

Assessment of dietary nutrient intakes: analysed vs calculated values

María Rodríguez-Palmero, ^{a*} Ana I. Castellote-Bargalló, ^a Carmen López-Sabater, ^a Carmen de la Torre-Boronat^a & Montserrat Rivero-Urgell^b

a University of Barcelona, Department of Human Physiological Sciences and Nutrition, Av. Joan XXIII s/n, 08028 Barcelona, Spain
b Scientific Department Laboratorios ORDESA, Barcelona, Spain b Scientific Department, Laboratorios ORDESA, Barcelona, Spain

(Received 10 February 1997; accepted 21 May 1997)

In this study we compared the nutrient composition of the diet of a nursing home calculated from food composition tables with that obtained from laboratory analysis of cooked mixed dishes. A total of 43 food samples were analysed. Analysed parameters comprised fat, fatty acids, cholesterol, α -tocopherol and eight minerals. We found that calculated values were a good estimation of energy, fat, polyunsaturated fatty acids (PUFA), cholesterol, potassium and phosphorus intakes. In contrast, other parameters showed differences between calculated and analysed values, which ranged from 11% for magnesium intake to 56% for sodium intake ($p < 0.05$). Values obtained from tables tended to underestimate saturated fatty acids, monounsaturated fatty acids, calcium, magnesium and sodium intakes, and overestimate α -tocopherol and iron intakes. Potential sources of error in both methods were studied. \odot 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Food composition data are used for many purposes in nutrition, food science and dietetics. They are crucial in assessing nutrient intakes in epidemiological studies that investigate the relation between diet and health, as well as in designing nutrient intervention studies. In addition, food composition information is needed when establishing policies and dietary guidelines for the general population according to health concerns; when formulating diets for population groups with special requirements, the pregnant, elderly, obese and physically active; in the food industry, for quality control of foods, development of new food products, food labelling or consumer information. These new applications of food tables have given rise to the need to test their validity. Scientists have emphasised the importance of improving the accuracy and reliability of food composition information. The need to use modern analytical methods for foods and also the inclusion of other components of foods in databases has also been stressed (Dwyer, 1994).

Comparison of intakes calculated from food composition tables with those obtained from laboratory analysis may provide an idea of the likely degree of accuracy of such tables for the prediction of food intake. In this sense, some studies have revealed differences between analysed and calculated data when assessing intakes of certain nutrients (Finglas et al., 1993; Bailey et al., 1994; Southon et al., 1994), suggesting that there is a risk of systematic errors when using food composition tables. However, while many studies have compared calculated and analysed intakes of specific nutrients (Mangels et al., 1990; Finglas et al., 1993; Bailey et al., 1994), few have compared total nutrient intakes.

The aim of this study was to compare lipid and mineral intakes in a group of elderly institutionalized people calculated from food tables with those obtained from chemical analysis of cooked mixed diets. In addition, we tried to identify potential sources of error when assessing dietary intakes by food tables, as well as potential nutrient groups susceptible to those errors.

MATERIALS AND METHODS

The study was carried out in a municipal nursing home for elderly people in Barcelona (Spain). Nutrient composition of the diet of the centre was studied. The diet, consisting of three weekly menus served in rotation, was designed by nutritionists to meet the Recommended Dietary Allowances (RDA) (National Research Council, 1989) and could be considered balanced and representative of the

^{*}To whom correspondence should be addressed.

western diet. Dishes were prepared according to usual recipes widely consumed in Spain (e.g. macaroni with tomato sauce, lentils with rice, mashed potatoes, omelette, turkey, oven cooked fish and chicken with potatoes). Meals were cooked by the staff of the centre following strict protocols of preparation in order to maintain a constant diet. Four meals per day were given and usually no additional food was eaten. Comparison between analysed and calculated data was based on intakes of 21 days (corresponding to the three weekly menus).

For the assessment of nutrient intake, the weights of all the ingredients of the diet were recorded. In addition, dishes served and returned by subjects at each meal were weighed for 6 consecutive weeks to determine the average quantity of every dish consumed by individuals. These weights were used to determine calculated and analysed intakes. Calculated mean daily nutrient intakes were determined from food composition tables by using the Tablas de composición de alimentos españoles (Mataix *et al.*, 1995). This food database is a compilation of data of other food tables and also includes analytical data. It was chosen because it is the most recent of the Spanish food tables and because it includes data from previous Spanish tables. McCance and Widdowson's The Composition of Foods (Holland et al., 1991) was also used for the composition of foods not present in the Spanish tables. On the other hand, the lipid fraction of every cooked dish and some ingredients was analysed and the following parameters were determined: total fat, fatty acids, cholesterol and α -tocopherol. The mineral content of cooked dishes and ingredients was also determined. The nutrient content of all the dishes consumed each day were combined and finally the results for all days were averaged to obtain daily intakes. Analysed daily energy intake was calculated as follows:

energy (kcal day⁻¹) = calculated protein (g day⁻¹) \times 4(kcal g⁻¹) + calculated carbohydrates $(g \, day^{-1}) \times 3.75(kcal g^{-1})$ + analysed fat $(g day^{-1}) \times 9(kcal g^{-1}),$

where protein, carbohydrate and fat were expressed as total ingestion in $g \, day^{-1}$. Calculated carbohydrate intake did not include dietary fibre.

Food analysis

Representative samples of each cooked dish served to the subjects were taken in duplicate on different days by one of the investigators, placed in sealed food containers and taken immediately to the food analysis laboratory. Each cooked dish with all the ingredients was ground and homogenized and when it was not analysed on the same day, it was stored under N_2 atmosphere at -20 °C. A total of 31 cooked dishes and 12 ingredients were analysed.

The total fat content of the samples was determined by acid hydrolysis followed by Soxhlet extraction with petroleum ether (Official Methods of Analysis, 1995a). This method can alter fat composition and so a qualitative lipid extraction was carried out using dichloromethane: methanol $(2:1, v/v)$ according to the method proposed by Chen et al. (1981). The extracted fat was used to determine the following parameters: fatty acid composition, cholesterol and α -tocopherol. Analyses were performed in duplicate.

Fatty acids were measured following Metcalfe et al. (1966). About 200 mg of fat was esterified with 5 ml of 0.5% sodium methoxide in a boiling water bath for 15 min. After cooling, 6 ml of boron trifluoride-methanol was added and the tubes were placed in the boiling bath for another 15 min. The fatty acid methyl esters were extracted with 2 ml of hexane and quantified by gas±liquid chromatography on an HP 5890A chromatograph (Hewlett-Packard, Palo Alto, USA) equipped with a $60 \text{ m} \times 0.25 \text{ mm}$ ID SP-2380 column (Supelco, Bellefonte, PA, USA) and a flame ionization detector. The temperature program was: initial oven temperature 160° C for 2 min, then increasing at a rate of 2° C min⁻¹ up to 235° C for 5 min. The injector and detector temperatures were 250 and 275° C, respectively. The linear velocity of the carrier gas was 20.5 cm s^{-1} .

Determination of cholesterol content was performed by capillary gas chromatography (Rodríguez-Palmero $et al., 1994$). The method consisted of saponification of 100 mg of sample with 0.5 ml of aqueous KOH solution (0.4 mg/ml) after addition of 8 ml of ethanolic butylhydroxyanisole (BHA) solution. After incubation in a water bath at 80° C for 15 min, the unsaponifiable matter was extracted twice with cyclohexane. After removal of solvent, bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) and pyridine were added as derivatizating agents. $5-\alpha$ -cholestane was used as an internal standard. Cholesterol was analysed in a Shimadzu GC-14A gas-liquid chromatograph (Kyoto, Japan) equipped with a flame ionization detector and a $30 \text{ m} \times 0.25 \text{ mm}$ I.D. fused-silica capillary column (Supelco, Bellefonte, PA, USA) coated with SPB-5 under the following conditions: oven temperature constant at 280° C, injector temperature at 290 \degree C, flame ionization detector at 310 \degree C, and linear velocity of carrier gas of 30 cm s^{-1} .

The α -tocopherol content of dishes was determined by the method proposed by Stancher and Zonta (1983). About 1 g of sample was weighed in a volumetric flask, and 0.1 g of ascorbic acid, 5 ml of distilled water and 5 ml of methanol were added. Samples were saponified overnight with 5 ml aqueous KOH solution (80%, v/v) at room temperature. After saponification, the sample was transferred to a separatory funnel with 60 ml of methanol:water $(20:40, v/v)$, and the unsaponifiable matter was extracted three times with 50 ml of diethyl ether, concentrated and redissolved in methanol. The internal standard was α -tocopheryl acetate. α -tocopherol

analysis was performed in a Hewlett Packard liquid chromatographic system (Hewlett Packard, PA, USA) equipped with an HP-1050 pump system. The detector was an HP-1040M photodiode-array detector. A Spherisorb ODS-2 $(250 \times 4.6 \text{ mm } I.D.; 5 \mu \text{m}$ particle size) column protected by a guard cartridge (C18, $5 \mu m$) system (Tracer, Analitica) was used. Isocratic elution at 50° C was performed with methanol:water (96:4) at a flow rate of 1 ml min⁻¹. A 10 μ l aliquot of sample was injected and α -tocopherol was detected at 292 nm.

For analysis of mineral content, portions of all cooked dishes were combined, ground and homogenized daily for 3 consecutive weeks. Duplicate samples of 0.5 g were digested in $HNO₃/HClO₄$ and were finally diluted to 25 ml with $HNO₃ 1%$ (Official Methods of Analysis, 1995b). Samples were analysed in triplicate. Three blanks were also digested with the samples as quality controls. Determination of minerals was performed by inductively coupled plasma (ICP). A Thermo Jarrell-Ahs 61E Polyscan was used in standard conditions, which was calibrated with three standards prepared in acid.

Identification and calculations

Fatty acids, cholesterol and α -tocopherol were identified by comparison of retention times with appropriate standards. All standards were purchased from Sigma (St. Louis, MO, USA). Fatty acids were quantified by internal normalization and their content was expressed as percentage of total fatty acids. The amount of cholesterol in each sample was calculated according to the following equation:

amount cholesterol (mg per gram fat) $=$

area $(X) \times$ amt (IS) \times RF (X) area (IS) \times sample weight (in g)

where area (X) = area of the cholesterol in the chromatogram of the sample; amt (IS) =amount of the internal standard added to the sample; area (IS) = area of the internal standard in the chromatogram of the sample; and $RF(X)$ = response factor of cholesterol relative to 5- α -cholestane (the value obtained experimentally was 1.12). The amount of α -tocopherol was calculated by peak area comparison with the internal standard $(\alpha$ tocopheryl acetate) using the calibration curve $y=4.631x - 0.139$.

Statistical methods

All dietary intake data were examined for normality and variance homogeneity before any further statistical analysis. Differences between analysed and calculated dietary data were tested using the unpaired t -test with $n=21$ (corresponding to the three weekly menus). A two-sided P value ≤ 0.05 was considered significant. For correlation analysis, parametric correlation analysis

(Pearson's r) was used. Analyses were performed by using Statgraphics v. 7.0 computer software program (STSC Inc and Statistical Graphics Corporation, US).

RESULTS

Validation of the analytical methods

Methods used for fatty acids, cholesterol and α -tocopherol analysis were validated by assessing the linearity of response, precision, accuracy and limits of detection and quantification. Precision was determined by analysing and injecting ten aliquots of a given sample and by determining coefficients of variation. The standard addition method was used to test the accuracy. Five levels of known standard amounts were added to a known mass of sample to obtain the recovery. Each level was analysed in triplicate. Limits of detection and quantification were calculated by determining the analytical background response for each compound by injecting ten blank samples into the chromatograph and calculating the standard deviation of this response (The United States Pharmacopeia, 1989).

Validation of the fatty acid analysis method was performed with five standards: C16:0, C18:1n-9, C18:2n-6, $C20:5n-3$, $C22:6n-3$. Coefficients of variation ranged from 1.10 to 1.36%. Mean recoveries were all greater than 93%. Detection and quantification limits ranged from 15 to 23 ng and from 17 to 25 ng, respectively. Validation of the cholesterol analysis method showed a coefficient of variation of 3.21% and a mean recovery of $94.06 \pm 5.20\%$. Limits of detection and quantification were 1.08 and 3.09 ng, respectively. The coefficient of variation obtained in the validation for α -tocopherol analysis method was 8.01%, which is lower than the value calculated by Horwitz (1982) for intralaboratory analysis. Mean recovery was $93 \pm 1.95\%$. Limits of detection and quantification for α -tocopherol were $7.28 \times 10^{-5} \mu$ g and $7.67 \times 10^{-4} \mu$ g, respectively. All methods showed good linearity $(r>0.999)$ in the range of concentrations studied.

Nutrient intakes

Mean daily dietary intakes of energy, total fat, fatty acids, cholesterol, α -tocopherol and minerals were determined by the two methods described above and the results are presented in Tables 1 and 2. We found that calculated values were a good estimation of energy, fat, polyunsaturated fatty acids (PUFA), cholesterol, potassium and phosphorus intakes. In contrast, values obtained from tables tended to underestimate saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), calcium, magnesium and sodium intakes, and overestimate α -tocopherol and iron intakes. Differences between mean calculated and analysed intakes ranged from 11% for magnesium intake to 56% for sodium

		Calculated			Analysed	
		Mean	SD		Mean	SD
Energy	kJ day ⁻¹	7536	612		7205	528
	kcal day ^{-1}	1803	146		1724	126
Protein	$\frac{6}{6}$ total energy)	13.5	1.0			
	$(g day-1)$	60.9	6.38			
Carbohydrate	(% total energy)	65.0	3.9			
	$(g \text{ day}^{-1})$	292.1	15.2			
Fibre	$(g \text{ day}^{-1})$	19.2	3.2			
Fat	$(\%$ total energy)	24.8	3.3		27.0	5.0
	$(g \text{ day}^{-1})$	50.1	10.3		52.0	11.9
Fatty acids	SFA (% total energy)	5.1	1.3	***	7.6	1.6
	$(g day-1)$	10.3	2.7	***	14.6	3.6
	MUFA (% total energy)	6.3	1.6	***	8.7	1.8
	$(g day-1)$	12.7	3.5	\ast \ast	16.6	4.1
	PUFA (% total energy)	10.6	1.4		9.6	2.8
	$(g day-1)$	21.5	3.0		18.5	6.0
	$n-6$ PUFA (% total energy)				9.2	2.8
	$(g \text{ day}^{-1})$				17.8	5.8
	$n-3$ PUFA (% total energy)				0.4	0.3
	$(g \text{ day}^{-1})$				0.7	0.6
	$n6/n3$ ratio				33.2	15.6
	<i>trans</i> FA (% total energy)				0.8	0.1
	$(g day-1)$				1.5	0.4
Cholesterol (mg day ⁻¹)		164.3	122.2		145.0	100.6
α -tocopherol (mg day ⁻¹)		16.6	4.2	***	8.5	3.8

Table 1. Calculated and analysed mean daily intake of nutrients in the nursing home

Values are means of 21 data (corresponding to the three weekly menus).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; trans FA, trans fatty acids. Calculated mean intakes were significantly different from those analysed (unpaired t-test): ** $P < 0.01$; ** $P < 0.001$.

intake. Cholesterol was the nutrient that showed the largest daily fluctuations, depending on consumption of eggs or egg products.

In addition, correlations between calculated and analysed nutrient intakes were examined. Table 3 shows that there was a significant positive relationship between calculated and analysed data for energy, fat, SFA, MUFA, PUFA, cholesterol, α -tocopherol, potassium and phosphorus, with r values ranging from 0.41 to 0.95.

Table 4 presents analysed mean contents of fat and

Table 2. Calculated and analysed mean daily intake of minerals in the nursing home

	Calculated		Analysed		
	Mean	SD	Mean	SD	
Ca (mg)	833.3	$114.3*$	990.5	236.8	
Fe (mg)	10.6	$2.1**$	6.8	5.0	
Y (mg)	0.1	0.0			
Mg (mg)	235.3	$32.4*$	262.0	50.6	
Zn (mg)	14.4	8.2			
Na (mg)	1726.6	*** 266.9	2687.6	289.8	
K (mg)	2406.8	231.2	2524.1	485.3	
P(mg)	1159.1	198.4	1089.5	252.8	
S(mg)			764.6	118.9	
Cu (µg)			1001.6	214.8	

Values are means of 21 data (corresponding to the three weekly menus).

Calculated mean intakes were significantly different from those analysed (unpaired t -test): $*P < 0.05$; $*P < 0.01$; *** $P < 0.001$.

other nutritional components, which are not normally presented in food composition tables, such as $n-6$ PUFA, *n*-3 PUFA and total *trans* fatty acids, of all cooked dishes and ingredients analysed in the present study. Fat content is also presented.

DISCUSSION

In this study we have compared lipid and mineral intakes of an elderly institutionalized population calculated from food composition tables with those from

Table 3. Correlations between calculated and analysed intakes

	r	P
Energy (kJ day ⁻¹)	0.82	${}_{0.001}$
Fat $(\%$ of total energy)	0.56	${}_{0.01}$
Fatty acids		
SFA (% of total energy)	0.61	${}_{0.01}$
$MUPA$ (% of total energy)	0.67	${}_{0.01}$
PUFA $(\%$ of total energy)	0.76	${}_{0.001}$
Cholesterol (mg day ⁻¹)	0.95	${}_{0.001}$
α -tocopherol (mg day ⁻¹)	0.52	${}_{0.05}$
Minerals		
K (mg day ⁻¹)	0.48	${}_{0.05}$
P (mg day ⁻¹)	0.41	${}^{<0.05}$

Only significant correlations are included.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; K, potassium; P, phosphorus; r , correlation coefficient.

PUFA, polyunsaturated fatty acids; trans FA, trans fatty acids. Values are means of duplicate samples and duplicate analysis.

direct laboratory analysis of cooked dishes. Dietary fat is particularly important not only as a source of essential nutrients, but also because of its role in the development of certain chronic diseases such as coronary heart disease, obesity, cancer, diabetes and immunological diseases (Meydani et al., 1990; Schaefer et al., 1995; Cave, 1996). In addition, minerals are essential nutrients which are involved in many physiological functions. Some recent studies have revealed a relationship between mineral intake and prevention of specific diseases, although present knowledge in this area is still rather limited (Wood et al., 1995). The present study focuses on these parameters because they are likely to be susceptible to variation, since fat contains labile nutrients that can suffer degradation during cooking and storage, whilst there are many factors affecting the mineral content of food.

The results of the present study show that there may be significant differences between calculated and analysed values when determining dietary intakes. The largest differences between calculated and analysed values were found for MUFA, SFA and α -tocopherol intakes $(38, 48 \text{ and } 49\% \text{ of difference, respectively}).$ Among minerals, the greatest differences were observed for iron

and sodium (36 and 56%, respectively). Nevertheless, we did not observe a definite pattern in such differences, and calculated values were higher for some nutrients, whereas analysed values were higher for others. These results are consistent with findings described elsewhere, in which differences between analysed and calculated dietary intakes were found (Southon et al., 1994; Hakala et al., 1996). Because the nursing home had an established diet and there were protocols for preparation of dishes, no method of recording diets was used in our study, thus reducing possible systematic errors. Therefore, the results allowed us to compare only differences between calculated and analysed values.

There are various possible sources of error which may explain the differences found between calculated and analysed values in the present study. First, it is known that nutrient composition can vary greatly in the same food due to variety, soil and season (vegetable foods) or age and sex (animal foods). It is also possible that in some cases the foods eaten differ considerably from those analysed when compiling the food tables (Dwyer, 1994). Moreover, the nutrient content of many manufactured products (e.g. sausages and hamburgers), which have a highly variable composition, is reported only in national food tables, but their composition may vary greatly even in the same country. Secondly, most nutrient values in food composition tables are based on the contents of raw foodstuffs, while preparation and cooking can generate important variations in the nutrient composition of foods. Furthermore, it has been reported that cooking can increase extraction of specific nutrients, producing discrepancies with raw foodstuffs in food composition tables. The Spanish food tables used in this study were based on the nutrient content of raw foodstuffs, and since we analysed nutrient content in cooked foods, this may be the main cause for differences found between calculated and analysed intakes. Currently, other food tables include the nutrient content of several cooked foods, which can reduce discrepancies between calculated and analysed intakes. Moreover, nutrient composition of several cooked dishes is presented in some food tables, but such data are not applicable to different ways of preparation and cooking.

Although $n-6$ and $n-3$ PUFA intakes were determined experimentally in the present study, the food tables used in this study did not include the $n-6$ or $n-3$ PUFA content of food. Indeed, to our knowledge only one Spanish food table does so (Martín Peña *et al.*, 1992). Such data should be taken into consideration, in the light of the increasing importance of these fatty acids and their role in the prevention of certain diseases (Kinsella *et al.*, 1990; Simopoulos, 1991). Moreover, the *trans* fatty acid content of foods is receiving increasing attention, since some studies have reported adverse effects of *trans* fatty acids on the risk of coronary heart disease. In Table 4, the mean content of fat, n-6 PUFA, n-3 PUFA and total trans fatty acids of all cooked dishes and ingredients analysed in this study is presented.

In conclusion, the present study shows that there may be significant differences between calculated and analysed values when determining dietary intakes. This should be taken into account in studies which rely on food composition information for determining dietary intakes.

ACKNOWLEDGEMENTS

This study was supported by a research grant 92/0139 from the Spanish CDTI (Technological and Industrial Development Centre) and from the Emissionat per a Universitats i Recerca of the Generalitat de Catalunya.

REFERENCES

- Bailey, A. L., Finglas, P. M., Wright, A. J. A. and Southon, S. (1994) Thiamin intake, erythrocyte transketolase (EC 2.2.1.1.) activity and total erythrocyte thiamin in adolescents. British Journal of Nutrition 72 , 111-125.
- Cave, W. T. (1996) Dietary omega-3 polyunsaturated fats and breast cancer. Nutrition 12, S39-S42.
- Chen, I. S., Shen, C.-S. J. and Sheppard, A. J. (1981) Comparison of methylene chloride and chloroform for the extraction of fats from food products. Journal of the Association of Official Analytical Chemists 58 , $599-601$.
- Dwyer, J. T. (1994) Future directions in food composition studies. Journal of Nutrition 124, 1783S-1788S.
- Finglas, P. M., Bailey, A., Walker, A., Loughridge, J. M., Wright, A. J. A. and Southon, S. (1993) Vitamin C intake and plasma ascorbic acid concentration in adolescents. Br Journal Nutr 69, 563-576.
- Hakala, P., Marniemi, J., Knuts, L.-R., Kumpulainen, J., Tahvonen, R. and Plaami, S. (1996) Calculated vs analysed nutrient composition of weight reduction diets. Food Chemistry 57 , $71-75$.
- Holland, B., Welch, A. A., Unwin, I. D., Buss, D. H., Paul, A. A. and Southgate, D. A. T. (1991) McCance and Widdowson's The Composition of Foods, 5th edn. The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, London.
- Horwitz, W. (1982) Evaluation of analytical methods for regulation. Journal of the Association of Official Analytical Chemists 65, 525-530.
- Kinsella, J. E., Lokesh, B. and Stone, R. A. (1990) Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. American Journal of Clinical Nutrition 52, 1-28.
- Mangels, A. R., Moser-Veillon, P. B., Andon, M. B. and Reynolds, R. D. (1990) Vitamin B-6 intakes of lactating women: Analyzed vs calculated values. Journal of the American Dietetic Association 90, 857-859.
- Martín Peña, G., Vigil Medina, L., Perianes Matesanz, J. F., Carreras Jiménez, R. and Ruiz Galiana, J. (1992) Tablas de composición de alimentos: una valoración práctica. Nutrition Hospitalatia $7,418–430$.
- Mataix, J., Mañas, M., Llopis, J. and Martínez de Vitoria, E. (1995) Tablas de Composición de Alimentos Españoles. Universidad de Granada, Granada.
- Metcalfe, L. D., Schmitz, A. A. and Pelka, J. R. (1966) Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chemistry 38, 514-515.
- Meydani, S. N., Barklund, M. P., Liu, S., Meydani, M., Miller, R. A., Cannon, J. G., Morrow, F. D., Rocklin, R. and Blumberg, J. B. (1990) Vitamin E supplementation

enhances cell-mediated immunity in healthy elderly subjects. American Journal of Clinical Nutrition 52, 557-563.

- National Research Council (1989) Recommended Dietary Allowances, 10th edn. National Academy Press, Washington, DC.
- Official Methods of Analysis (1995a) 16th edn, AOAC Int., Airlington, VA, sec. 963.15. Fat in cacao products. Soxhlet extraction method, chapter 31, p. 10.
- Official Methods of Analysis (1995b) 16th edn, AOAC Int., Airlington, VA, sec. 984.27. Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc in infant formula, chapter 50, p. 14.
- Rodríguez-Palmero, M., de la Presa-Owens, S., Castellote-Bargallo, A. I., López-Sabater, M. C., Rivero-Urgell, M. and de la Torre-Boronat, M. C. (1994) Determination of sterol content in different food samples by capillary gas chromatography. Journal of Chromatography \hat{A} 672, 267–272.
- Schaefer, E. J., Lichtenstein, A. H., Lamon-Fava, S., McNamara, J. R. and Ordovas, J. M. (1995) Lipoproteins, nutrition, aging, and atherosclerosis. American Journal of Clinical Nutrition 61(Suppl.), 726S-740S.
- Simopoulos, A. P. (1991) Omega-3 fatty acids in health and disease and in growth and development. American Journal of Clinical Nutrition 54, 438-463.
- Southon, S., Wright, A. J. A., Finglas, P. M., Bailey, A. L., Loughridge, J. M. and Walker, A. D. (1994) Dietary intake and micronutrient status of adolescents: effect of vitamin and trace element supplementation on indices of status and performance in tests of verbal and non verbal intelligence. Br Journal Nutr 71, 897-918.
- Stancher, B. and Zonta, F. (1983) High-performance liquid chromatography of fat soluble vitamins. Simultaneous quantitative analysis of vitamins D2, D3 and E. Study of percentage recoveries of vitamins from cod liver oil. Journal of Chromatography $256, 93-100$.
- The United States Pharmacopeia. (1989) Ed XXIII (USP XXIII), Mack Printing, Easton.
- Wood, R. J., Suter, P. M. and Russell, R. M. (1995) Mineral requirements of elderly people. American Journal of Clinical Nutrition 62, 493-505.