Allergy 2008: 63: 425–431

DOI: 10.1111/j.1398-9995.2008.01627.x

Original article

Urban/rural differences in diet and atopy in South Africa

Background: There are large differences in the prevalence of atopic disease between urban and rural areas of developing countries, without good explanation. Diet has been associated with atopic disease, but studies of specific nutrients are contradictory, cross-sectional studies often being unsupported by trials. We investigated diet as an explanation for the difference in the prevalence of atopy between urban and rural areas in South Africa.
Methods: A cross-sectional analysis of food frequency questionnaires and allergen skin tests from 698 children aged 8–13 years, recruited from 24 schools in Cape Province, South Africa, who were taking part in a case–control study of exercise-induced bronchospasm. Food frequency data were analysed with a principal components analysis (PCA).
Results: The first two principal components of diet explained 25% of the variance, and discriminated almost perfectly between urban and rural subjects. The 'urban' component of diet was strongly associated with positive skin tests even after adjusting for urban residence. There were no significant associations between individual foods or nutrients and positive skin tests, allowing for multiple testing.
Conclusions: Diet explains part of the difference in prevalence between urban and rural areas. The ability to demonstrate this using PCA, but not by exhaustive analysis of all foods, reflects the value of reducing the number of dietary dimensions. The number of foods and nutrients which can be assessed, and the possibility of confounding and effect modification, make it difficult to identify the features of diet most directly implicated in disease. This may explain inconsistencies in dietary studies.

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Key words: children; dietary patterns; food frequency questionnaire; skin tests; South Africa.

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Accepted for publication 25 November 2007

It has been known since at least the 1970s that atopy manifests far less in rural than in urban areas of poorer countries (1–3). This is not due to lack of allergic sensitization in rural areas (4), and the explanation is not yet clear. Lower levels of atopy have been linked to infections in early childhood [the so-called 'hygiene hypothesis' (5)] as well as parasite infestation (6), but there is no evidence that parasites are less common in urban areas of poor, tropical countries than in rural areas, or that conditions in rural areas are 'less hygienic'. In a randomized controlled trial carried out in schools in Ecuador, treatment against geohelminth parasites had no effect on atopy (7).

There is accumulating evidence that diet affects the prevalence of asthma and possibly of atopic disease in general. Areas of specific interest have been dietary antioxidants (8), dietary lipid (9) and dietary electrolytes (10), as well as the consumption of probiotics (11). Interpretation of the evidence is difficult, however, and many of the findings described in observational studies have not been confirmed in trials, possibly because of the wide potential for confounding and effect modification in nutritional studies (12). Methods of data analysis are available for dealing with questions of confounding and interaction of dietary exposures, but the sheer number of features of diet that can be measured will often defeat a comprehensive investigation of this kind. Over the last decade, researchers have moved away from the analysis of individual foods and nutrients, and towards the extraction of a small number of dietary 'patterns' using data analytic methods – most popularly principal components analysis (PCA) (13). PCA of a multivariate dietary assessment, such as a food frequency questionnaire (FFQ), proceeds by standardizing food weights to have the same variance, and forming the weighted linear combination of these standardized food weights with the greatest variance, that is the dimension of diet along which there is the most variation. This is the first principal component. Each subsequent principal component is derived as the dimension of diet which shows the most variation subject to being uncorrelated with all previous components (14).

Several studies have used PCA to research relationships between diet and disease, including coronary heart

disease (15, 16) and colon cancer (17, 18). In this paper, we use PCA of FFQ data from South African schoolchildren to assess the influence of diet on skin test sensitivity, and to investigate whether diet can explain urban/rural differences in atopy.

Methods

Study population

Schoolchildren in this study were participating in a case–control study of exercise-induced bronchospasm (EIB) in Cape Province, South Africa, which is described in detail elsewhere (19). The study was designed to have 80% power at the 5% significance level to detect a relative risk of EIB of 2.0 for serological evidence of infection with parasites, assuming that 80% of the population overall had evidence of infection. This required a sample of 282 cases and 282 controls. From a pilot study, the prevalence of EIB was estimated conservatively at 8%, and it was therefore planned to test 3500 children.

Subjects were aged 8–13 years, and were all black South Africans from the Xhosa population. They were recruited from two distinct geographical areas: one rural and one urban. All eligible schools in the rural area were approached to take part. In the urban area, schools were enrolled in their order of construction, starting with the most recent, with sufficient schools enrolled to achieve the required sample size. EIB was assessed by measuring forced expiratory volume in one second (FEV_1) and forced expiratory flow at 25–75% of forced vital capacity ($FEF_{25–75}$), before and after an exercise challenge. Cases were those children with a fall in FEV_1 greater than 15% or a fall in $FEF_{25–75}$ greater than 26%. Controls were selected randomly from children with no response to the exercise challenge (fall in FEV_1 no greater than 10% and fall in $FEF_{25–75}$ no greater than 20%). The sampling fraction used for selecting controls varied between schools. Recruitment was staggered between the rural and urban areas in blocks of 3–4 weeks over a 9-month period from January to September 1999, to reduce differences in climate between areas at the time of testing.

The project proposal was approved by the University of Cape Town Biomedical Research Ethics Committee. All parents provided informed consent.

Dietary assessment

Information on children's diet was obtained using a semi-quantitative FFQ (20). The 138 foods included were compiled from data gathered during previous dietary surveys in the Western Cape (21, 22), and based on the advice of local experts (a full list of foods is available from the authors). The FFQ was completed at an interview at which both child and parent/guardian were present. Respondents were presented with nine frequency choices for each food ranging from 'almost never' to 'more than six times a day'. Using a selection of bowls and plates used by interviewees, subjects were asked to choose a plate most similar to the one they used when eating, and to indicate the level to which this receptacle was filled. Average portion sizes calculated in this way were used to convert food frequencies into intakes in g/week. Weights of different foods were used to calculate total energy intake and other nutrient intakes using South African tables (23). To assess reliability of the FFQ, 33 subjects were given a repeat interview 6 weeks later. The mean weighted kappa across all foods was 0.47 (i.e. moderate – the range was 0.33–0.74).

Statistical analysis

Principal components analysis was applied to the FFQ data. We extracted the first two components only, because the scree plot of eigenvalues did not suggest any natural choice beyond two components, and selecting two components made graphical representation of the data particularly straightforward. Independent samples *t*-tests were used to see how the first two principal components related to age group, sex and urban/rural location. We considered orthogonal rotations of the first two principal components, including a varimax rotation, using the correlations between rotated components and individual foods (the factor loadings) to aid their interpretation.

Because of the design of the case–control study, our sample included an unrepresentatively high proportion of EIB cases, which had the potential to bias the results of our analysis. To investigate this, we repeated the PCA using only data from control subjects, and re-calculated the two dimensions of diet for the full sample based on the first two principal components obtained from the controls. These correlated almost perfectly with the principal components based on the whole sample (both correlations 1.00 to 2 decimal places).

Skin test sensitivity, the main outcome, was defined as a positive skin test [a response greater than to the negative control (24)] to any one of the following locally prevalent allergens: dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis*), moulds (aspergillus, cladosporium and alternaria), grasses (timothy and Bermuda), cockroach, cat and dog. Associations between skin test sensitivity and the two dimensions of diet obtained from PCA were analysed using a logistic regression, adjusting for effects of total energy intake (on a log scale), age, sex and urban/rural location. Probability weights were used in these analyses to allow for the different sampling proportions of EIB cases and controls. This method was also used to analyse the association between skin test sensitivity and each foodstuff from the 138-item FFQ in turn, using Simes' method to adjust for multiple comparisons, controlling the false discovery rate at 5% (25). These results were displayed using a 'smile' plot of *P*-values against standardized regression coefficients. The same approach was used to analyse 33 nutrient intakes calculated from the FFQ.

All analyses were carried out using STATA 9 (StataCorp, College Station, TX, USA) and SPSS 11 (SPSS Inc., Chicago, IL, USA).

Results

Twenty-four eligible schools were identified in the rural area, and 18 agreed to participate, the commonest reason for nonparticipation being turmoil in the community. Six schools were approached in the urban area, and all agreed to participate. Figure 1 summarizes the recruitment process. A total of 5111 children from urban and rural schools were eligible to take part, and 3304 (64.6%) of these completed an exercise challenge. A total of 773 were selected for further assessment, of whom 698 (90.3%) completed an FFQ. Table 1 shows characteristics of these 698 children.

Principal components analysis

The first two principal components explained 25% of the variance in the original 138 food items. Figure 2A is a

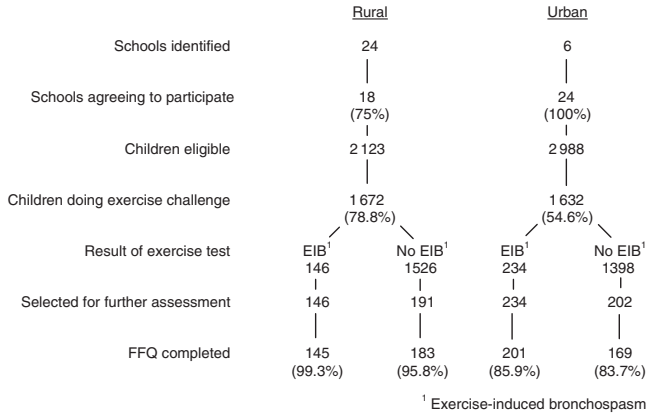

Figure 1. Sampling flowchart (percentages show proportion of previous total).

scatterplot showing how individual children scored on these two components, illustrating that between them these two components discriminated almost perfectly between urban and rural children.

An orthogonal rotation of the first two principal components retains the same dietary information but may make interpretation of the dimensions easier. In our example, a varimax rotation did not improve interpretability (results not shown). We chose instead to rotate the principal components clockwise through 30°, as shown in Fig. 2B. This rotation, which we labelled the 'convenience' rotation, separates out the urban and rural children along the vertical axis, which can therefore be interpreted as an urban/rural dimension of diet. The second rotated dimension reflects other aspects of diet uncorrelated with the urban/rural dimension. Correla-

Table 1. Characteristics of the sample

	Rural		Urban		
	EIB[2], *n* = 145	No EIB[2], *n* = 183	EIB[2], *n* = 201	No EIB[2], *n* = 169	Total[1] *n* = 698
Age (years)					
Mean (SD)	10.3 (1.2)	10.5 (1.2)	10.5 (1.2)	10.5 (1.2)	10.5
% Male	46.9	47.0	39.6	48.3	44.6
Energy intake (kcal)					
Mean (SD)	2957 (1215)	2836 (1158)	3854 (1228)	3834 (1246)	3302
% Skin test positive	22.4	16.7	37.3	30.2	23.7

[1]Total summary statistics are weighted to adjust for sampling fractions.
[2]Exercise-induced bronchospasm.

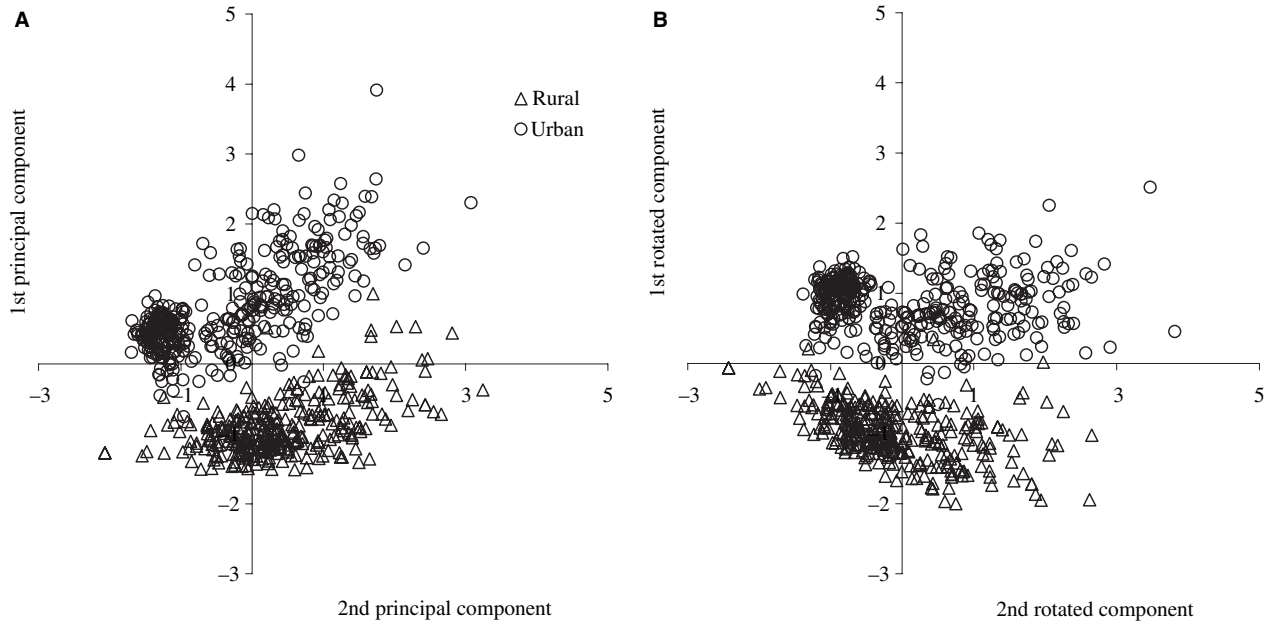

Figure 2. Two-component solution from principal components analysis of food frequency questionnaire: (A) first two principal components; (B) convenience rotation of first two principal components.

Table 2. Correlations between food intakes and each of the two convenience-rotated dimensions of diet (only correlations > 0.40 or < −0.40 are included in the table)

	First convenience-rotated dimension of diet (urban/rural dimension)	Second convenience-rotated dimension of diet
Oat porridge	0.52	–
Sugar-coated cereal and milk	0.43	–
White rice	0.58	–
Corn on the cob	−0.41	–
Fried dry corn	−0.48	–
Jam	–	0.56
Peanut butter	–	0.55
Hard margarine as a spread	–	0.54
Carrots	0.64	–
Fried potatoes	0.62	–
Tinned peas	0.54	–
Tinned beans	0.51	–
Tomato	0.48	–
Nonhot peppers	0.47	–
Avocado	0.44	–
Boiled potatoes	0.43	–
Dried peas	–	0.51
Spring onion	−0.55	–
Nonsweet melon	−0.58	–
Young pumpkin	−0.62	–
Wild leaves	−0.63	–
Cabbage	−0.64	–
Pumpkin leaves	−0.75	–
Tinned fruit salad	0.67	–
Currants, raisins, sultanas	0.58	–
Grapes	0.56	0.47
Pineapple	0.53	–
Plums	0.49	0.45
Bananas	0.44	0.52
Apricot	0.40	–
Apples	–	0.64
Oranges	–	0.59
Pears	–	0.53
Peaches	–	0.47
Umsobo (wild berries)	−0.67	–
Umswi (wild berries)	−0.79	–
Sausage	0.71	–
Fried chicken	0.63	–
Chicken	0.63	–
Chicken in breadcrumbs	0.59	–
Tripe	0.59	–
Mutton	0.58	–
Polony (processed meat)	0.51	–
Beef	0.46	–
Beef burger	0.45	–
Meat pie	0.43	0.45
Tinned fish in brine	–	0.52
Fish	–	0.40
Fried egg	0.41	–
Boiled egg	–	0.40
Yoghurt	0.61	–
Processed cheese	0.58	–
Ice cream	0.56	0.40
Jelly	0.71	–
Packet custard	0.67	–
Chocolate bar	0.48	–
Peanuts	0.42	0.46
Potato crisps	–	0.60
Niknaks (synthetic snacks)	–	0.64
Hard margarine for cooking	0.54	–
Maize cooking oil	–	0.42
Hard white fat for cooking	–	0.50
Chicken fat for cooking	–	0.42
Orange squash	–	0.54
Tea	–	0.41
Samp (dried corn kernels) drink	−0.45	–
Fermented mealie meal and flour	−0.50	–

tions between individual foods and the convenience-rotated dimensions of diet are shown in Table 2. Foods which were strongly negatively correlated with the urban/rural dimension of diet (characteristic of rural diets) were pumpkin leaves, young pumpkin, cabbage and wild leaves and berries. Foods which were strongly positively associated with the urban/rural dimension of diet (characteristic of urban diets) were fried potatoes, carrots, tinned fruit salad, chicken, sausages, yoghurt, packet custard and jelly.

Association between diet and skin test sensitivity

Eleven children had a missing skin test result. Of the remainder, 187 (27.2%) had sensitivity to at least one of the allergens tested. As expected, the prevalence of skin test sensitization was higher in urban than in rural children (Table 1). When the two convenience-rotated dimensions of diet were entered together into a logistic regression analysis with sensitivity as the dependent variable, adjusting for total energy intake, age, sex and urban/rural location (Table 3), there was strong evidence of an effect of the urban/rural dimension of diet ($P = 0.009$) but not of the second dimension of diet ($P = 0.51$). A difference of 1 SD on the urban/rural

Table 3. Effects of diet and potential confounding variables on odds of skin test sensitivity: results of multivariable logistic regression

	Adjusted odds ratio	95% CI	*P*
Urban/rural dimension of diet (per SD)	2.1	1.2–3.7	0.009
Second convenience-rotated dimension of diet (per SD)	1.2	0.7–1.8	0.52
Age (per year)	1.1	0.9–1.3	0.47
Male sex	0.8	0.5–1.3	0.41
Urban location	0.6	0.2–1.8	0.35
Energy intake (per doubling)	0.7	0.3–1.8	0.49

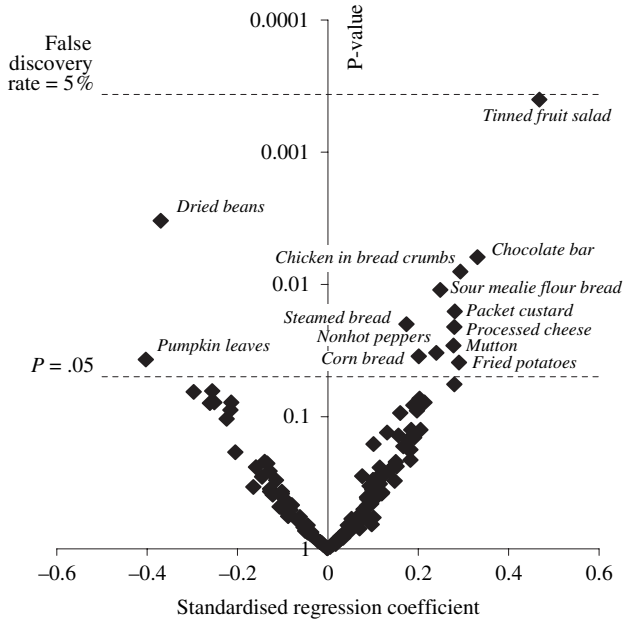

Figure 3. Association of foods with atopy: smile plot showing *P*-value against standardized logistic regression coefficient for different foods. Points on the right represent positive associations; points on the left represent negative associations. To be considered significant, controlling the false discovery rate at 5%, points must lie above the higher dotted line.

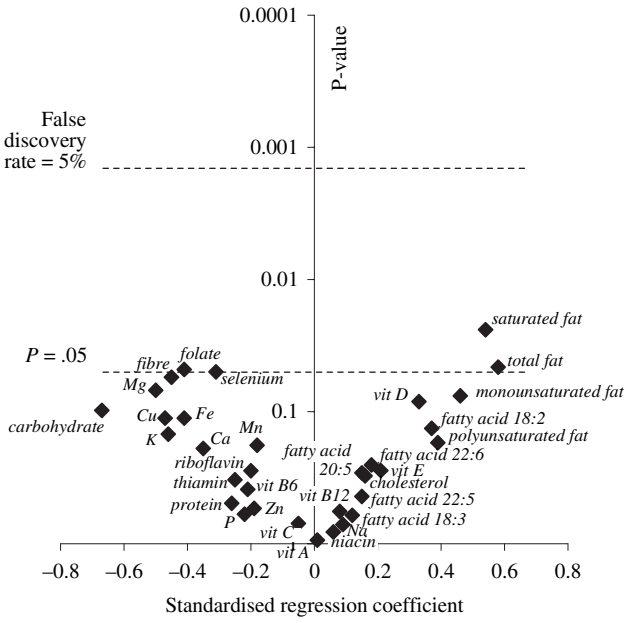

Figure 4. Association of nutrients with atopy: smile plot showing *P*-value against standardized logistic regression coefficient for different nutrients. Points on the right represent positive associations; points on the left represent negative associations. To be considered significant, controlling the false discovery rate at 5%, points must lie above the higher dotted line.

dietary scale doubled the odds of skin test sensitivity. The results of entering each of the 138 individual food items separately into logistic regression analyses are shown in the smile plot in Fig. 3. To be considered significant (with the false discovery rate controlled at 5%), an individual result here needs to be above the upper horizontal line. Hence, there was no convincing evidence of a link between any of the individual foodstuffs and skin test reactivity, after taking proper account of the number of comparisons. Figure 4 shows the smile plot looking at different nutrient intakes. Again, there was no convincing evidence of a link between any of the nutrient intakes and skin test reactivity.

Discussion

The first two principal components of diet between them discriminated almost perfectly between urban and rural populations. A more urban diet was associated with an increased likelihood of a positive skin test even after adjusting for the place of residence of the individuals. This association was not convincingly demonstrated by analysing all 138 food items separately, and was found in spite of the fact that the first two principal components accounted only for 25% of the variance in intake of the 138 foodstuffs.

We assessed diet using an FFQ rather than repeated 24-h recall. A review of nutritional assessments in children and adolescents has concluded that FFQs can be sufficiently reliable to permit diet to be related to health outcomes in these groups (26). The validity and reliability of FFQs in adults has also been confirmed by Katsouyanni et al. (27). Although the latter found a tendency for FFQs to overestimate frequency of consumption, this kind of bias will not affect our PCA as it depends only on correlations between food intakes.

We have shown that a typically urban diet was associated with an increased prevalence of atopy, but the roles played by individual nutrients are less clear. None of the associations in Fig. 3 is significant after adjusting for the number of comparisons, although the directions of these associations are mostly in line with current hypotheses.

Most interest in lipids has focused on the pro-inflammatory properties of the n-6 fatty acids and the competitive effects of the n-3 fatty acids, largely derived from marine sources (28), although experimental support for this being a major influence on clinical disease is still weak (29). In our study all lipids, including both omega-6 (n-6) fatty acids, such as linoleic acid (18 : 2), and omega-3 (n-3) fatty acids, such as docosohexaenoic acid (DHA or 22 : 6) and eicosapentaenoic acid (EPA or 20 : 5), were positively associated with atopic disease. The associations with total and saturated fat would have been significant had we not taken the multiple comparisons into account.

Associations with low intake of folate and selenium would have also been marginally significant if the multiple testing had not been taken into account. Consistent with the apparently protective role of folate found in this analysis, a recent study has suggested that dietary folate and riboflavin may protect against atopy, particularly in those with a variant of the methylenetetrahydrofolate reductase gene (30). Selenium has an antioxidant role and intake has also been associated with reduced levels of asthma (12). However, there is so far very little evidence from trials that enhancing selenium intake reduces the risk of asthma (31–33).

Vitamins A and E were positively associated with atopy in our study, which is unexpected as both have antioxidant properties. Vitamin E in particular has been associated in some studies with a reduction in atopy (12), although trials of vitamin E supplementation in asthma either alone (34) or in combination (33) failed to show an effect. The positive association between the other fat soluble vitamin, vitamin D and atopy is less surprising, as infant supplementation with vitamin D has previously been reported to be linked to higher rates of asthma and allergic rhinitis (35). However, maternal intake of vitamin D during pregnancy has also been associated with a reduced risk of wheeze in early childhood (36), and in the USA the geographic distribution of anaphylaxis exhibits a strong north–south gradient consistent with a protective effect of vitamin D through sun exposure (37).

Apart from vitamins A and E, the other nutrients with an antioxidant role were all negatively associated with allergy in our study. These include vitamin C, which is an antioxidant, and copper, zinc and manganese, which, like selenium, all play important antioxidant roles. This is consistent with the hypothesis that dietary antioxidants are important in protecting against atopic disease (38), although an intervention study in adults found no effect of supplementation with antioxidants, including vitamins C and E with β-carotene and selenium, on immune responses (33).

Reasons for the strong association between atopy and the principal component representing a rural diet, and the failure to show a significant association between atopy and any of the more specific components of diet could be explained in several ways. Most obviously, it may be that the important component of diet is not represented in the nutrients we analysed. It might be argued that the effects of different nutrients or foods will be more apparent in combination with one another than they are separately – either because their effects are cumulative, as could happen, say, with antioxidants, or because adding together the contributions of several foods give a measure with improved reliability. Nevertheless, there were individual foods and nutrients which we would have considered statistically significant if we had an *a priori* reason to look at them alone, and some of these – for example saturated fat – were significant even after adjusting for urban/rural diet. The problem is that we had less statistical power for looking at effects of individual foods or nutrients, as we had to allow for multiple testing. PCA does not lead us to the most useful dietary predictor of atopy, but it does offer an objective way to reduce the number of dietary dimensions analysed, while retaining as much dietary information as possible.

In conclusion, this is the first strong evidence that large urban/rural differences in skin sensitivity in developing countries can partly be explained by differences in diet. The ability to demonstrate this, using principal components of diet, but not by exhaustive analysis of all foods, reflects the value of reducing the number of dietary dimensions.

Acknowledgements

This study was carried out as part of a research programme funded by the Department of Health's Policy Research Programme. James Calvert was supported by a Wellcome Training Fellowship in Clinical Tropical Epidemiology. The authors acknowledge the contributions of Lesley Bourne and Petro Wolmerans from the Medical Research Council (MRC) in South Africa, who advised on foods to be included in the FFQ, and Thabisile Hlatshwayo-Moleah, University of the Transkei, who advised on foods included in rural diets, as well as assisting in the design of questionnaires and in the training of staff in the administration of nutritional surveys.

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