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 women, 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 % 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

Abbreviations: EGR, erythrocyte glutathione reductase activity; ETK, erythrocyte transketolase activity; RDA, recommended dietary allowance.

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sizes (< 10 000, 10 000–100 000 and > 100 000 inhabi-
tants) 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 theoreti-
cal 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 indi-
vidual 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 ill-
ness 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 photo-
graphs 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 inter-
view, and cross-checked to detect systematic differences.

Food intake 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 col-
lected with a structured questionnaire developed by the
National Health Survey (Ministerio de Sanidad y Con-
sumo, 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 (Veno-
test; Terumo Corporation, Leuven, Belgium). The samples
were centrifuged at 3 000 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 trans-
ketolase activity (ETK) and erythrocyte glutathione
reductase activity (EGR) to assess vitamins B1 and B2
respectively, in accordance with the procedures described
by Vuilleumier 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 (Vuilleumier 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) (Vuilleumier 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
for independent samples. Vitamin intakes were
adjusted for total energy using the energy-adjusted
method (Willett & Stampfer, 1986), and means were com-
pared 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, smok-
ing and alcohol drinking. All analyses were done with ver-
sion 10.0 of the Statistical Package for Social Sciences
(SPPS 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 B1 intake, and
milk, meat, grain products and fruit supplied the largest
percentage intakes (70·0 %) of vitamin B2.

Table 1 shows mean intakes of energy, vitamin B1 and
B2 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 B2. In men, intakes
were below two-thirds of the recommended dietary allow-
ance (RDA) in 7·80 % of the participants for vitamin B1
and in 18·00 % for vitamin B2. In women the percentages
were slightly lower at 4·50 for vitamin B1 and 11·70 for
vitamin B2.

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.
The percentage of the population with an acceptable nutritional status was 86·40 for vitamin B1 (α<1·16) and 89·20 for vitamin B2 (α<1·20). The percentage of participants who were deficient (α<1·25) for vitamin B1 (6·40) was similar to the 5·30 value found for vitamin B2 deficiency (α<1·40). Thus the percentages of the population at moderate risk were 7·20 for vitamin B1 (α25–1·16) and 5·50 for vitamin B2 (α1·40–1·20). ANOVA revealed a significant interaction only between age and gender for vitamin B2 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 B2, whose values were significantly higher in the 40–49-year-old group than in the 25–39-year-old group (Table 2).

Lower educational levels were associated with lower vitamin B2 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 1·45 (SD 0·54) mg vitamin B2/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 B1 and B2 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-smokers (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 B1/d, 1·52 (SD 0·52) mg vitamin B2/d) were lower than in non-smokers (n 1965) (1·48 (SD 0·53) mg vitamin B1/d, 1·58 (SD 0·52) mg vitamin B2/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 B1 (r 0·69; P<0·01), and with vitamin B2 (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; \( P < 0·001 \)). 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; Hiraoaka, 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 (Hiraoaka, 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 \( \alpha \)EGR 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 vs. 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 (aETK<1.16) in 13.6% of our study population for vitamin B1, and in 10.8% for vitamin B2 (aEGR<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.

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