Assessment of thiamin (vitamin B$_1$) and riboflavin (vitamin B$_2$) status in an adult Mediterranean population

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The aim of the present study was to assess the nutritional status for thiamin (vitamin B$_1$) and riboflavin (vitamin B$_2$) in an adult Mediterranean population, in order to identify patterns of intake, groups at risk for deficiency and factors that might influence this risk. A cross-sectional survey was carried out in Andalusia, a western Mediterranean region in southern Spain. Nutrient intakes were studied in a random sample of 3390 subjects (1746 men, 1644 women) who were between 25 and 60 years of age. Blood samples were obtained for biochemical assays in a random subsample of 372 subjects (181 men, 191 women). Food consumption was assessed by 48 h recall. Vitamin B$_1$ and B$_2$ were measured as erythrocyte transketolase and as erythrocyte glutathione reductase activation coefficients, respectively. Energy and vitamin intakes were significantly higher in men than in women. Intakes were below two-thirds of the recommended dietary allowance for vitamin B$_1$ in 7·80 % of the men and 4·50 % of the woman, and were below this level for vitamin B$_2$ in 18·00 % of the men and 11·70 % of the women. Age, educational level, alcohol use and smoking were also associated with differences in the intake of these nutrients. Biochemical analyses showed that vitamin B$_1$ and B$_2$ status was deficient in 6·40 and 5·30 % of the population, respectively. Although factors such as gender, age, level of education, drinking and smoking can have an effect on the risk of inadequate intake of these nutrients, these factors did not affect biochemical indices of nutritional status in the present study.

Vitamin B$_1$ and B$_2$ status: Micronutrient status: Adults: Mediterranean region

Abundant information is currently available regarding food and nutrient intakes in Spain (Varela *et al.* 1985, 1995; Moreiras *et al.* 1995; Ministerio de Agricultura Pesca y Alimentación, 2000). However, information regarding biochemical status for vitamins in the adult population in Spain, particularly for thiamin (vitamin B$_1$) and riboflavin (vitamin B$_2$), is scarce. The only data available are from a few studies that were local in scope (González-Gross *et al.* 1991; Carbajal *et al.* 1996; Sánchez *et al.* 1999; López-Sobaler *et al.* 2002), a regional study (Serra-Manjem *et al.* 1996) and a meta-analysis of the results of earlier reports (Ortega *et al.* 2001). However, there are no data on the biochemical status for thiamin and riboflavin in the resident population of southern Spain.

The aim of the present study was to assess the nutritional status for vitamins B$_1$ and B$_2$ in the adult population of the region of Andalusia (a western Mediterranean region in southern Spain), identify groups at risk for dietary deficiency and suggest factors that may influence nutritional status for these nutrients. It is hoped that this information will be useful in developing future health interventions based on dietary habits.

Methods

Participants

The data reported here were obtained within the framework of a wide-ranging study in the region of Andalusia (southern Spain). A cross-sectional epidemiological survey was conducted from 1998 to 2000, with a representative random sample of adults living in the region of Andalusia, an 87 597 km^2 area with 7 305 117 inhabitants (Instituto de Estadística de Andalucía, 2001). The participants were between 25 and 60 years old at the time of the study. Sampling was probabilistic and stratified, and took place in several stages. The primary sampling unit was cities and towns (municipalities), the secondary unit was homes and the tertiary unit was individuals of either gender. In each of the eight provinces (domains) that make up the region the sample was distributed proportionately between men and women, and between three age groups: 25–39 years; 40–49 years; 50–60 years.

Municipalities were chosen randomly in each province with adequate measures to ensure that towns of different

sizes ($<$ 10 000, 10 000–100 000 and $>$ 100 000 inhabitants) were accurately represented in the sample. Secondary sampling units (homes) were selected with a random walk procedure.

The population of inhabitants between the ages of 25 and 60 years at the time of the study was about 2 900 000 (Instituto de Estadística de Andalucía, 2001). The theoretical sample size was 3680 subjects for a sampling error of less than 5 % and estimated at the 95 % confidence level. Ten municipalities were chosen in each province, forty-six homes were chosen in each municipality, and one individual was chosen in each household. The actual sample consisted of 3390 individuals (1746 men, 1644 women), for a participation rate of 92·12 % with valid observations. Participants were asked if they had any acute or chronic illness and were included if they were (or appeared to be) in good health; pregnant and lactating women were excluded.

Blood samples were taken for biochemical analysis from a random subsample of 372 subjects (181 men, 191 women), who comprised approximately 10 % of the sample.

Food consumption was assessed by a 24 h recall method in which participants recalled in an interview all foods consumed during the immediate previous 48 h (Cameron & Van Staveren, 1988). The data were obtained by eight dietitians with the aid of an open questionnaire and photographs as a reference for portion size. The pictures showed fresh foods or foods prepared according to usual recipes for dishes that are widely consumed in the area. All dietitians were trained to use a standardized procedure for the interview, and cross-checked to detect systematic differences.

Food intakes were converted into energy and nutrients with the help of the Spanish food composition table (Mataix *et al.* 1998). The composition database was used under AYS44 Diet Analysis software from ASDE, SA (Valencia, Spain).

Information about lifestyle factors (smoking and alcohol drinking, physical exercise and level of education) was collected with a structured questionnaire developed by the National Health Survey (Ministerio de Sanidad y Consumo, 1997). The study protocol was approved by the Medical Ethical Committee of the Health Council of the Andalusian Regional Government, and informed consent was obtained from each subject.

Analytical methods

In the morning, after the participants had abstained from eating or drinking overnight, blood was collected (6 ml) in tubes that contained 1 ml anticoagulant citrate-dextrose (citric acid 38 mmol, sodium citrate 74·8 mmol, D-glucose 123·6 mmol in water to make 1000 ml)-stabilizer (Venoject; Terumo Corporation, Leuven, Belgium). The samples were centrifuged at 3000 g for 15 min at 20°C to separate plasma, and erythrocytes washed with isotonic saline (9 g NaCl/l) were stored at $-$80°C until analysis. Biochemical studies consisted of the measurement of erythrocyte transketolase activity (ETK) and erythrocyte glutathione reductase activity (EGR) to assess vitamins B_1 and B_2 respectively, in accordance with the procedures described by Vuilleuimier *et al.* (1983). ETK was measured with

and without added thiamin pyrophosphate (Sigma, St Louis, MO, USA), and EGR was measured with and without added FAD (Sigma). The activation coefficient (α) for ETK (αETK) and EGR (αEGR) was taken as the ratio of activity with added thiamin pyrophosphate or FAD:activity without thiamin pyrophosphate or FAD, respectively (Vuilleuimier *et al.* 1983). The CV of αETK and αEGR were 4·65 (mean 1·01) % and 4·15 (mean 1·05) %, respectively, in fifteen blood samples analysed in duplicate for day-to-day analysis.

The cut-off points for αETK were deficient (high risk; $>$1·25), low (moderate risk; 1·16–1·25) and acceptable (low risk; $<$1·16) (Vuilleuimier *et al.* 1983; Sauberlich, 1999), and the corresponding values for αEGR were deficient ($>$1·40), low (1·40–1·20) and acceptable ($<$1·20) (Institute of Medicine, 1998; Sauberlich, 1999).

Statistical analysis

The crude experimental data were subjected to Student's t test for independent samples. Vitamin intakes were adjusted for total energy using the energy-adjusted method (Willett & Stampfer, 1986), and means were compared with the *post hoc* method of Bonferroni. ANOVA (2×3 way) was used to test the differences in intake and analytical values between gender and age groups, and to look for interactions between age (as a three-level variable) and gender. Linear regression analysis was used to find bivariate correlations; Pearson's correlation coefficient was calculated for 95 % confidence levels. Multiple logistic regression analysis was used to estimate the degree of association between intake or analytical values (dependent variable) and energy, gender, age, educational level, smoking and alcohol drinking. All analyses were done with version 10.0 of the Statistical Package for Social Sciences (SPSS Institute Inc., Chicago, IL, USA). Differences were considered significant at the 5 % probability level.

Results

In the present study population meat, grain products, fruit and pulses supplied 70·3 % of the vitamin B_1 intake, and milk, meat, grain products and fruit supplied the largest percentage intakes (70·0) of vitamin B_2.

Table 1 shows mean intakes of energy, vitamin B_1 and B_2 and mean biochemical values of the two vitamins, together with their percentile distributions. Energy and vitamin crude intakes were significantly higher in men than in women. However, when vitamin intake was adjusted for total energy intake, the difference between gender was significant only for vitamin B_2. In men, intakes were below two-thirds of the recommended dietary allowance (RDA) in 7·80 % of the participants for vitamin B_1 and in 18·00 % for vitamin B_2. In women the percentages were slightly lower at 4·50 for vitamin B_1 and 11·70 for vitamin B_2.

The results of the biochemical analyses showed that blood levels of the two vitamins were within normal limits in each gender (low risk). There were no significant differences between gender in biochemical values for vitamins.

Table 1. Mean intakes and biochemical status for thiamin (vitamin B$_1$) and riboflavin (vitamin B$_2$)

(Mean values and standard deviations)

	Total (n 3390)		Men (n 1746)					Women (n 1644)				
	Mean	SD	Mean	SD	P25	P50	P75	Mean	SD	P25	P50	P75
Intake												
Energy (MJ/d)	9·28	3·46	10·50	3·69	8·00	10·08	12·41	7·94*	2·61	6·03	7·68	9·39
B$_1$ (mg/d)	1·46	0·70	1·61	0·77	1·07	1·46	1·98	1·29*	0·56	0·89	1·17	1·59
			1·44†	0·54				1·48†	0·53			
B$_2$ (mg/d)	1·57	0·71	1·69	0·80	1·22	1·56	1·96	1·48*	0·53	1·07	1·37	1·72
			1·53†	0·58				1·62*†	0·61			
Biochemical status (n 372)§												
B$_1$ (αETK)	1·01	0·17	1·01	0·17	0·92	1·02	1·10	1·02	0·19	0·94	1·00	1·05
B$_2$ (αEGR)	1·04	0·25	1·05	0·29	0·92	1·00	1·14	1·04	0·21	0·97	1·00	1·10

P, percentile; αETK, erythrocyte transketolase activity coefficient; αEGR, erythrocyte glutathione reductase activity coefficient.

* Mean values were significantly different from those for the men ($P < 0.001$).

† Mean values adjusted for energy intake.

§ 181 men and 191 women.

The percentage of the population with an acceptable nutritional status was 86·40 for vitamin B$_1$ (α < 1·16) and 89·20 for vitamin B$_2$ (α < 1·20). The percentage of participants who were deficient (α < 1·25) for vitamin B$_1$ (6·40) was similar to the 5·30 value found for vitamin B$_2$ deficiency (α < 1·40). Thus the percentages of the population at moderate risk were 7·20 for vitamin B$_1$ (α1·25–1·16) and 5·50 for vitamin B$_2$ (α1·40–1·20). ANOVA revealed a significant interaction only between age and gender for vitamin B$_2$ status ($P < 0.01$).

Energy and vitamin crude intakes were significantly higher in the youngest group (25–39 years). When vitamin intake was adjusted for energy intake, the difference between age groups was not significant (Table 2). The only significant difference in the biochemical indices of nutritional status was found for vitamin B$_2$, whose values were significantly higher in the 40–49-year-old group than in the 25–39-year-old group (Table 2).

Table 2. Mean daily intakes and biochemical status for thiamin (vitamin B$_1$) and riboflavin (vitamin B$_2$) in different age groups

(Mean values and standard deviations)

Age...	25–39 years (n 1763)		40–49 years (n 748)		50–59 years (n 879)	
	Mean	SD	Mean	SD	Mean	SD
Intake						
Energy (MJ/d)	9·76	3·68	9·09*	3·28	8·49*†	3·00
B$_1$ (mg/d)	1·51	0·75	1·43*	0·64	1·36*†	0·61
	1·45‡	0·50	1·46‡	0·52	1·47‡	0·50
B$_2$ (mg/d)	1·61	0·74	1·54*	0·62	1·48*	0·57
	1·58‡	0·59	1·56‡	0·57	1·58‡	0·56
Biochemical status (n 189)§						
B$_1$ (αETK)	1·01	0·18	1·02	0·18	1·04	0·17
B$_2$ (αEGR)	1·01	0·23	1·11*	0·39	1·05	0·17

αETK, erythrocyte transketolase activity coefficient; αEGR, erythrocyte glutathione reductase activity coefficient.

* Mean values were significantly different from those for 25–39 years ($P < 0.05$).

† Mean values were significantly different from those for 40–59 years ($P < 0.05$).

‡ Mean values adjusted for energy intake.

§ Seventy-one men and 112 women.

Lower educational levels were associated with lower vitamin B$_2$ intakes adjusted for energy: mean intake was 1·61 (SD 0·54) for participants with university level education (n 614), 1·59 (SD 0·53) for those with secondary level education (n 782) and 1·54 (SD 0·54) mg vitamin B$_2$/d for those with primary level education (n 1902) or no schooling (n 92) ($P < 0.05$ in both cases). However, the results of the biochemical analyses showed no significant differences between subgroups compared according to educational level.

The possible effect of alcohol use and smoking was also examined on the intake of these vitamins. In the population who drank alcohol (n 1675), vitamin B$_1$ and B$_2$ intakes adjusted for energy were 1·49 (SD 0·76) and 1·59 (SD 0·52) mg/d respectively; these values were significantly higher than in non-drinkers (n 1715) (1·42 (SD 0·78) and 1·52 (SD 0·53) mg/d, respectively) ($P < 0.05$ in both cases). In smokers (n 1425) intakes adjusted for energy for both vitamins (1·42 (SD 0·51) mg vitamin B$_1$/d, 1·52 (SD 0·52) mg vitamin B$_2$/d) were lower than in non-smokers (n 1965) (1·48 (SD 0·53) mg vitamin B$_1$/d, 1·58 (SD 0·52) mg vitamin B$_2$/d) ($P < 0.05$ in both cases).

In contrast, the results of the biochemical analyses showed no significant differences between subgroups of alcohol drinkers (47·8 % of the subsample used for biochemical analyses) v. non-drinkers for either vitamin. Likewise, smoking (43·8 % of the subsample used for biochemical analyses) was not associated with significant differences in the biochemical findings in comparison with non-smokers.

Despite these differences, mean values for both vitamins were within normal limits (denoting low risk) in all subgroups analysed in the present study (three different levels of education, alcohol drinkers, non-drinkers, smokers and non-smokers).

The values obtained for intake and biochemical indices were subjected to bivariate analysis to search for linear correlations between intakes of the different nutrients, and between nutrient intake and biochemical level. Energy intake appeared to correlate significantly with the intake of vitamin B$_1$ (r 0·69; $P < 0.01$), and with vitamin B$_2$ (r 0·63; $P < 0.01$). Alcohol intake correlated with energy

intake (r 0·39; $P<0.01$), vitamin B_1 intake (r 0·17; $P<0.01$), vitamin B_2 intake (r 0·13; $P<0.01$) and the number of cigarettes smoked per d (r 0·24; $P<0.01$). Moreover, the number of cigarettes smoked per d correlated directly with energy intake (r 0·16; $P<0.01$), vitamin B_1 intake (r 0·06; $P<0.01$) and vitamin B_2 intake (r 0·04; $P<0.05$). Vitamin B_1 intake correlated with vitamin B_2 intake (r 0·54; $P<0.01$).

The multiple logistic regression model adjusted for energy, age and gender showed that vitamin B_2 intakes were associated with lower educational levels (elementary school studies or no formal education) (odds ratio 1·43, 95 % CI 1·15, 1·77; $P<0.001$, university education being designated 1 as the reference).

Discussion

In our study population, mean intake of vitamin B_1 was higher than the RDA for the adult population in Spain, and mean intake for vitamin B_2 was similar to the RDA (Varela, 1994). Our values were higher than the RDA established by the Institute of Medicine (1998).

The linear correlations between energy intake and intake of each of the two vitamins support the notion that the greater vitamin intake in men is related to their greater energy intake. When the results were adjusted for energy intake, no significant differences were found between men and women for vitamin B_1 (Table 1). The intake of vitamin B_2 adjusted by energy was significantly higher in women than in men (Table 1). A greater dietary density of vitamins in women was also noted by other authors (Hercberg *et al.* 1994). However, although the difference in vitamin B_2 intake between men and women was significant, it may not have been large enough to be translated into a difference in biochemical status.

The intakes of each of the two vitamins in southern Spain were similar to the mean values reported for Spain nationally (Moreiras *et al.* 1995). However, vitamin B_1 and B_2 intakes in our study population were lower than in the adult population of other industrialized countries (Institute of Medicine, 1998; Hiraoka, 2001).

The results of the biochemical analyses reflected adequate intakes of both vitamins in each gender (Table 2). The percentage of individuals in our sample (6·40) in whom vitamin B_1 was deficient was lower than that found in a sample of Spanish women aged 21 to 30 years (Carbajal *et al.* 1996), in an elderly British population (Bailey *et al.* 1997), in Australian blood donors (Booth *et al.* 1998) and in the population of the Seychelles (Bovet *et al.* 1998), but higher than that found in French adults (de Carvalho *et al.* 1996).

Although a correlation has been reported between vitamin B_1 intake and biochemical status (Hiraoka, 2001), our results, as those of others (Fidanza *et al.* 1984; Bailey *et al.* 1994; Alberti-Fidanza *et al.* 1998), failed to substantiate this. The differences between these studies may reflect differences in the composition of the study population and in the techniques used by different authors.

Our value for the prevalence of vitamin B_2 deficiency in southern Spain (see p. 663) was similar to that reported for a population of independently living elderly Spanish

individuals (López-Sobaler *et al.* 2002), and lower than that found for an elderly British population (Bailey *et al.* 1997), elderly Guatemalan individuals (Boisvert *et al.* 1993) and a Saudi population (El-Hazmi & Warsy, 1987).

Although studies with deficient rats showed a clear relationship between riboflavin intake and EGR (Prentice & Bates, 1981), our results for a southern Mediterranean population showed no significant correlation between vitamin B_2 intake and biochemical status. An earlier study by González-Gross *et al.* (1991) likewise failed to detect such a correlation. In our study population, individuals with an αEGR greater than 1·2 did not have lower vitamin intakes (González-Gross *et al.* 1991).

The significant differences in vitamin B_1 and B_2 intakes between age subgroups reflect the greater mean daily intake of energy in the youngest group (25–39 years). When intakes were adjusted for total energy intake, the differences became non-significant (Table 2).

The greater time spent on leisure-time physical exercise in younger age groups (1·60 (SD 3·37) h/week in the 25–39-year-old group *v.* 0·83 (SD 2·60) h/week in the 40–49-year-old group; $P<0.001$) may have increased vitamin B_2 requirements (Manore, 2000) and may thus account for the worse biochemical status for vitamin B_2 in these subjects (Table 2). However, in the present study thiamin status was not affected by physical exercise, in agreement with the results of Fogelholm *et al.* (1992).

Educational level is known to influence nutrient intake (Quandt, 1998). The lower riboflavin intake among individuals with the lowest levels of education (see p. 663) may reflect the lower mean consumption of dairy products in this group. Logistic regression analysis confirmed this result; it was found that the subgroups with lower levels of education were at higher risk for inadequate riboflavin intake. However, the higher intakes of this vitamin in individuals with secondary school or university-level education were not reflected as a significantly higher biochemical status for this nutrient.

The greater energy-adjusted intakes of vitamin B_1 and B_2 in alcohol drinkers reflected greater meat consumption in this subgroup. Our results are consistent with the linear correlations between alcohol and vitamin intakes.

In chronic alcohol abusers requirements for vitamin B_1 (Herve *et al.* 1995; Ambrose *et al.* 2001) and B_2 (Pinto *et al.* 1987) are higher. The moderate mean intakes for alcohol in our population (one to two drinks/d) may help explain why no impairment was found in vitamin status among alcohol drinkers in the present study. An earlier report likewise failed to find any correlation between blood levels of this vitamin and alcohol intake (Fidanza *et al.* 1984).

The greater energy-adjusted intakes of vitamin B_1 and B_2 in non-smokers may reflect their greater consumption of milk, fruits and vegetables, even though smokers ate more meat.

One earlier study found lower levels of riboflavin in smokers than in non-smokers (Benton *et al.* 1997); however, no significant differences were found between these two subgroups in our study population.

The prevalence of methylenetetrahydrofolate reductase C677T polymorphism in Spain is among the highest in

Europe and is similar to the prevalence in Italy (Guillén *et al.* 2001). The increased plasma total homocysteine associated with this mutation is thought to occur only with poor riboflavin status (McNulty *et al.* 2002). Because this trend might favour the increase in mortality from cerebrovascular disease and IHD in this region (Instituto de Estadística de Andalucía, 2001), further research will be needed to discover the exact relationship between nutritional status for riboflavin and risk for these significant public health problems.

Although the interpretation of data from survey studies can be complex, our results provide a precise estimate of the nutritional status for thiamin and riboflavin in the adult population of southern Spain. Nutritional status was not acceptable (αETK < 1·16) in 13·6 % of our study population for vitamin B$_1$, and in 10·8 % for vitamin B$_2$ (αEGR < 1·20). Factors such as gender, age, level of education, alcohol consumption and smoking can have an effect on the risk of inadequate intake of these nutrients, although these factors did not affect biochemical indices of nutritional status in the present study.

Acknowledgements

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