22 (SD 1.20) mmol/24 h) than that of the vegetarians ($P = 0.014$). It is suggested that the higher protein intake of the omnivores resulted in an increase in urinary total acid excretion, which may explain the higher rate of Ca excretion.

Omnivores: Vegetarians: Acid–base status: Titratable acid output: Calcium

Short-term severe dietary manipulation is known to alter blood acid–base status (Greenhaff *et al.* 1987*a,b*). By ingesting a diet which is high in fat and protein and low in carbohydrate (CHO) it is possible to induce an acute mild metabolic acidosis. It is believed that the cause of the metabolic acidosis is an increase in circulating non-volatile weak acids resulting from the increased dietary intake of protein (Greenhaff *et al.* 1988). These studies were, however, conducted only over a 3 or 4 d period, and whether a long term alteration in acid–base status would persist if such a dietary regimen were to be maintained is unknown. A vegetarian diet can be categorized under one of three classifications: a demivegetarian diet is a vegetable diet supplemented with fish, milk, dairy products and eggs; a lacto-ovo vegetarian diet contains vegetables supplemented with milk, dairy products and eggs; a pure

* Present address: Neuromuscular Biology Group, Crewe and Alsager Faculty, Manchester Metropolitan University, Hassall Road, Alsager, ST7 2HL.

vegetarian diet, also referred to as a vegan diet, contains no animal products (Wilson *et al.* 1975; Draper *et al.* 1990). There have been several reports of differences in the composition of the diet between omnivores and vegetarians, with omnivores having a higher daily protein intake than vegetarians (Taber & Cook, 1980; Shultz & Leklem, 1983; Tylavsky & Anderson, 1988). Increases in dietary protein intake while eating a mixed diet have also been reported to exert an acidifying effect on urine (Hunt, 1956; Lutz, 1984; Breslau *et al.* 1988). This is characterized by an increase in titratable acid output (Hunt, 1956; Lutz, 1984; Breslau *et al.* 1988) and in some cases by a decrease in urinary pH (Hunt, 1956; Kurtz *et al.* 1983).

Sherman & Gettler (1912) suggested that the acidity or alkalinity of the residual ash of dietary components following combustion should allow assumptions to be made concerning the effect of diet on urine acid–base status. The food composition tables from which these assumptions were made had been used, without question, for almost 75 years. Dwyer *et al.* (1985) re-assessed the validity of the assumptions of Sherman & Gettler (1912) using data based on current food composition tables. The alkaline- and acid-ash contents of the three different diets were calculated from data derived from several different food composition tables (cf. Dwyer *et al.* 1985). It was reported that vegetarian and vegan diets had a higher alkaline ash than omnivore diets but that the estimates were not sufficiently reliable to allow assumptions to be made concerning urinary pH (Dwyer *et al.* 1985).

To our knowledge the blood and urine acid–base status of vegetarians has not been investigated. The present study was designed to compare two groups of individuals who had long-term differences in dietary habits, vegetarian and omnivorous, and the effect of those diets on whole-body acid–base status.

MATERIALS AND METHODS

Subjects

Thirty-three healthy females volunteered to participate in the present study. Their age, height, weight and estimated percentage body fat are given in Table 1. Of this total group of females, twenty regularly ate meat whilst the remaining thirteen were classified as vegetarians (i. e. they consumed some dairy products but no meat). Although two of the vegetarian subjects occasionally ate fish, neither did so during the week of the study. The study had been approved by the local ethics committee and each subject provided written informed consent before participating in the study, after having been given a full explanation of all experimental procedures and of the need to adhere to the stringent experimental criteria.

Experimental protocol

All subjects weighed and recorded their intake of food (including drinks) for a period of seven consecutive days; this included all alcohol consumed and in addition any vitamin supplements and medication that they were taking. From these records their daily intake of food was assessed using the computerized microdiet system based on McCance and Widdowson's food composition tables (Paul & Southgate, 1983). Subsequent analysis was carried out to determine the contribution of the macronutrients to the diet as a percentage of total energy intake.

Table 1. *Physical characteristics and daily dietary intake of the omnivorous and vegetarian women participating in the study*

(Mean values and standard deviations)

| | Omnivores (n 20) | | Vegetarians (n 13) | | Statistical significance of difference between groups: P |
|-----------------------|------------------|------|--------------------|------|---|
| | Mean | SD | Mean | SD | |
| Age (years) | 23 | 5 | 24 | 5 | 0.24 |
| Height (m) | 1.66 | 0.06 | 1.64 | 0.08 | 0.58 |
| Wt (kg) | 60.9 | 4.5 | 57.7 | 2.1 | 0.71 |
| Body fat (%) | 25.9 | 3.5 | 25.0 | 5.0 | 0.97 |
| Energy intake (MJ) | 9.1 | 1.5 | 7.2 | 1.1 | 0.0003 |
| Protein (g) | 69.9 | 8.8 | 55.1 | 10.0 | 0.0002 |
| Fat (g) | 88.3 | 18.0 | 65.4 | 22.2 | 0.0054 |
| CHO (g) | 279 | 69.1 | 227 | 26.1 | 0.024 |
| Alcohol (g) | 5.9 | 6.6 | 7.9 | 6.7 | 0.51 |
| Protein (% of energy) | 12.9 | | 12.8 | | 0.60 |
| Fat (% of energy) | 36.6 | | 34.1 | | 0.16 |
| CHO (% of energy) | 48.2 | | 49.5 | | 0.31 |
| Alcohol (% of energy) | 2.3 | | 3.6 | | 0.16 |

Body composition

On their first visit to the laboratory each subject's height and weight were measured. An estimation of their percentage body fat was made from the sum of four skinfold measurements (triceps, biceps, supra-iliac and subscapular) using the procedures and the equations described by Durnin & Womersley (1974).

Blood sample collection and analysis

On two separate occasions during the week of their weighed food intake, subjects were asked to report to the laboratory in the morning after an overnight fast. An arterialized venous blood sample (Forster *et al.* 1972) was taken from a superficial vein on the dorsal part of the heated hand; before the sample was collected, subjects rested for at least 15 min in a seated position with the hand immersed in water maintained at a temperature of 42°. A sample (2.5 ml) of blood was collected anaerobically in a heparinized syringe, capped and kept on iced water and analysed within 2 h for pH, partial pressure (p) for CO₂ and pO₂, using a Radiometer BMS3 MK2 blood gas analyser (Radiometer, Copenhagen, Denmark). It should be noted that the use of arterialized venous blood will consistently overestimate pCO₂, by approximately 0.13 kPa, and underestimate pH, by 0.005 units, compared with values observed from arterial blood (Forster *et al.* 1972). Plasma bicarbonate (HCO₃⁻) and blood base excess (BE) values were calculated according to Siggaard-Andersen (1963).

A further 10 ml blood sample was collected: of this, one portion (2.5 ml) was mixed with K₃EDTA (1 mg/ml). This sample was used to determine haemoglobin concentration by the cyanomethaemoglobin technique (Drabkin & Austin, 1935) and packed cell volume by the microcapillary centrifugation method (Hawksley, Lancing, Sussex). Erythrocyte and leucocyte counts were measured using a Coulter Counter (Coulter Electronics, Dunstable, Beds.). The remaining 7.5 ml blood was put into a plain tube and allowed to clot; the serum was then separated by centrifugation and analysed for total cholesterol and HDL-cholesterol content (kit no. 07 1106 3; Roche Diagnostics, Welwyn Garden City, Herts.),

total protein concentration (Boehringer Corp, Lewes, Sussex) and serum albumin (kit no. 07 1472 0; Roche Diagnostics).

Urine samples

Subjects were asked to provide two 24 h urine samples during the week of their weighed intake. Each collection began in the morning on the day before the blood sampling and finished on the morning of the blood sample. Urine was collected in plain containers. The volume of urine was measured using a 1 litre measuring cylinder and then thoroughly mixed in order to re-suspend any precipitate. The pH of the urine sample was measured using a Russell electrode (type CTWL; Russell Electrodes, Fife) and a Corning 140 pH meter (Corning Science Products, Halstead, Essex). Total acid content was determined by titration using the method described by Jorgensen (1957). Two 20 ml portions were stored: one of these was acidified with 1M-HCl to a pH below 3 and both were frozen. The samples were later analysed for Ca (kit no. 07 1028 8; Roche Diagnostics) and Mg concentration (kit no. E36742; Ciba-Corning, Halstead, Essex).

Statistical analysis

The CV was calculated for several analytical methods from duplicate measurements; blood pH n 50, CV 0.0%; pCO₂ n 50, CV 1.6%; blood haemoglobin n 50, CV 0.7%; plasma protein n 50, CV 1.1%; urine pH n 55, CV 0.3%; urine titratable acid n 40, CV 2.1%, urine Ca n 55, CV 1.7%, urine Mg n 55, CV 2.7%. Before performing any inferential statistical test, data were checked for normality of distribution. Statistical analysis was by Student's t test for paired and unpaired data as appropriate. Data are expressed as means and standard deviations except in Fig.1 in which, for reasons of clarity, values are expressed as means with their standard errors.

RESULTS

Anthropometric characteristics

Statistical analysis of the physical characteristics of the two groups did not reveal any differences between them with respect to height, weight, or percentage body fat (Table 1).

Dietary intake and composition

Analysis of the dietary records provided by the two groups of subjects demonstrated that there were differences of approximately 2 MJ/d in overall energy intake (Table 1); this was a direct result of a difference in macronutrient intake. The total energy intake for the omnivores was calculated to be 9.1 (SD 1.5) MJ/d, which was greater ($P=0.0003$) than the intake (7.2 (SD 1.1) MJ/d) of the vegetarians.

The mean daily fat intake of the omnivores was 88 (SD 18) g/d and was found to be higher ($P=0.0054$) than that of the vegetarian subjects (65 (SD 22) g/d). Similarly, the protein intake of the omnivorous group (70 (SD 9) g/d) was greater ($P=0.0002$) than that of the vegetarian subjects (55 (SD 10) g/d). The amount of CHO reported to be consumed daily by the omnivores was 279 (SD 69) g/d and this was also found to be greater ($P=0.024$) than that (227 (SD 26) g/d) of the vegetarians (Table 1). There was no difference in alcohol intake between the omnivores (5.9 (SD 6.6) g/d) and vegetarians (7.9 (SD 6.7) g/d). When the macronutrient intakes of the respective groups are expressed as a percentage of the total energy intake, the diet composition of the two groups was not different (Table 1).

Comparison of the daily dietary intake of Ca and P failed to show any differences between the two groups despite the observed differences in total energy intake (Table 2); however, the dietary intake of Mg was higher in the vegetarian subjects ($P=0.05$). The estimated N intake of the omnivorous group was 11 (SD 2) g/d, which was higher ($P=0.013$) than that (9 (SD 2) g/d) of the vegetarian subjects; this is a direct result of the greater protein intake of the omnivores. There were three subjects (one vegetarian and two omnivores) who at the time of the study were taking dietary supplements; two subjects supplemented their diet with multi-vitamins and Fe, while the remaining subject took evening primrose (*Oenothera biennis*) oil and ginseng. One subject (vegetarian) was taking medication (antibiotics) during the course of the study.

An estimation of the alkaline- and acid-ash contents of the diets was made (Sherman & Gettler, 1912; Hunt, 1956; Dwyer *et al.* 1985) based on the sum of the cations minus the anion content of the diets. The cations and anions were converted into mmol from mg by dividing by their atomic mass and then to mEq by multiplying the mmol value by the respective valence. An average value of 1.8 was used for the valence of P since it can be found in mono-, di-, and tri-valent forms in body fluids (Shohl, 1923). Comparison of the diets showed that there was a large individual variation in both groups; the vegetarian diet had a higher alkaline-ash content (51.8 (SD 25.1) mEq/24 h) than the omnivorous diet (42.4 (SD 24.1) mEq/24 h), but following log transformation of these data, statistical analysis failed to show a significant difference ($P=0.098$).

Blood acid-base status

The day-to-day variation in acid-base status of each group was compared before making comparison between the two groups. The day to day variation with respect to each of the acid-base variables was small and not statistically significant within either of the two groups. The plasma HCO_3^- (mmol/l) on each of the 2 d for the omnivores was 19.5 (SD 1.4) on day 1 and 19.9 (SD 1.1) on day 2 and for the vegetarians 19.9 (SD 1.7) on day 1 and 19.8 (SD 1.1) on day 2. Blood BE values on days 1 and 2 were -4.1 (SD 1.2) and -3.7 (SD 0.9) mmol/l respectively for the omnivores and -3.7 (SD 1.4) and -4.1 (SD 0.9)

Table 2. Daily intake of dietary minerals (mmol/24 h) and urinary excretion of calcium and magnesium (mmol/24 h) of premenopausal omnivorous and vegetarian women*

(Mean values and standard deviations)

| | Omnivores (n 20) | | Vegetarians (n 13) | | Statistical significance of difference between groups: P |
|-----------------------|------------------|------|--------------------|------|--|
| | Mean | SD | Mean | SD | |
| Dietary intake of: | | | | | |
| Na | 117 | 29 | 93 | 32 | 0.039 |
| K | 72 | 17 | 78 | 13 | 0.47 |
| Ca | 24.4 | 6.1 | 25.4 | 7.7 | 0.63 |
| Mg | 10.9 | 3.3 | 12.8 | 2.3 | 0.05 |
| P | 40.4 | 2.4 | 40.4 | 2.4 | 0.95 |
| S | 29.0 | 8.9 | 25.6 | 6.1 | 0.19 |
| Cl | 115 | 24 | 99 | 27 | 0.083 |
| Urinary excretion of: | | | | | |
| Ca | 3.87 | 1.34 | 3.22 | 1.20 | 0.014 |
| Mg | 4.10 | 1.43 | 3.67 | 1.61 | 0.12 |

* For details of subjects and procedures, see Table 1 and pp. 684–686.

mmol/l respectively for the vegetarians. The mean values for each of these variables were subsequently used for statistical analysis.

A small but significant difference ($P=0.05$) was found between the groups with respect to blood pH. The blood pH of the omnivorous group of subjects was higher (7.41 (SD 0.02)) compared with that of the vegetarians (7.40 (SD 0.01); Table 3). The other measured and calculated blood acid-base variables were not different between the two groups (Table 3).

Haematological and plasma variables

There were no differences between the day 1 and day 2 samples with respect to haemoglobin, packed cell volume, total cholesterol, HDL-cholesterol or the concentration of total protein or albumin. With the exception of plasma cholesterol there was no difference between the two groups with respect to these variables. The vegetarians had a significantly higher plasma total cholesterol concentration than did the omnivores ($P=0.023$; Table 3). In the present study, LDL-cholesterol concentration was not measured and there was no difference in HDL-cholesterol concentration between groups.

Urine acid-base status

Comparison of the variation between sample days 1 and 2 in urinary pH and titratable acid of the two groups did not reveal any statistically significant differences between the two measurements. Although there was no significant difference in urinary pH between the two groups, the omnivores tended to have a lower urinary pH (day 1 6.26 (SD 0.11), day 2 6.26 (SD 0.11)) than the vegetarians (day 1 6.52 (SD 0.15), day 2 6.38 (SD 0.16); $P=0.17$). The urinary titratable acid output of the omnivores was 48.9 (SD 20.3) mEq/24 h, compared with 35.3 (SD 23.3) mEq/24 h for the vegetarians ($P=0.014$; Fig. 1).

Urinary excretion of calcium and magnesium

Comparison of the urinary excretion of Ca and Mg did not reveal a difference between the two 24 h collections for the whole subject population. When the values were compared between groups, the omnivores had a higher daily urinary excretion of Ca than the vegetarians ($P=0.014$; Table 2). The urinary excretion of Mg was found not to be different between the two groups (Table 2).

DISCUSSION

The findings of the present experiment demonstrate significant differences in total energy intake and dietary macronutrient content between the vegetarian and omnivorous subjects, although the composition of the diet expressed as the percentage contribution of the macronutrients to total energy intake was not different between the two groups. There were also differences in blood and urinary acid-base status between the groups, with the vegetarians demonstrating a more alkaline urine. Furthermore, the daily urinary excretion of Ca by the omnivorous subjects was higher than that of the vegetarian group, despite the dietary intake of Ca being the same. It is postulated that the higher dietary protein intake of the omnivorous group was responsible for their higher urinary acid output and that this caused an increased rate of Ca excretion.

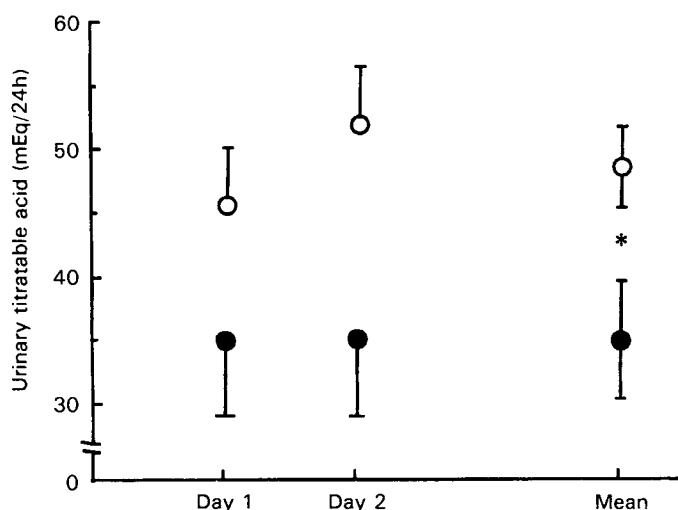


Fig. 1. Variability between sampling days 1 and 2 and mean values for both days for urinary titratable acid output (mEq/24 h) for premenopausal omnivorous (○) and vegetarian (●) women. Values are means with their standard errors represented by vertical bars for twenty omnivores and thirteen vegetarians. Mean values were significantly different between groups: * $P=0.014$. For details of subjects and procedures, see Table 1 and pp. 684–686.

The total energy intake of the two groups was different ($P=0.0003$), with the energy intake of the omnivores being almost 2 MJ/d higher. The total energy intake of the vegetarian subjects was reported as 7.2 (SD 1.1) MJ/d. Although this value for total energy intake appears low, previously-reported energy intakes of vegetarians are generally similar. The energy intake of Seventh-day Adventist vegetarians, based on a 3 d dietary record, was 7.4 (SD 1.7) MJ/d; this was not different from the energy intake (7.7 (SD 1.6) MJ/d) of non-

Table 3. Haematological, plasma protein and lipid variables and resting plasma pH, partial pressure (p) of carbon dioxide, bicarbonate ions and base excess (BE) for premenopausal omnivorous and vegetarian women*

(Mean values and standard deviations)

| | Omnivores ($n=20$) | | Vegetarians ($n=13$) | | Statistical significance of difference between groups: P |
|---------------------------|----------------------|------|------------------------|------|--|
| | Mean | SD | Mean | SD | |
| Hb (g/l) | 115 | 9 | 116 | 11 | 0.43 |
| PCV (%) | 39.4 | 2.0 | 39.4 | 2.5 | 0.79 |
| Total protein (g/l) | 70.9 | 6.6 | 70.9 | 6.3 | 0.85 |
| Albumin (g/l) | 47.2 | 4.4 | 47.9 | 4.5 | 0.10 |
| Cholesterol (mmol/l) | 4.03 | 0.73 | 4.54 | 0.62 | 0.023 |
| HDL (mmol/l) | 1.58 | 0.26 | 1.63 | 0.35 | 0.69 |
| pH | 7.41 | 0.02 | 7.40 | 0.01 | 0.05 |
| $p\text{CO}_2$ (kPa) | 4.21 | 0.32 | 4.32 | 0.23 | 0.47 |
| HCO_3^- (mmol/l) | 19.7 | 1.3 | 19.9 | 1.3 | 0.78 |
| BE (mmol/l) | -3.8 | 1.2 | -3.8 | 1.1 | 1.0 |

Hb, haemoglobin; PCV, packed cell volume.

* For details of subjects and procedures, see Table 1 and pp. 684–686.

vegetarian Seventh-day Adventists (Shultz & Leklem, 1983). One other study also found that energy intakes were not different between vegetarians and omnivores, based on 3 d food records and food-frequency questionnaires (Taber & Cook, 1980). Demi-vegetarians were reported to have an average daily energy intake of 8.0 (SE 2.6) MJ/d and lacto-ovo-vegetarians of 7.7 (SE 2.5) MJ/d based on a 3 d weighed intake of food (Draper & Wheeler, 1990). Vegetarians completing a 7 d weighed intake of food were reported to have an average energy intake of 8.1 (SE 0.9) (Roberts & Mathers, 1990). In addition, a recent study (Ramsdale & Bassey, 1994) based on a 3 d weighed intake reported energy intakes of 7.9 (SE 0.3) MJ/d for a group of women of a similar age range (18–44 years) but with a higher body mass (67.3 (SE 1.0) kg) compared with those in the present study.

The low dietary energy intake of the vegetarians highlights the difficulties encountered by the present study and those of other authors in obtaining a reliable estimate of energy intake based on a record of the 7 d weighed intake of food. Using the doubly-labelled-water technique discrepancies between reported energy intake and energy expenditure in men and women have been reported (Livingstone *et al.* 1990). Calculation of the BMR from age, height and body mass (Schofield *et al.* 1985) demonstrates that the BMR of the two present groups should be similar; 5.87 (SE 0.49) MJ/d for the omnivore subjects and 5.69 (SD 0.44) MJ/d for the vegetarians. The minimum energy expenditure of sedentary individuals is reported to be $1.3 \times \text{BMR}$ (Prentice *et al.* 1985). The reported energy intake of the subjects in the present study was calculated to be 1.55 (SD 0.25) \times estimated BMR for the omnivorous subjects and 1.27 (SD 0.20) \times BMR for the vegetarians. The low energy intakes of the vegetarians may be a case of under-reporting or may be the result of an unconscious change in their normal dietary pattern. The alternative explanation is that they do indeed have a lower energy requirement, perhaps related to a lower level of physical activity. Since the importance of recording everything they ate was emphasized to the subjects, the low energy intake reported is more likely to be a consequence of an unconscious change in dietary pattern.

It is widely reported that omnivores have, on average, a higher daily protein intake than vegetarians (Taber & Cook, 1980; Shultz & Leklem, 1983; Tylavsky & Anderson, 1988). In the present study the mean dietary intake of protein of the omnivores (70 (SD 9) g/d) was almost 15 g/d greater than that of the vegetarians. An acute increase in dietary protein intake is reported to have an acidifying effect on blood (Kurtz *et al.* 1983; Greenhaff *et al.* 1987*a,b*, 1988). The diet regimen that was employed by Greenhaff *et al.* (1987*a,b*) was high in protein (> 29% of total energy), high in fat (> 64%) and low in carbohydrate (< 10%) and the diets were isoenergetic with each subject's normal intake; adherence to this regimen resulted in inducing a mild metabolic acidosis. The increases in circulating β -hydroxybutyrate and free fatty acid concentration while consuming these high-fat-high-protein diets are similar to the changes in metabolite concentration following a 24 h fast (Maughan, 1978). The reported changes in these metabolites have been described as ketoacidosis normally found during fasting (Rose, 1977). Altering dietary composition by reducing the CHO content and increasing the protein content of two experimental diets for 10 d was reported to reduce plasma HCO_3^- concentration and lower plasma pH while subjects consumed the lower CHO diet (Kurtz *et al.* 1983). Despite the significant differences in fat and protein intake between the two groups of subjects in the present study, the only difference in blood acid-base status was the lower plasma pH of the vegetarians; this small difference, although statistically significant, is probably of little biological importance.

Increases in dietary protein intake have also been reported to exert an acidifying effect on urine (Hunt, 1956; Lutz, 1984; Breslau *et al.* 1988). This is characterized by an increase

in titratable acid output (Hunt, 1956; Lutz, 1984; Breslau *et al.* 1988) and in some cases by a decrease in urinary pH (Hunt, 1956; Kurtz *et al.* 1983). As reported in the present study the titratable acid output of the omnivores was on average 13.3 mEq/d higher than that of the vegetarians. The acidifying effect of the increased dietary protein intake is probably a direct result of the metabolism of the S-containing amino acids, cysteine, cystine and methionine (Sherman, 1941; Hunt, 1956, Lemann & Relman, 1959).

Not all the net urinary acid excretion can be accounted for by the metabolism of the S-containing amino acids (Lemann & Relman, 1959). The alkaline- and acid-ash theory of dietary components influencing urinary acid-base status was first proposed by Sherman & Gettler (1912) but was subsequently questioned by Hunt (1956) and Lemann *et al.* (1965) when they reported that the alkaline- or acid-ash content of diet failed to fully account for all acid production from the oxidation of the diet. Halperin (1982) identified the need to take into account the metabolism of other amino acids that consume H⁺ (glutamate and aspartate) and the metabolism of organic anions such as acetate, citrate, malate and tartrate. In the present study, it was calculated from the cation and anion content of the diets that the alkaline-ash content of the vegetarian diet appeared to be 18% greater than that of the omnivore diet. The absolute difference between the two diets was calculated to be about 9 mEq/d. Titratable acid output of the omnivores was 28% higher than that of the vegetarians; an absolute difference of 13.6 mEq/d.

The difference in dietary protein intake between the diets of the two subject groups was 14.8 g/d. The amino acid composition of vegetable and animal proteins also differs. Animal protein has a higher cystine and methionine content than vegetable protein and this difference should be reflected, therefore, in a higher dietary content of S. The S content of the diet of omnivorous subjects was calculated to be 12% greater than that of the vegetarian diet. The absolute difference in S content between the two diets was calculated to be 3.4 mEq/d. These data demonstrate that the higher titratable acid output of the omnivores may be related as much to the lower alkaline ash content of the diet as to the higher protein and S content.

Several dietary factors are known to cause hypercalciuria in both human subjects and animals; these include protein, Na and S intake (Block *et al.* 1980; Linkswiler *et al.* 1981; Breslau *et al.* 1988; Hu *et al.* 1993; Chan & Swaminathan, 1994). The urinary excretion of Ca is reported to increase when the dietary protein intake is increased (Wachman & Bernstein, 1968; Allen *et al.* 1979; Hegsted & Linkswiler, 1981; Linkswiler *et al.* 1981; Lutz, 1984; Anderson *et al.* 1987; Kerstetter & Allen, 1990). The link between an increase in urinary excretion of Ca and dietary protein intake has been postulated to be related to the metabolism of the S-containing amino acids and the excretion of the strong inorganic acids so formed. It is well documented that an increase in the S content of the diet produces a hypercalciuric effect (Hunt, 1956; Lemann & Relman, 1959; Block *et al.* 1980). The suggestion that changes in acid-base status can affect the excretion of Ca does not appear to be too tenuous considering the circumstantial evidence which demonstrates a reduction in urinary calcium excretion following the administration of alkalizing agents (Sakahee *et al.* 1983; Lutz, 1984; Lemann *et al.* 1989). Despite a similar Ca intake between the two groups, the urinary excretion of Ca was higher in the omnivore group. This could also be related to a higher intake of NaCl. The daily intake of Na in the omnivores was 117 (SD 29) mmol/24 h which was 25% higher ($P < 0.05$) than the intake of the vegetarians (93 (SD 32) mmol/24 h). It is known that Na has a hypercalciuric effect (Chan & Swaminathan, 1994) that is thought to be due to a reduction in renal Ca absorption accompanying a reduction in Na re-absorption (Chan *et al.* 1992). In rats a significant interaction between a high protein intake and high Na intake was reported to reduce the renal absorption of Ca (Chan &

Swaminathan, 1994). In the present study both protein and Na intake were higher in the omnivorous subjects compared with the vegetarians and the interaction between these two factors could explain the increased excretion of Ca despite similar dietary intake.

The present findings support earlier reports which have shown that acidification of urine results in an increased Ca excretion. In the present study, however, the differences between subject groups are due to differences in the self-selected diets. The higher dietary intake of protein by the omnivores was the possible cause of the increased urinary output of titratable acid and, hence, the elevated excretion of Ca despite similar dietary intakes of Ca.

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