

This is the peer reviewed version of the following article:

Greater dietary variety is associated with better biochemical nutritional status in Spanish children: the Four Provinces Study.
Royo-Bordonada MA, Gorgojo L, Ortega H, Martín-Moreno JM, Lasunción MA, Garcés C, Gil A, Rodríguez-Artalejo F, de Oya M; Investigators of the Four Provinces Study.
Nutr Metab Cardiovasc Dis. 2003 Dec;13(6):357-64.

which has been published in final form at:

[https://doi.org/10.1016/s0939-4753\(03\)80004-2](https://doi.org/10.1016/s0939-4753(03)80004-2)

“Greater dietary variety is associated with better biochemical nutritional status in Spanish children: The Four Provinces Study”

Miguel A. Royo-Bordonada¹, Lydía Gorgojo¹, Henar Ortega², José M. Martín-Moreno³, Miguel A. Lasunción^{2,4}, Carmen Garcés⁵, Angel Gil⁶, Fernando Rodríguez-Artalejo⁷, Manuel de Oya⁵ on behalf of the investigators of the Four Provinces Study.[†]

¹ Instituto de Salud Carlos III. Ministerio de Sanidad y Consumo. Madrid

² Servicio de Bioquímica-Investigación, Hospital Ramón y Cajal, Madrid

³ Dirección General de Salud Pública. Ministerio de Sanidad y Consumo. Madrid

⁴ Departamento de Bioquímica y Biología Molecular, Universidad de Alcalá de Henares. Madrid

⁵ Unidad de Lípidos. Fundación Jiménez Díaz. Universidad Autónoma de Madrid. Madrid

⁶ Facultad de Ciencias de la Salud. Universidad Rey Juan Carlos. Madrid

⁷ Departamento de Medicina Preventiva y Salud Pública. Universidad Autónoma de Madrid

[†] Investigators of the Four Provinces Study: Mercedes Benavente, Rafael Rubio, José L. del Barrio, Alexis Studer (Fundación Jiménez Díaz), Ovidio Fernández (Hospital Cristal Piñol, Orense), Alfonso Macías, Alipio Mangas (Universidad de Cádiz), Jacinto Fernández Pardo (Hospital General Universitario, Murcia), Diego Gómez-Coronado Cáceres (Hospital Ramón y Cajal).

KEYWORDS: Diet variety, children, nutrients, diet, food surveys.

Address for correspondence:

Miguel Angel Royo Bordonada

Area de Jefatura de Estudios.

Escuela Nacional de Sanidad. Instituto de Salud Carlos III
C/ Sinesio Delgado 8
28029 Madrid

Tel: +34 91 3877857

Fax: +34 91 3877862

E-mail: mroyo@isciii.es

Short title: Dietary variety and nutritional status in children

ABSTRACT

Background and Aim. Although dietary variety has been associated with a better nutritional profile, its possible role in obesity makes its overall health benefits doubtful. In this study we examine the association between dietary variety and anthropometric variables, food intake, and various biomarkers of this intake in Spanish children.

Methods and Results. This is a cross-sectional study of 1112 children between 6 and 7 years old, from Cadiz, Murcia, Orense and Madrid, selected through random cluster-sampling of schools. Information on food and nutrient intake was obtained through a food frequency questionnaire (FFQ). The dietary variety index (DVI) was calculated based on the number of different foods consumed more than once-a-month. The anthropometric variables (weight and height) and the plasma lipid and vitamin levels were determined using standardized methods. Our results show that the body mass index (BMI) did not vary substantially as a function of DVI. Average BMI was 16.9 in the lowest tertile of DVI and 17.2 in the highest ($p=0.20$). Unlike BMI, DVI was positively associated ($p< 0.05$) with plasma levels of α and β -Carotene, lycopene, retinol, α -tocopherol, and vitamin E, with energy intake and with most of the foods, particularly vegetables, fruits, and sausages (coefficients of correlation: 0.43, 0.26, and 0.23 respectively).

Conclusions. **Dietary variety is associated with a better food and nutritional profile in Spanish children. Nevertheless, the presence of a positive association between DVI and energy intake, and consumption of sausages and pre-cooked products calls for the recommendation of a varied diet with respect to healthy foods, such as cereals (especially whole grains), fruits, and vegetables.**

INTRODUCTION

Traditionally, nutritional epidemiology has focused on the health effects of specific nutrients and foods. To investigate their effects on health, some studies have analyzed the role of specific foods or nutrients such as garlic(33) or linolenic acid(16). Other studies have grouped foods or nutrients according to a series of common characteristics, for example fruits and vegetables(46) or monounsaturated fatty acids(24). Nevertheless, diet is a collection of diverse foods, nutrients and other chemical substances whose possible interactions cannot be analyzed in all their complexity using the aforementioned approaches(48).

Therefore, the growing development of studies that look at diet from a global perspective, using dietary patterns or food variety, could contribute to our understanding of the mechanisms by which diet affects health(9) and this could be useful when making nutritional guidelines and policies(12).

Dietary guidelines encourage a varied diet, a reasonable recommendation if we consider that a human being needs to consume more than 40 different essential nutrients, found widely distributed among foods(44, 49). Furthermore, not only a more healthy nutritional profile has been observed among individuals with a more varied diet(15, 20, 21, 26, 35, 42), but also the indices that measure dietary diversity and variety have been correlated with greater longevity(18, 19) and a reduced risk of vascular problems(51) and some types of cancer(11, 25). Even so, the possible influence of dietary variety on the development and persistence of obesity raises doubts about its possible health benefit(36). Furthermore, recent evidences seem to indicate that the protective health effect associated with dietary diversification is more closely related to intake of specific foods considered healthy(17, 32).

This study, which analyzes the diet of school-age children in four Spanish cities with different demographic and sociocultural characteristics, complements the available information on the validity of varied food choices as an indicator of dietary quality. To this end, we examined the correlations between dietary variety and anthropometric variables, caloric intake, intake of principal food groups and various biomarkers of this intake.

METHODS

Study subjects

We selected representative samples of school children, ages 6-7, in four regions in Spain (Cadiz, Madrid, Orense and Murcia) over the period 1998-1999. These regions were chosen mainly because of the large difference in cardiovascular mortality between them. More detailed information about the design of the study is available in previous publications (37, 38).

Children were selected by means of random cluster-sampling in schools, and stratified by sex and type of school (i.e., public versus private). Type of school was an indicator of socio-economic level. Sampling was carried out in two stages: in the first, schools were selected from lists supplied by the regional educational authorities; and in the second, classrooms and pupils were selected. In each region six schools were selected. Approximately 50 children were invited to participate in each school. (38) All children reported by parents to be suffering from metabolic, endocrine, liver or kidney disorders were excluded so as to rule out the possibility of the values of any of the variables of interest being altered.

The study protocol complied with Helsinki Declaration guidelines and Spanish statutory provisions governing clinical research on humans(1), and was formally approved by the Clinical Research Ethics Committee of the Jiménez Díaz Foundation in Madrid (Spain).

Data collection and study variables

The study was orally presented to the Board of Governors (Consejo Escolar) of each of the schools. Following this, a letter was circulated to the parents of all children invited to participate in the study, outlining the study goals and procedures, and securing their written authorization. In addition, this same letter urged all parents to seek the necessary consent from their children.

Food and nutritional data:

At each school, data were collected by a field team, comprising a physician, a nurse and a group of persons purpose-trained in the use of a food frequency questionnaire (FFQ), who conducted the survey and obtained the information from the children's mothers or the people responsible for their food.

The FFQ, initially developed for use on adults and previously validated in Spain by Martín Moreno et al.(28),(28) was adapted for a primary-school population by amending and downscaling the list of foods and portions consumed, eliminating alcoholic beverages and including some foods frequently found in a child's diet (e.g., pizzas, hamburgers, etc.). These amendments were based on a recent, systematic, in-depth review of child-population food surveys in Spain(13). The final version of the FFQ included a total of 77 food items grouped under 11 headings by affinity in nutrient content. For each food, the usual size of the serving eaten was defined (e.g., 1 cup of milk equivalent to 170 cc.; a dish of lentils equivalent to 60 g. dry weight) and the mean frequency of consumption of such servings over the previous year ascertained. The FFQ provided 5 consumption frequency scales (never, annually, monthly, weekly and daily). Standard Spanish food-composition tables were used to convert

the foods into nutrients and total caloric intake for each of the subjects included in the study(29, 34).

Anthropometric variables

Measurements were taken with the children lightly dressed and barefoot. Height was measured to the last millimeter using a portable stadiometer, and weight was measured to the last 0.1 kg using a standardized electronic digital scale. From these measurements, body mass index (BMI)(weight in kilograms divided by the square of the height in meters: kg/m^2) was then computed. Prevalence of overweight and obesity was calculated as the percentage of children exceeding BMI cut-off points proposed in a recent synthesis of international studies(6).

Biochemical data

Fasting (12-hours) venous blood samples were obtained by venipuncture into a Vacutainer tube containing EDTA. Samples were kept on ice and sent to the study's central laboratory for analysis. Once centrifuged, the fractions were separated and frozen at -70°C . Cholesterol and triglycerides were measured enzymatically (Menarini Diagnostics, Italy) with an RA-1000 Autoanalyzer. The cholesterol bound to high density lipoproteins (HDL-C) was determined after precipitation of lipoproteins containing apoB with phosphowolframic acid and Mg (Boehringer Mannheim, Germany). The cholesterol bound to low density lipoproteins (LDL-C) was calculated using the Friedewald formula. Concentrations of retinol, α -tocopherol, γ -tocopherol, α -carotene, β -carotene and lycopene in plasma were measured by High-performance liquid chromatography (HPLC), using a modified form of the method described by Schafer y

Walldius(41).

Dietary variety index (DVI)

Dietary variety has traditionally been measured on the basis of the number of different foods consumed during a 3-day period(10, 23, 29). In this study, however, the FFQ used allowed the measurement of habitual intake and contained a fixed number of food items, some of which included several distinct nutrients. For example, the same item included foods such as eggplants, zucchini, cucumber, carrot, pumpkin and peppers. Consequently, in accordance with a method proposed by other authors(31), dietary variety was calculated using the number of different types of foods (items of the questionnaire) eaten more than once a month. Furthermore, similar foods were grouped as one single item for their evaluation as a component of dietary variety as suggested in the USDA Center for Nutrition Policy and Promotion guidelines(50). For example, whole milk and 1% fat milk were grouped in the same item, as were roasted, boiled or fried potatoes. Thus, the final number of items was 72.

Statistical data analysis

After tabulation and basic descriptive analysis of the data, a t-test and analysis of variance were used to compare the mean values between groups. The Pearson correlation coefficients were used to examine the linear association between quantitative variables. To calculate these, variables that didn't follow a normal distribution were transformed

logarithmically. Linear regression was used to calculate the magnitude of this association. Analysis of covariance was used to adjust the means for caloric intake. The analysis was carried out for all nutrients with and without adjusting for total caloric intake(27). All comparisons were two-sided at a 0.05 significance level. Statistical analyses were performed using the Statistical Analysis System computer software package(5).

RESULTS

The rate of response was 85%, with little variation among the four cities. The sample generated included 1112 individuals, 557 (50.1%) boys and 555 (49.9%) girls, with an average age of 6.7. The DVI fell in a range of 21 to 60, from a possible 0 to 72. The mean value was 42. The BMI did not vary significantly as a function of dietary variety, its values were 16.9 in the lowest tertile and 17.2 in the highest tertile ($p=0.20$). Although the percentages of overweight and obese children were higher in the group of children from the upper tertile of DVI (31.9%, and 14.5% respectively) than in the lower tertile (30.7%, and 11.3% respectively), these differences were not statistically significant (Table 1).

In table 2 a positive and statistically significant correlation ($p<0.05$) can be seen between DVI and caloric intake, as well as consumption of oils and fats, meat and fish, legumes and cereals, fruits, vegetables, and pre-cooked foods. The strongest associations were seen with consumption of vegetables ($CC=0.43$), fruits ($CC=0.26$) and sausages ($CC=0.23$). However, in all cases the strength of the association was reduced when adjusted for total caloric intake. For dairy products, the positive association seen in the raw analysis became negative when adjusted for total caloric intake. Finally, there was not a statistically significant association between consumption of carbonated beverages and DVI. As for salt, the weak correlation found was negative.

To calculate the magnitude of the slope of caloric intake as a function of DVI, we used a linear regression model. On a percentile scale, the coefficient of linear regression of the DVI was 0.32 ($p<0.0001$). In other words, as variety increased so did caloric intake but at a lower than proportional rate.

As seen in table 3, after adjusting for total caloric intake, the DVI showed a positive and statistically significant correlation ($p<0.05$) with plasma levels of α and β -carotene, lycopene, retinol, α -tocopherol and vitamin E. Children in the upper tertile of DVI showed higher plasma levels of HDL-C and lower of triglycerides, although these differences were of small magnitude and did not reach statistical significance.

repiSälud
Repositorio Institucional en Salud

DISCUSSION

The results of this study suggest that a varied diet is generally associated with a more healthy dietary pattern and biochemical profile in Spanish children. Dietary variety was positively correlated with the intake of principle food groups and negatively with salt intake. A more varied diet was accompanied by higher plasma levels of HDL-C, carotenes, lycopene, retinol, α -tocopherol and vitamin E and slightly lower levels of triglycerides. Furthermore, although caloric intake and that of oils and fats increase with dietary variety, the differences observed in BMI and prevalence of excess weight and obesity were small to moderate in magnitude and not statically significant.

When interpreting the results obtained, the inherent limits of the measuring instrument and the fact that the main source of information was the memory of the children's mothers must be kept in mind. Although some studies show that questionnaires on frequency of food consumption produce an overestimation of caloric intake in children(45), others indicate that it is possible to adequately measure habitual intake of energy and nutrients with this type of questionnaires(14, 47). Also, there is evidence that mothers inform adequately about meals that children have at home(22, 31). On the other hand, it should be noted that the impact of a specific type of food on dietary variety depends on the number of different foods included in the same item of the FFQ. In general, if more foods are included in the same item that item will have a greater contribution to dietary variety. Because of this, the different structure of the FFQ used in different studies could explain some of the differences in the results. Finally, since this is a cross sectional study, its impossible to infer directly that a change in dietary variety caused by a clinical or community intervention will translate into

a specific change in the consumption of specific foods, or vice-versa. Nevertheless, this study is useful to characterize which foods contribute most to variety in the diet of most Spanish children. According to our results (table 2) these foods are fruits, vegetables and sausages.

There is evidence that the amount of food needed to feel satiated and therefore the amount consumed vary with the type(39) and number of different foods eaten(36). According to our results the relationship between variety and energy intake is not quite proportional, which suggests that subjects reduce the total intake of each food as dietary variety increases. However, in the case of high-calory foods, such as certain condiments (butter, ketchup, margarine, mayonnaise, sauces, dressings, etc.), carbonated beverages, and baked goods the increase in dietary variety is associated with a higher degree of obesity and an increase in body fat(30, 36). Unfortunately, in recent decades foods of these type have become much more available in the supermarkets of the developed world(36). Consequently, it is not surprising that, in the populations of western countries, greater dietary variety comes from the consumption of more diverse foods of all types, including processed and high-calory foods(30). However, in our study it was the consumption of fruits and vegetables which presented the strongest association with DVI. This phenomena could explain the weak association found between the degree of obesity and dietary variety. On the other hand, the positive association between DVI and energy intake could merely represent differences on physical activity, as seen previously in other context. Furthermore, the DVI showed a very weak correlation with the intake of baked goods and no correlation with carbonated beverages consumption. Therefore it is still possible that the absence of a clear association between DVI and degree of obesity could be due to an underestimation of the actual effect.

Nutrients are found widely distributed among foods and no one food group provides all essential nutrients. The fact that dietary variety is positively associated with the consumption of a majority of the food groups, explains the positive association observed with plasma levels of all micronutrients studied. This is consistent with the results of previous studies that show a higher intake of various vitamins in individuals with more varied diets(15, 20, 21, 26, 35, 42). Plasma carotene levels are considered reliable markers of vegetable intake(8). Consequently, the association observed between DVI and plasma carotene and lycopene levels, provides additional evidence confirming that vegetable intake is one of the main elements that contributes to increasing the dietary variety of Spanish children.

The total intake of fat, lipids and olive oil was higher in the upper tertile of dietary variety. In any case, the quality of the fat consumed was somewhat better in Spanish children with a more varied diet. These children showed a lower consumption of saturated fatty acids and a higher consumption of unsaturated fatty acids(40). In spite of these observations, the plasma lipid profile in the children with a more varied diet was not clearly more healthy. This contrasts with the notable improvement in lipid profile seen in a previous study of the elderly population, particularly when the index used only considered the variety of fruit and vegetable intake(4). On the other hand, salt intake showed a negative correlation with dietary variety, a previously observed phenomenon(7). The excess of salt intake of Spanish children and adolescents(13, 38, 43) could be related to the high blood pressure values observed in this population(2) and the elevated prevalence of hypertension among the

middle age population(3). Therefore, in this specific aspect, the promotion of a varied diet could contribute, although very modestly, to controlling an important cardiovascular risk factor.

Summing up, our results show that dietary variety tends to be associated with a better nutritional profile in Spanish children. Nevertheless, we should be cautious when making recommendations, since our results show a positive association between DVI and energy intake (including a parallel correlation with consumption of sausages and pre-cooked products), and this could lead to possible overweight and/or obesity. In this sense, we share the general concern about the potential risks of a varied diet in populations with predominant intake of high-calory foods. We are in agreement with the basic recommendation towards a varied diet with respect to healthy foods, such as cereals (especially whole grains), fruits, and vegetables(36, 48).



Acknowledgement of funding: This study was partly funded by grants from the Madrid Regional Authority (*Comunidad de Madrid 08.4/0006/1997*) and Pedro Barrié de la Maza Foundation.

LITERATURE CITED

1. Boletín Oficial del Estado (1993) Royal Decree (Real Decreto) 561/1993, of 16 April: governing the requirements for and manner in which clinical drug trials are to be conducted. BOE number 114, may 13th of 1993, pp. 14346-14364.
2. Grupo Cooperativo Español para el Estudio de los Factores de Riesgo Cardiovascular en la Infancia y Adolescencia (1995) Factores de riesgo cardiovascular en la infancia y adolescencia en España. Estudio RICARDIN II: valores de referencia. An. Esp. Pediatr. 43: 11-7.
3. Banegas JR, Rodríguez-Artalejo F, de la Cruz JJ, Guallar P, del Rey J. (1998) Blood pressure in Spain. Distribution, awareness, control, and benefits of a reduction in average pressure. Hypertension 32: 998-1002.
4. Bernstein M, Tucker KL, Ryan N, O'neill E, Clements K, Nelson M, Evans W. (2002) Higher dietary variety is associated with better nutritional status in frail elderly people. J. Am. Diet. Assoc. 102: 1096-104.
5. Cody RP, Smith JK. Applied Statistics and the SAS programming language. New Jersey: Prentice-Hall, 1991.
6. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 320: 1240-3.
7. Drewnowski A, Ahlstrom S, Driscoll A, Rolls BJ. (1997) The dietary variety score: assessing diet quality in healthy young and older adults. J. Am. Diet. Assoc. 97: 266-71.
8. Drewnowski A, Rock Ch, Henderson SA, Shore AB, Fischler C, Galan P, Preziosi P, Hercberg S. (1997) Serum beta-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. Am. J. Clin. Nutr. 65: 1796-802.
9. Erickson K. (2002) Dietary pattern analysis: a different approach to analyzing and old problem, cancer of the esophagus and stomach. Am. J. Clin. Nutr. 75: 5-7.
10. Fanelli MT, Stevenhagen KJ. (1985) Characterizing consumption patterns by food frequency methods: Core foods and variety of foods in diets of older Americans. J. Am. Diet. Assoc. 85: 1570-6.

11. Fernández E, D'Avanzo B, Negri E, Franceschi S, La Vecchia C. (1996) Diet diversity and the risk of colorectal cancer in Northern Italy. *Cancer Epidemiol. Biomarkers Prev.* 5: 433-6.
12. Gibney MJ, Serra LI, Kearney JM, Becker W, Löwik MRH, Wiseman M, de Henauw S, Haraldsdóttir J, Flynn MAT, Graça P, Kafatos A, Koenig J, Leclercq C, Lambe J, Ribas L, Román B, Valsta L, Volatier J, Wearne S. (2000) Por una política de nutrición en Europa en el siglo XXI: desarrollo de unas guías alimentarias basadas en el consumo de alimentos. *Nutr. Clín.* 20: 29-46.
13. Gorgojo L, Guallar E, Martín-Moreno JM, López-Nomdedeu C, Vázquez C, Martí-Henneberg C, Serrano-Ríos M. (1999) Encuestas alimentarias en los niños españoles de edad escolar: análisis del período 1984-1994. *Med. Clín. (Barc.)* 112: 368-74.
14. Hammond J, Nelson M, Chinn S, Rona RJ. (1993) Validation of a food frequency questionnaire for assessing dietary intake in a study of coronary heart disease risk factors in children. *Eur. J. Clin. Nutr.* 47: 242-50.
15. Hatloy A, Torheim LE, Oshaug A. (1998) Food variety-a good indicator of nutritional adequacy of the diet? A case study from an urban area in Mali, West Africa. *Eur. J. Clin. Nutr.* 52: 891-8.
16. Hu FB, Stampfer MJ, Manson JE, Rimm EB, Wolk A, Colditz GA, Hennekens CH, Willet WC. (1999) Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *Am. J. Clin. Nutr.* 69: 890-7.
17. Kant AK, Schatzkin A, Graubard BI, Schairer C. (2000) A prospective study of diet quality and mortality in women. *JAMA.* 283: 2109-15.
18. Kant AK, Schatzkin A, Harris TB, Ziegler RG, Block G. (1993) Dietary diversity and subsequent mortality in the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Am. J. Clin. Nutr.* 57: 434-40.
19. Kant AK, Schatzkin A, Ziegler RG. (1995) Dietary diversity and subsequent cause-specific mortality in the NHANES I epidemiologic follow-up study. *J. Am. Coll. Nutr.* 14: 233-8.
20. Kant AK, Schatzkin A, Ziegler RG, Nestle M. (1991) Dietary diversity in the US population, NHANES II, 1976-1980. *J. Am. Diet. Assoc.* 91: 1526-31.
21. Kant AK, Thompson FE. (1997) Measures of overall diet quality from a food frequency questionnaire: National Health Interview Survey, 1992. *Nutr. Res.* 17: 1443-56.

22. Klesges RC, Klesges LM, Brown G, Frank GC. (1987) Validation of the 24-hour dietary recall in preschool children. *J. Am. Diet. Assoc.* 87:1383-5.
23. Krebs-Smith SM, Smiciklas-Wright H, Guthrie HA, Krebs-Smith J. (1987) The effects of variety in food choices on dietary quality. *J. Am. Diet. Assoc.* 87:897-903.
24. Kris-Etherton PM. (1999) Monounsaturated fatty acids and risk of cardiovascular disease. *Circulation* 100: 1253-8.
25. La Vecchia C, Muñoz SE, Braga C, Fernández E, Decarli A. (1997) Diet diversity and gastric cancer. *Int. J. Cancer* 72: 255-7.
26. Marshall TA, Stumbo P, Warren J, Xian-Jin X. (2001) Inadequate nutrient intakes are common and are associated with low dietary variety in rural, community-dwelling elderly. *J. Nutr.* 131: 2192-6.
27. Martín-Moreno JM. (1993) Adjustment for total caloric intake in nutritional studies: an epidemiologic perspective. *Eur. J. Clin. Nutr.* 47(suppl 2): S51-S52.
28. Martín-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, Willet WC. (1993) Development and validation of a food frequency questionnaire in Spain. *Int. J. Epidemiol.* 22: 512-9.
29. Mataix J et al. (1998) Tabla de composición de alimentos españoles. Editorial Universidad de Granada, Campus Universitario de Cartuja, Granada.
30. McCrory MA, Fuss P, McCalum J, nken A, Berts S. (1999) Dietary variety within food groups: association with energy intake and body fatness in men and women. *Am. J. Clin. Nutr.* 69: 440-7.
31. McCullough ML, Feskanich D, Rimm EB, Giovannucci EL, Ascherio A, Variyam JM, Spiegelman D, Stampfer MJ, Willet WC. (2000) Adherence to the dietary guidelines for americans and risk of major chronic disease in men. *Am. J. Clin. Nutr.* 72: 1223-31.
32. Michels KB, Wolk A. (2002) A prospective study of variety of healthy foods and mortality in women. *Int. J. Epidemiol.* 31: 847-54.
33. Milner JA. (2001) A historical perspective on garlic and cancer. *J. Nutr.* 131: 1027S-31S.
34. Moreiras-Varela O, Carbajal A, Cabrera L. (1999) Tablas de composición de alimentos. Ediciones Pirámide, Madrid.
35. Randall E, Nichaman M, Contant Ch. (1985) Diet diversity and nutrient intake. *J. Am. Diet. Assoc.* 85: 830-6.

36. Raynor HA, Epstein LH. (2001) Dietary variety, energy regulation, and obesity. *Psicol. Bull.* 127: 325-41.
37. Rodríguez-Artalejo F, Garcés C, Gil A, Lasunción MA, Martín-Moreno JM, Gorgojo L, de Oya M. (1999) Estudio Cuatro Provincias: principales objetivos y diseño. *Rev. Esp. Cardiol.* 52: 319-26.
38. Rodríguez-Artalejo F, Garcés C, Gorgojo L, López E, Martín-Moreno JM, Benavente M, del Barrio JL, Rubio R, Ortega H, Fernández O, de Oya M. (2002) Dietary patterns among children aged 6-7 years in four Spanish cities with widely differing cardiovascular mortality. *Eur. J. Clin. Nutr.* 56: 1-8.
39. Rolls BJ, Fedoroff IC, Guthrie JF, Laster LJ. (1990) Foods with different satiating effects in humans. *Appetite* 15: 115-26.
40. Royo-Bordonada MA, Gorgojo L, Martín-Moreno JM, Garcés C, Rodríguez-Artalejo F, Benavente M, Studer A, de Oya M. (2003) Variedad y diversidad de la dieta de niños españoles: Estudio Cuatro Provincias. *Med. Cín. (Barc.)* 120: 167-71.
41. Schafer L, Walldius G. (1992) Simultaneous measurement of serum probucol and lipid-soluble antioxidants. *J. Lipid. Res.* 33: 131-7.
42. Schuette L, Song W, Hoerr S. (1996) Quantitative use of Food Guide Pyramid to evaluate dietary intake of college students. *J. Am. Diet. Assoc.* 96: 453-7.
43. Serra-Majem LI, García-Closas R, Ribas L, Perez-Rodrigo C, Aranceta J. (2001) Food patterns of Spanish schoolchildren and adolescents: The enKid Study. *Public Health Nutr.* 4: 1433-8.
44. Sociedad Española de Nutrición Comunitaria (2001) Guías alimentarias para la población española. Recomendaciones para una dieta saludable. IM&C, Madrid.
45. Stein AD, Shea S, Basch Ch, Contento IR, Zybert P. (1992) Consistency of the Willet semiquantitative food frequency questionnaire and 24-hour dietary recalls in estimating nutrient intakes of preschool children. *Am. J. Clin. Nutr.* 135: 667-77.
46. Steinmetz K, Potter J. (1991) Vegetables, fruit, and cancer. I. *Epidemiology. Cancer Causes Control* 2: 325-57.
47. Treiber FA, Leonard SB, Frank G, Davis H, Levy M. (1990) Dietary assessment instruments for preschool children: Reliability of parental responses to the 24-hour recall and a food frequency questionnaire. *J. Am. Diet. Assoc.* 90: 814-20.

48. Tucker KL. (2001) Eat a variety of healthful foods: old advice with new support. *Nutr. Rev.* 59: 156-8.
49. US Department of Agriculture and US Department of Health and Human Services (2000) Nutrition and your health: Dietary Guidelines for Americans. 5th. Home and Garden Bulletin No. 232, 1-39. US Government Printing Office, Washington, DC.
50. US Department of Agriculture (1995) The healthy eating index. US Department of Agriculture, Washington, DC.
51. Wahlqvist ML, Sam Ch, Myers K. (1989) Food variety is associated with less macrovascular disease in those with type II diabetes and their healthy controls. *J. Am. Coll. Nutr.* 8: 515-23.