

Bioavailability of energy, nitrogen, fat, zinc, iron and calcium from rural and urban Mexican diets*

BY JORGE L. ROSADO, PATRICIA LOPEZ, MARIA MORALES, ELSA MUNOZ AND LINDSAY H. ALLEN

Departamento de Fisiologia de la Nutricion, Instituto Nacional de la Nutricion 'Salvador Zubiran', Vasco de Quiroga #15, Tlalpan, Mexico DF 14000 and the Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269-4017, USA

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The availabilities of nutrients from a representative rural Mexican diet (RMD) and a representative urban Mexican diet (UMD) were evaluated by balance experiments in sixteen Mexican women. Compared with UMD, the plant-based RMD led to a higher number of defaecations and higher faecal excretion of dry matter, fat, nitrogen and energy. Apparent digestibility of N from RMD was only 67% compared with 90% from UMD. N balance was 0.4 and 2.6 g/d with RMD and UMD respectively ($P < 0.001$). Apparent digestibility of energy was 89 and 95% from RMD and UMD respectively ($P < 0.001$). Calculation of the metabolizable energy (ME) using Atwater's (Atwater & Bryant, 1900) general factors overestimates the determined ME in RMD by 8%. The Food and Agriculture Organization/World Health Organization/United Nations University (World Health Organization, 1985) recommended factors for correction of digestibility of fibre intake overestimate energy and protein absorption from RMD. The diets provided similar amounts of zinc, and its apparent absorption from RMD was 0.5 mg/d (4.6%) and its balance was 0.1 mg/d. This compared with values for UMD of 1.6 mg/d (16%) and 1.2 mg/d respectively. Iron intake was higher from RMD (17.4 v. 11.6 mg/d; $P < 0.01$), but apparent absorption was 17 v. 35% and balance was 2.7 and 3.8 mg/d ($P < 0.001$) for RMD and UMD respectively. RMD also contained more calcium (745 v. 410 mg/d) but apparent absorption from RMD was negative (–136 v. 15 mg/d) and balance was more negative (–197 v. –77 mg/d; $P < 0.05$). Thus, the content of these minerals is not low in the rural diet but their bioavailabilities are poor.

Bioavailability: Dietary fibre: Macronutrients: Minerals: Mexico

A large proportion of the world's population, especially in developing countries, consumes diets consisting primarily of plant foods. It has been reported (Instituto Nacional de la Nutricion, 1974; Bourges, 1980; Madrigal *et al.* 1986) that 80–85% of Mexicans, representing a significant part of the urban population and most of the less-developed rural population, consume diets based on corn tortillas, beans, vegetables and fruits. Tortillas and beans provide most of the dietary energy and protein. Inclusion of animal products is occasional and highly variable in both population groups and regions.

Diets typical of rural Mexico, and of many developing countries, contain a considerable amount of dietary fibre which may affect availability of several nutrients including energy (Calloway & Kretsch, 1978; Kelsay *et al.* 1978), protein (Southgate & Durnin, 1970; Kelsay *et al.* 1978; Kies & Fox, 1978; Cornin & Delpeuch, 1981), fat (Southgate & Durnin, 1970; Prynne & Southgate, 1979; Kelsay *et al.* 1981; Gallaher & Schneeman, 1985), 'available'

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carbohydrates (Chapman *et al.* 1985; Wolever *et al.* 1973) and minerals (Reinhold *et al.* 1975; Kelsay *et al.* 1979; Behall *et al.* 1987). Fibre may also induce satiety (Blundell & Burley, 1987; Rigaud *et al.* 1987) leading to lower nutrient intakes. Finally, dietary fibre may be used as a source of energy subsequent to metabolic activities of the bacteria in the colon (Cummings, 1984).

Those rural Mexican children who consume diets high in traditional plant foods and low in animal products are at most risk of delayed growth and development. This has been hypothesized to be related to the probable poor availability of nutrients such as iron and zinc (Allen *et al.* 1987, 1991). No information is available on Zn intake in Mexico, nor on the availability of Zn, Fe or calcium from representative diets. The protein and energy (but not mineral) utilization of a rural Guatemalan diet, which is similar to the Mexican rural diet, was investigated by Calloway & Kretsch (1978). Compared with that from a fibre-free egg formula, the digestibilities of energy, protein and fat were reduced.

The present research was designed to compare the availability of energy, protein, fat, Zn, Fe and Ca from diets representing the usual dietary compositions of rural and urban populations in Mexico. The implication for nutrient requirements and for dietary recommendations when such diets are ingested is discussed.

SUBJECTS AND METHODS

Subjects

The study was divided into two metabolic periods in which either a typical rural Mexican diet (RMD) or urban Mexican diet (UMD) was fed in a randomized cross-over design. From 14 to 32 d elapsed between each period. Each period consisted of an initial stage (lasting 4–12 d) in which the subjects became equilibrated to the diet, judged by constancy of polyethylene glycol excretion, followed by a 6 d balance period. The sixteen adult female subjects were young, middle-class Mexican students of nutrition and medicine with a mean age of 23.4 (range 16–27) years. The nature, purpose and potential risks of the study were explained to the subjects who then signed consent forms. The study protocol was approved by the Committee on Biomedical Research on Humans of the National Institute of Nutrition (INNSZ).

The nutritional status of the subjects was evaluated by anthropometry and several biochemical indicators in order to screen for nutrient deficiencies. Anthropometry, including weight, height, triceps skinfold thickness and upper-arm circumference, was measured at the beginning and end of each metabolic period. Weight-for-height and skinfolds were compared with mean standard values reported by Frisancho (1984). Biochemical determinations, including serum albumin, serum globulin, serum total protein, haemoglobin, packed cell volume and serum alkaline phosphatase (EC 3.1.3.1), were performed on samples collected at the beginning of each metabolic period following standard methods (Simmons, 1955). Subjects were confined during the two metabolic periods in the Metabolic Unit of INNSZ and were ambulatory and engaged in light activity. They were asked to maintain, as far as possible, their normal activity level. Those who engaged in regular exercise or strenuous activity were allowed daily exercise (10–30 min) on a stationary bicycle.

Experimental diets

RMD and UMD were given to each of the sixteen subjects in a cross-over design. RMD resembled the diet typically consumed in rural communities and UMD resembled one from more 'modernized' urban areas (see Table 1) and they were based on findings of previous

Table 1. *Types and amounts of foods included in each 3 d menu cycle*
(Mean values and standard deviations for sixteen subjects)

Urban Mexican diet			Rural Mexican diet		
Food	Amount (g/d)		Food	Amount (g/d)	
	Mean	SD		Mean	SD
Animal protein					
Beef	127	22			
Chicken	125	44			
Tuna	77	25			
Ham	64	15			
Egg	160	57			
American cheese	51	12			
Oaxaca cheese	41	9	Oaxaca cheese	38	8
Cereals and legumes					
Potato	310	59	Maize tortillas	502	108
Bread and cookies	344	81	Maize elotes (cobs)	322	68
Wheat tortillas	111	20	Maize beverage	53	16
Rice	136	26	Wheat pasta	87	18
			Black beans	180	39
			(<i>Phaseolus vulgaris</i>)		
Vegetables					
Celery	67	15	Mushrooms	80	17
Lettuce	201	123	Lettuce	16	3
Tomato	114	34	Tomato	595	128
Green tomato	45	8	Green tomato	189	60
Avocado	91	17	Squash (<i>Cucurbita pepo</i>)	120	25
Chile poblano (<i>Capsicum</i> spp.)	36	16	String beans (<i>Phaseolus coccineus</i>)	80	17
			Chile poblano (<i>Capsicum</i> spp.)	120	25
			Nopales*	435	137
Fruits					
Fruit juice	498	161	Papaya	312	89
Banana	130	46	Watermelon	202	197
Melon	155	125	Melon	121	146
Papaya	113	71	Orange	80	17
Watermelon	228	248	Jicama (<i>Pachyrrhizus palmatilobus</i>)	195	56

* Nopales are the leaves of the nopal cactus (*Opuntia* spp.).

dietary surveys (Instituto Nacional de la Nutricion, 1974; Bourges, 1980; Madrigal *et al.* 1986). In RMD the foods provided 70% of the total energy as carbohydrates, 11% as protein and 19% as fat. Most of the protein in this diet was from plant sources. Maize provided 40% of the total protein, black beans (*Phaseolus vulgaris*) 28%, vegetables and fruits 22%, wheat pasta 5.6%, and cheese 4.2%. Maize in RMD was consumed as tortillas (60%), 'elotes' (cobs; 30%) and a small amount (10%) in a low-energy-density beverage known as 'atole'. UMD on the other hand, consisted of foods that were combined to provide 50% of the energy from carbohydrate, 15% from protein, and 35% from fat. Protein in UMD was provided by beef, chicken or tuna (about 49%), eggs 10%, cereals 23%, vegetables and fruits 9%, and desserts 3%.

For both diets, three different daily menus with similar food and nutrient compositions

were prepared. The menus differed only in the form of preparation and were fed in 3 d cycles in order to add variety. The similarity of the nutrient compositions of the three menus was confirmed initially by direct analysis of diets in two subjects (values not shown). Table 1 also shows the average amount of each food consumed during each 3 d cycle. Each of the daily menus was prepared in duplicate for each subject so that the duplicate could be analysed for nutrient content (see below).

Food servings were calculated to provide 151 kJ/kg per d at the onset of each metabolic period. For the first 3 d all food eaten and left by each subject was weighed, and their average energy intake calculated. The subject was then fed this amount of energy throughout the remainder of that metabolic period. Daily menus were divided into three meals and no foods or drinks other than deionized water were allowed between meals.

Metabolic balances

Starting from the first day of the experimental periods and continuing every subsequent day, 3000 mg polyethylene glycol 4000 (PEG; Merck, Mexico City) were added daily to each subject's diet. The PEG was divided among the three daily meals by dissolving 1000 mg/meal in the accompanying drink. PEG was used both to determine the point at which pre-experimental diet residues had been eliminated and to correct for incomplete faecal collections (Allen *et al.* 1979). Starting on the second day, each defaecation was collected as a separate sample. PEG/g dry matter was analysed immediately in every sample until a similar concentration (i.e. difference < 10% of total PEG concentration) was found in at least two consecutive samples. The time from the first day the PEG was fed until the constancy of its concentration in dry faecal solids was considered to be the equilibration period. This period lasted (mean (SD)) 5.5 (0.2), range 4–7 and 8.9 (0.5), range 5–12 d with RMD and UMD respectively. The end of this period indicated complete elimination of pre-experimental diet and was the time at which subjects were considered to be in 'equilibrium'.

Metabolic balances were carried out for a 6 d period after equilibrium was reached. In eight subjects faeces were collected for a 9 d period but as results were not significantly different between 6 and 9 d balances the 6 d period was used in all cases. Menstruation was avoided by starting each experimental period immediately after bleeding stopped. During each balance period faeces were collected into acid-washed containers on a 24 h basis, refrigerated immediately after defaecation, then weighed and pooled in 3 d samples for analysis. Urine was also collected on a 24 h basis using acid-washed plastic bottles containing 20 ml 5 M-hydrochloric acid. Stool weight, dry matter excretion, and number of faecal samples (laxation rate) were recorded daily during the balance periods.

Biochemical analyses

The duplicate samples of diets were pooled for 3 d periods and homogenized using a colloidal blender (Probest and Clark, West Germany). Homogenized samples were analysed for: dry solids by drying the sample in a vacuum oven at 60° until constant weight; PEG by an adaptation of the method of Malawer & Powell (1967) reported by Allen *et al.* (1979); nitrogen by the automated Kjeldahl method (Kjeltec Auto System, Denmark); and ash and fat (extracted by diethyl ether) by standard methods (Association of Official Analytical Chemists, 1984). A portion of the homogenized sample was freeze-dried and analysed for energy in an adiabatic bomb calorimeter calibrated with benzoic acid (Parr Instruments, USA). Homogenized samples of food were freeze-dried and digested before mineral analysis as follows: 0.5 g freeze-dried samples were digested with 5 ml concentrated

nitric acid at room temperature for at least 2 h, then solutions were heated to 60–70° for 1 h. After cooling and addition of 2 ml perchloric acid (700 ml/l) they were heated at 150° for about 1.5 h until clear and most of the perchloric acid had evaporated. Then 2 ml concentrated HCl was added, the solution warmed for 3–5 min, 10 ml deionized water added and the resultant solution quantitatively filtered. Deionized water was added to volume (25 ml) and the samples analysed for Zn, Fe and Ca by atomic absorption spectrophotometry (Perkin Elmer, Norwalk, CT).

Pooled faeces were diluted with deionized water (1:1, w/v), then homogenized (Hobart Dayton Mexicana, Mexico) and analysed for PEG and dry solids as described for diets, and for total fat after saponification and extraction with light petroleum (b.p. 40–60) (van de Kamer *et al.* 1949). N, energy and minerals were measured in freeze-dried faeces as described previously for diets.

Daily urine samples were analysed for total volume and creatinine (Bonsnes & Taussky, 1945). Portions were then combined into 3 d pools for analysis of N by micro-Kjeldahl digestion and, after freeze-drying to < 40 g water/kg, for energy content in the bomb calorimeter. Effectiveness of combustion was confirmed by measuring the recovery of benzoic acid. Urinary minerals were measured directly using appropriate dilutions. Samples for Ca determination were diluted with lanthanum chloride solution to a final concentration of 10 ml/l. Recovery of all three minerals from diet, faeces and urine ranged between 96 and 103%.

Calculations and statistics

Metabolic balances were computed from analysed dietary intakes and the average urinary and faecal excretion during the last 6 d of each experimental period. Faecal excretion of nutrients was corrected to account for day-to-day variation in faecal flow using the formula: (faecal nutrient (mg/d) × (diet PEG (mg/d)/faecal PEG (mg/d))). This correction was made on each 3 d pool on a dry weight basis. Apparent digestibility of each nutrient was calculated as intake minus faecal excretion. N balance (N intake – faecal N – urinary N – miscellaneous losses) was calculated using a value of 8 mg/kg per d to approximate skin and other miscellaneous losses (World Health Organization, 1985).

Metabolizable energy (ME) values were calculated by two different methods: (1) by applying Atwater's (Atwater & Bryant, 1900) general factors of 16.7 (4), 37.6 (9) and 16.7 (4) kJ (kcal)/g crude fat, crude protein and carbohydrate (by difference) respectively that was consumed during the balance period; (2) by applying Atwater's specific energy values for foods (Merrill & Watt, 1955) to the intakes of fat, protein and carbohydrates from each food item as calculated from food composition tables (Hernandez *et al.* 1967). Determined values of ME were obtained in the balance experiment as follows: gross energy intake – (gross energy of faeces + gross energy of urine). The determined values were also corrected for N balance using the formula: ME = diet energy – faecal energy – 6.28 × (dietary N – faecal N) (Bernstein *et al.* 1955; Calloway & Kretsch, 1978).

Data from each dietary treatment were compared by analysis of variance and Student's paired *t* test using each subject as their own control (Snedecor & Cochran, 1980) and employing SAS software (SAS Institute Inc., Cary, NC, USA).

RESULTS

All the subjects were well-nourished based on the following average values and ranges: weight/height, 99 (range 91–109)% of standard; haemoglobin 148 (range 128–160) g/l and packed cell volume 43.9 (range 38.2–49.2). Weight averaged 54.5 (SD 4.2, range

Table 2. *Faecal characteristics of sixteen women consuming the rural (RMD) and urban (UMD) Mexican diets**
(Means with their standard errors)

	RMD		UMD		Statistical significance of difference: <i>P</i> <
	Mean	SE	Mean	SE	
Food intake (g/d):					
Total wt	2275	84	1896	70	0.001
Dry matter	1210	74	1159	52	0.31
Faecal excretion (g/d):					
Total wt	184.6	24	51.2	5.5	0.001
Dry matter	36.6	3.6	13.3	1.1	0.001
Water	148.0	21	38.0	4.5	0.001
Water in faeces (g/kg)	780	10	720	10	0.001
Laxation rate (defaecations/d)	1.4	0.1	0.9	0.1	0.001

* For details, see Table 1.

49.0–61.8) kg, and heights 157 (SD 5.5, range 150–168) cm. Overall weight loss was 0.9 kg with RMD and 0.7 kg with UMD. There were no abnormal values for serum albumin, globulin or alkaline phosphatase.

Values for fibre intake and digestibility, as well as the intake of other nutrients, have been reported elsewhere (Rosado *et al.* 1991). Comparing RMD with UMD, intakes of neutral-detergent fibre, acid-detergent fibre, cellulose, hemicellulose and lignin were three to four times higher with RMD. RMD contained approximately fifteen times more phytic acid (1.62 *v.* 0.09 mmol/d).

Faecal characteristics are presented in Table 2. The weight of faecal matter was 133 g/d higher when RMD was fed. An increase in faecal water was responsible for 78% of this difference, and there was a highly significant ($P < 0.001$) 2.8-fold increase in faecal dry matter. The higher faecal weight was associated with a significantly increased daily number of defaecations.

PEG recovery was 79 (SE 2)% with RMD and 65 (SE 6)% with UMD. In the eight subjects for whom the balance period was extended to 9 d, PEG recovery averaged 99 (SE 4)% (RMD) and 79 (SE 5)% (UMD) indicating that incomplete recovery is due to delayed excretion of the test diet rather than methodological problems with the PEG.

Table 3 shows intakes, excretion and apparent absorption of energy, N and fat. Gross energy intake was significantly lower from RMD by 694 kJ/d. In addition, apparent digestible energy was only 6769 kJ/d compared with 7780 kJ/d with faecal energy almost twice that from UMD. UMD provided significantly more N while faecal N was less than half that for RMD, the apparent digestibility being 65 and 90% for RMD and UMD respectively. Of the sixteen subjects, seven were in slightly negative N balance when consuming RMD, and all subjects were in positive N balance with UMD. Faecal fat excretion from RMD was twice that from UMD, and this combined with a lower intake led to a significant decrease in the total amount of fat digested when RMD was consumed ($P < 0.001$).

Table 4 shows ME intakes derived by the different methods. From the balance values, ME was 1065 kJ/d higher from the urban diet. Even when corrected for differences in N balance between the two diets, the ME in UMD remained 994 kJ/d higher (Table 4). For

Table 3. *Intake, excretion, apparent absorption and balance of energy, nitrogen and fat in sixteen women consuming rural (RMD) and urban (UMD) Mexican diets**

(Mean values with their standard errors)

	RMD		UMD		Statistical significance of difference: $P <$
	Mean	SE	Mean	SE	
Energy (MJ/d)					
Intake	7.60	0.46	8.29	0.42	0.04
Faecal excretion	0.83	0.07	0.41	0.02	0.001
Urinary excretion	0.23	0.01	0.27	0.01	0.002
Apparently digested	6.77	0.42	7.88	0.41	0.003
Apparent metabolizable energy	6.54	0.41	7.60	0.39	0.004
Apparent digestibility (% of intake)	89	0.9	95	0.3	0.001
N (g/d)					
Intake	7.6	0.6	10.5	0.4	0.001
Faecal excretion	2.5	0.3	1.1	0.1	0.001
Urinary excretion	4.7	0.5	6.8	0.4	0.001
Apparently digested	5.1	0.6	9.4	0.4	0.001
Balance†	0.4	0.4	2.6	0.4	0.001
Apparent digestibility (% of intake)	67	3	90	0.4	0.001
Fat (g/d)					
Intake	34.4	2.5	55.2	3.2	0.001
Faecal excretion	3.6	0.4	1.6	0.1	0.001
Apparently digested	30.8	2.4	53.6	3.1	0.001
Apparent digestibility (% of intake)	89.5	1.1	97.1	0.2	0.001

* For details, see Table 1.

† Calculated using 8 mg N/kg per d to approximate sweat and other miscellaneous losses (World Health Organization, 1985).

Table 4. *Comparison of metabolizable energy (ME; MJ/d) of rural (RMD) and urban (UMD) Mexican diets† calculated by different methods‡*

(Mean values with their standard errors for sixteen subjects)

Method	RMD		UMD	
	Mean	SE	Mean	SE
Gross energy intake	7.60	0.46	8.29	0.42
Measured ME‡	6.54	0.41	7.60	0.39
Atwater's§ general factors	7.03**	0.42	7.39	0.33
Specific energy factors	6.64	0.34	7.73	0.38

Mean value was significantly different from measured ME: ** $P < 0.01$.

† For details, see Table 1.

‡ If corrected for nitrogen balance, these values are 6.64 for RMD and 7.63 for UMD (Bernstein *et al.* 1955; Calloway & Kretsch, 1978).

§ Atwater & Bryant (1900).

Table 5. *Mineral intake, excretion, apparent absorption and balance of sixteen women consuming the rural (RMD) and urban (UMD) Mexican diets**

(Mean values with their standard errors)

	RMD		UMD		Statistical significance of difference: $P <$
	Mean	SE	Mean	SE	
Zinc					
Intake (mg/d)	10.9	1.0	10.0	1.9	NS
Faecal excretion (mg/d)	10.4	1.0	8.4	0.5	0.02
Faecal (% intake)	96	6	84	4	0.01
Apparent absorption (mg/d)	0.5	1.4	1.6	1.9	NS
Urinary excretion (mg/d)	0.3	0.05	0.4	0.04	NS
Balance (mg/d)	0.1	1.4	1.2	2.0	NS
Iron					
Intake (mg/d)	17.4	0.7	11.6	0.5	0.01
Faecal excretion (mg/d)	14.5	1.2	7.6	0.2	0.001
Faecal (% intake)	83	9	65	5	0.01
Apparent absorption (mg/d)	2.9	0.8	4.0	1.6	0.05
Urinary excretion (mg/d)	0.2	0.02	0.2	0.02	NS
Balance (mg/d)	2.7	0.9	3.8	1.6	0.001
Calcium					
Intake (mg/d)	745	70	410	30	0.001
Faecal excretion (mg/d)	881	92	395	27	0.001
Faecal (% intake)	118	18	96	8	NS
Apparent absorption (mg/d)	-136	65	15	15	NS
Urinary excretion (mg/d)	61	6	92	11	0.01
Balance (mg/d)	-197	88	-77	22	0.05

NS, not significant.

* For details, see Table 1.

UMD the application of Atwater's (Atwater & Bryant, 1900) general factors resulted in a similar value to that obtained using the balance data. By contrast, the use of Atwater's (Atwater & Bryant, 1900) general factors with the RMD significantly overestimated ME by about 8% compared with the metabolic balance data. The application of Atwater's (Atwater & Bryant, 1900) specific energy conversion factors to determine ME resulted in a similar value to that estimated from the balance experiment for both diets.

Zn intake was similar from the two diets (Table 5). Urinary excretion was about 3 and 4% of the intake for UMD and RMD respectively. Faecal excretion was significantly ($P < 0.01$) higher from RMD as subjects absorbed on average 1.6 mg/d (16% of intake) Zn from UMD and only 0.5 mg/d (4.6% of intake) from RMD. Zn balance was 1.2 mg/d with UMD, but was close to zero with RMD. Due to high variability between subjects these differences in apparent absorption and balance were not statistically significant from zero.

With RMD, Fe intake was about 50% higher (Table 5). Urinary excretion was less than 2% of intake on both diets. Faecal excretion of Fe was twice as high with RMD. Subjects apparently absorbed an average of 4.0 mg/d (34% of intake) from UMD compared with 2.9 mg/d (17% of intake) from RMD. Fe retention was about 15 and 33% of intake with RMD and UMD respectively, and the difference was highly significant ($P < 0.001$).

RMD provided 82% more Ca (Table 5). Urinary excretion was 22 and 8% with UMD and RMD respectively. Faecal Ca was more than twice as high from RMD ($P < 0.001$) but expressed as a percentage of intake this difference was not statistically significant. Apparent

absorption was 3% of intake for UMD and was negative from RMD. Ca retention was negative with both diets and was about 2.5 times lower from RMD ($P < 0.05$).

DISCUSSION

Nutrient intake

There was close agreement between analysed macronutrient composition and composition calculated from dietary surveys (Instituto Nacional de la Nutricion, 1974; Bourges, 1980; Madrigal *et al.* 1986) for both RMD and UMD. Subjects ingested less gross energy (by 697 kJ/d) and ME (1065 kJ/d) with RMD. The higher fibre content may have induced satiety. The effects of dietary fibre on satiety (Blundell & Burley, 1987; Levine *et al.* 1989) have been attributed to a decrease in the rate of gastric emptying (Schwartz *et al.* 1982) and an effect on insulin levels (Evans & Miller, 1975) or release of peptides, or both, that could modify feeding behaviour (Ali *et al.* 1982). An important characteristic of diets consumed in rural areas in Mexico and some other countries is that they normally include relatively few foods. Although some variety was introduced here by cooking the same foods in different ways, the rather monotonous rural diet may have contributed to the reduced intake observed. The decreased energy (ME) intake from RMD should have sustained a decrease in body-weight of 71 g/d in our subjects, but this was not observed.

Faecal output

Consuming RMD produced similar effects to those reported after the experimental addition of various types of fibre to diets. These include increases in stool number and faecal dry weight and water. Increased faecal weight with RMD was mostly explained by the higher water content of the stools and a lesser, but highly significant, increase in faecal dry matter.

Nutrient absorption and utilization

RMD produced significantly more faecal N and a decreased N balance due to both higher faecal N and a lower N intake. Others have reported increased faecal N due to dietary fibre (Southgate & Durnin, 1970; Kelsay *et al.* 1978). Calloway & Kretsch (1978) studied the effect of Guatemalan foods on energy and protein digestibility by six American men. Apparent digestibility of protein (69%) was very similar to our results with RMD.

The two-fold higher faecal energy excretion with RMD reflected an apparent digestibility of energy of 89% compared with 95% with UMD. Similarly, Calloway & Kretsch (1978) found an 89% apparent absorption of energy from a 'Guatemalan' diet compared with 97% from an egg-formula diet. Faecal fat excretion was 2.3 times greater with RMD, explaining only a small proportion of the higher faecal energy. A lower apparent digestibility of fat has also been observed by other investigators using different sources of dietary fibre (Southgate & Durnin, 1970; Calloway & Kretsch, 1978; Kelsay *et al.* 1978). ME was 1065 kJ/d higher with UMD because more energy (697 kJ/d) was consumed and less energy (412 kJ/d) was excreted in faeces. The combination of reduced intake and increased faecal excretion of energy may have deleterious effects on the nutrition of individuals consuming plant-based diets, especially if the dietary supply of energy is low.

Zn

The Zn content of many foods in RMD is unknown and food composition tables in Mexico (Hernandez *et al.* 1967) and other Latin American countries (Instituto de Nutricion de Centroamerica y Panama, 1974) do not contain information for Zn. To our knowledge this

is the first report on Zn intake from Mexican diets. Zn intake was similar from RMD and UMD, comparable with that of adults in the United States (Greger & Sciscoe, 1977; Holden *et al.* 1979; Hunt *et al.* 1979) but slightly lower than that reported with a typical Italian diet (Carnovale *et al.* 1987).

Faecal excretion of Zn with RMD was higher than with UMD by about 2 mg/d. Possibly this is due to the significant amount of phytic acid in the plant-based RMD, with phytic acid:zinc molar ratios of 9.7 (SE 1) for RMD and only 0.6 (SE 0.2) for UMD. Inhibitory effects of phytic acid on Zn absorption have been reported previously (Reinhold *et al.* 1973; Sandstrom *et al.* 1987).

Fe

Although the Fe content of UMD was low it was efficiently absorbed, possibly due to a high content of haem-Fe and ascorbic acid (Monsen *et al.* 1978). However, the 2.9 mg Fe/d retained from RMD cover the 1.5 mg daily requirement for absorbable Fe for adult women (National Research Council, 1989). This surprisingly adequate retention of Fe could be attributed to the substantial intake of both Fe, mostly from beans and tortillas, and ascorbic acid from fruits. Kelsay *et al.* (1979) reported an Fe balance of 3.8 mg/d, when Fe intake from a mixed diet was 21.8 mg/d, which rose to 4.6 mg/d when dietary fibre intake was increased with fruits and vegetables. Fe absorption from mixed diets was not affected by rice fibre (Cullumbine *et al.* 1950) or by the presence of phytic acid (Walker *et al.* 1948) when Fe intakes were between 11 and 26 mg/d. RMD contained greater amounts of dietary fibre and phytic acid, both potential inhibitors of Fe absorption (Lynch, 1984), which suggests that food factors in a mixed diet interact; the solubilizing effects of ascorbic acid to some extent counterbalance the negative effects of dietary fibre and phytic acid. The Mexican dietary surveys on which the composition of RMD and UMD were based include information from tropical regions where ascorbic acid intake from fruit and vegetables is high. Dietary levels of the vitamin in many rural areas are much lower than those in the current study. For example, in a rural highland area near Mexico City the ascorbic acid intake of adult women averaged only 50 mg/d and the prevalence of Fe deficiency anaemia was high (Allen *et al.* 1987).

Ca

Subjects fed on RMD ingested about 500 g maize tortillas/d. In Mexico tortillas are prepared with lime-soaked maize and a calcium hydroxide solution is added at the time of cooking. The average maize tortilla contains about 1100 mg Ca/kg (Hernandez *et al.* 1967) and contributes over 500 mg Ca/d from RMD. Ca intake from UMD was about half that from RMD, and was lower than that reported for the average Mexican diet (Bourges, 1980), several Western diets (Solomons, 1986), and the subjects' usual intakes before the study (878 (SE 297) mg/d). We excluded milk (but not cheese) from UMD which lowered Ca intake to a level well below present recommendations (National Research Council, 1989) and contributed to their negative balance for Ca (−77 mg/d).

The highly negative Ca balance observed with the plant-based RMD is similar to that reported by Reinhold *et al.* (1973) for individuals consuming the traditional unleavened wholemeal bread of Iran (−200 to −300 mg/d). Similarly, McCance & Widdowson (1942) demonstrated a reduction in dietary Ca absorption when bread made from high-extraction brown flours replaced that made from white flours in a normal diet. More recently, Cummings *et al.* (1979) observed a similar effect when fibre intake was increased from 22 to 53 g/d by replacing white flour products with whole-wheat products and high-bran foods. The negative Ca balance caused by whole-wheat products has been attributed to the formation of unabsorbable Ca–phytate complexes (Allen, 1982). However, fibre itself also impairs Ca absorption. Kelsay *et al.* (1979) observed that increasing fibre intake by the

addition of fruits and vegetables to a basal diet decreased Ca balance from 72 to -122 mg/d. Thus, the higher concentration of dietary fibre, phytates, and perhaps other constituents in the plant-based RMD may have lowered the bioavailability of Ca.

It is also possible that Ca is not well absorbed from the alkaline $\text{Ca}(\text{OH})_2$ added to maize during the preparation of tortillas, which were the major source of Ca in RMD. The low incidence of eclampsia in pregnant Guatemalan women who habitually consume lime-treated corn tortillas has been attributed to their high Ca intake (Villar *et al.* 1983), but this hypothesis should be re-examined given the poor absorption of Ca from this source. The relatively high prevalence of osteoporosis in Guatemala, in spite of high dietary Ca, has been used as evidence against a protective role for Ca in the etiology of this disease (Garn, 1970). The low bioavailability of this Ca may explain why no protective effect is seen.

Subjects in the present study consumed each diet for only 10–21 d. Long-term adaptation in Ca retention has been demonstrated in subjects consuming high phytate (Walker *et al.* 1948) diets or a low Ca intake (Spencer *et al.* 1969). Further studies are needed to determine Ca balance in subjects who consume plant-based RMD habitually. Nonetheless, the Ca in tortillas is obviously present in a poorly absorbable form.

Implications for energy and protein recommendations

The Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) has set the safe level of intake of proteins with a digestibility similar to that of milk, egg or meat at 0.75 g/kg per d for adults (World Health Organization, 1985). For foods and diets with a lower N digestibility it was suggested that the safe level be corrected by a digestibility factor. Suggested factors for specific foods range from 78 to 96%. Factors of 85 or 95% are suggested for mixed diets based on whole grains and vegetables or refined cereals respectively. We found an apparent N absorption of 65% from a diet moderately rich in fibre and similar to that typically consumed by the population in rural Mexico. This value was 90% with a typical more refined urban diet. Recommended correction factors for plant-based mixed diets may need to be revised.

ME from RMD and UMD in the present study was determined by three different methods: using values obtained in the balance experiment, using Atwater's (Atwater & Bryant, 1900) general factors, and specific energy conversion factors used in US food tables (Merrill & Watt, 1955) with the foods consumed in RMD and UMD. According to the FAO/WHO/UNU Expert Committee (World Health Organization, 1985), the energy calculated using Atwater's (Atwater & Bryant, 1900) general factors for diets moderately rich in dietary fibre should be multiplied by 0.975. For diets containing larger amounts of dietary fibre the suggested factor is 0.95. Although RMD could be classified as having moderate amounts of dietary fibre, even the highest correction suggested with Atwater's (Atwater & Bryant, 1900) general factors will overestimate daily energy availability by about 5%. In contrast, energy availability calculated with the specific factors for foods agreed well with the values obtained in the balance experiment for both diets. This suggests that the energy conversion factors for specific foods may better estimate the ME in diets moderately rich in dietary fibre such as those consumed by the majority of the population in Mexico. Similar results have been reported recently for high-fibre Western diets (Goranzon & Forsum, 1987).

In general, it is apparent that RMD is not low in its content of Zn, Fe, or Ca but that the bioavailability of these nutrients is relatively poor. The World Health Organization (Wolever *et al.* 1973) recommends that Zn intake for adult females should be about 5.5, 11 or 22 mg/d if Zn bioavailability is 40, 20 and 10% respectively in order to meet requirements, calculated to be about 2.5 mg/d. Zn absorption from UMD (16%) is adequate to meet current recommendations (National Research Council, 1989), but Zn

intake or absorption from RMD would have to be greatly increased in order to meet requirements. The surprisingly high Fe content of RMD combined with the very high ascorbic acid intake supported Fe balance in the young women, although availability of Fe was less compared with the urban situation. Finally, the surprisingly poor absorbability of Ca from RMD deserves further attention, given the interest in the prevalence of pre-eclampsia and osteoporosis in population groups that are habitual consumers of large amounts of Ca in tortillas.

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