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ORIGINAL COMMUNICATION

Nutritional assessment, health markers and lipoprotein profile in postmenopausal women belonging to a closed community

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Objective: To assess the dietary characteristics of a closed community and their relationship with several health markers and lipid and lipoprotein values in postmenopausal women.
Design: Energy and nutrient intake, serum lipids, lipoproteins, antioxidants, peroxides and low-density lipoprotein (LDL) peroxides in addition to several health markers were measured in a closed, postmenopausal female community consuming a diet without meat, meat products and alcoholic beverages.
Setting: Departamento de Nutrición and Sección Departamental de Química Analítica and Escuela de Especialización de Análisis Clínicos, Universidad Complutense de Madrid, Spain and Lerma, Burgos, Spain.
Results: Cereals, vegetables, legumes and fruit, together with milk and eggs, constituted the most important ingredients of the diet consumed. Dietary carbohydrates contributed 42%en and lipids 46.4%en. The SFA/MUFA/PUFA ratio was 1/2/1 and the n-3/n-6 ratio 0.05 (SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids). The study community diet was monotonous and made for possible deficiencies of iron, magnesium, zinc, vitamin B_6 and vitamin D, in variable proportions. Routine biochemical and haematological normality markers indicate that only one woman presented hyperglycaemia and hyperuricaemia. Two women had haemoglobin levels <12 g/dl, but their mean corpuscular volume or mean corpuscular haemoglobin was normal. The prevalence of high cholesterol values (>6.21 mmol/l) was 42.8%, while that of high LDL-cholesterol levels (>3.88 mmol/l) was 35.7%, but none of the women displayed levels of high-density lipoprotein (HDL)-cholesterol <1.16 mmol/l, triglycerides >1.2 mmol/l or an LDL-cholesterol/HDL-cholesterol ratio >3. Only one woman had apolipoprotein (Apo) B levels >1.5 g/l, while most of the women presented Apo B values <1.2 g/l and an ApoA-1/ApoB ratio <1.1. Plasma and LDL-peroxide levels, together with the tocopherol and carotene intakes, suggest a good antioxidant status in this population.
Conclusions: The diet of the study group seems compatible with a healthy life-profile and permits a more-than-acceptable degree of cardiovascular disease protection. However, the consumption of certain nutrients should be improved.
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Introduction

The traditional Mediterranean diet has been associated with low both total and cardiovascular mortality and morbidity rates (Keys *et al*, 1986). The Mediterranean diet pyramid suggests that vegetables, fruit, cereals and olive oil should be consumed daily. Consumption of eggs, poultry and fish is only recommended a few times a week and that of meat only once a month or somewhat more frequently in the case of small portions (Willett *et al*, 1995). Daily exercise and wine during meals are also included in this pyramid. For social

and economic reasons, several variants of the classical Mediterranean diet actually coexist in Spain. Thus, although consumption of olive oil is traditional in Mediterranean countries, it has been partially displaced by that of other oils, such as sunflower oil, in domestic and institutional settings (Parras & Torres, 1995).

The aged, and mainly those residing in rural areas, are reported to be at greater risk of suffering malnutrition than younger individuals (Suzana *et al*, 2002). Numerous physiological and psychological changes make them vulnerable to health and nutritional problems. In addition, cultural and religious influences can alter dietary patterns and limit the intake of several essential nutrients.

The present paper aims to assess the diet of a closed community of postmenopausal women consuming a Mediterranean diet including no meat, meat products or alcohol and in which the culinary oil used was a mixture of olive and sunflower oils (SO). In order to understand the possible long-term benefits of this diet, its consumption was related to several health markers and to lipid and lipoprotein profiles. The prevalence of cardiovascular disease (CHD) risk factors was also assessed.

Methods

Subjects

A total of 14 nuns from an enclosed convent were studied. Subjects were chosen because of their age, dietary habits and orderly lifestyle. Age and some anthropometrical characteristics of this population are defined in Table 1. None of the subjects presented previous cardiovascular, metabolic or systemic disease or took any medication that might affect lipoprotein metabolism. The study protocol was approved by an Ethics Committee of the Universidad Complutense de Madrid and performed in accordance with the Helsinki Declaration.

Dietary assessment and total energy expenditure

Food intake of each individual was assessed by the 'precise weighing method' during a 14-day period. Energy and nutrient intakes were calculated using food composition tables for the raw weights of foodstuffs (Moreiras *et al*, 2001). As their culinary fat, the nuns used a homogeneous mixture (1:1, vol/vol) of olive and refined sunflower oils. A high proportion of this oil mixture ($\sim$45%) was consumed raw in salad dressing, while the rest was used for sautéing, frying, pot-roasting and to prepare fish, eggs, vegetables and stews. Energy and nutrient intakes were compared with the Spanish recommended dietary intakes (RDI) (Departamento de Nutrición, 2001). Energy expenditure was calculated according to the factorial method suggested by the FAO/WHO/UNU Expert Consultation Report (1985), in which the basal metabolic ratio was calculated according to several formulas. Physical activity was recorded for a week and converted to energy using the Buskirk and Mendez (1980) tables. The Crist *et al* (1980) formula was used for calculating the average thermogenic effect of the diet.

Anthropometric parameters

Using standardised methodology, body weight and standing height were measured twice a week. Body mass index (BMI)(weight (kg)/height^2 (m)) was also calculated. Systolic

Table 1 Anthropometrical characteristics, energy expenditure, routine biochemical and haematological markers, and serum and lipoprotein lipids in postmenopausal women

	Median$\pm$*s.d.*	*Minimum–maximum*		*Median*$\pm$*s.d.*	*Minimum–maximum*
Age (y)	62.9$\pm$11.2	46–76	Glucose (mmol/l)	5.37$\pm$0.58	4.45–7.11
Weight (kg)	54.3$\pm$9.3	40.7–70.3	Urea (mmol/l)	5.59$\pm$1.04	3.83–8.00
Height (cm)	153$\pm$7.0	142–164	Uric acid (mmol/l)	0.21$\pm$0.05	0.15–0.30
Body mass index (kg/m^2)	23.2$\pm$3.4	19.6–29.3	AST (IU/l)*	29.1$\pm$8.8	16–49
Total energy expenditure (kJ)	7548$\pm$778	5995–9272	ALT (IU/l)*	18.5$\pm$6.6	4–30
Basal metabolic rate (BMR) (kJ)	5121$\pm$502	4347–5966	Creatinine (μmol/l)	75.7$\pm$15.0	58.4–99.9
Physical activity over BMR (kJ)	1799$\pm$304	1356–2310	GGT (IU/l)*	17.5$\pm$5.5	11–29
Thermogenic effect (kJ)	670$\pm$68	540–825	ALP (IU/l)*	72.2$\pm$16.9	42–111
Total cholesterol (TC) (mmol/l)	6.41$\pm$1.15	4.73–8.84	Total bilirubin (μmol/l)	8.2$\pm$ 1.9	5.1–10.3
Triglycerides (mmol/l)	0.83$\pm$0.19	0.53–1.20	Leukocytes (10^9/l)	6.19$\pm$1.35	4.0–8.6
LDL-cholesterol (mmol/l)	3.78$\pm$0.80	2.84–5.87	Erythrocytes (10^{12}/l)	4.470$\pm$0.500	3.270–5.070
HDL-cholesterol (mmol/l)	1.88$\pm$0.40	1.19–2.59	Haemoglobin (g/dl)	13.0$\pm$1.1	11.0–14.6
TC/HDL-cholesterol	3.47$\pm$0.54	2.70–4.28	Haematocrit (%)	40.1$\pm$3.1	33.3–45.3
LDL-cholesterol/HDL-cholesterol	2.07 $\pm$0.48	1.51–2.84	MCV (fl)	87.9$\pm$3.7	83–95
Apolipoprotein (Apo) A-I (g/l)	1.56 $\pm$0.21	1.25–1.98	MCH (pg)	28.7$\pm$1.5	27–31
Apolipoprotein (Apo) B (g/l)	1.08 $\pm$0.21	0.75–1.62	MCHC (g/dl)	32.4$\pm$0.6	31–33
Apo A-I/Apo B	1.47$\pm$0.23	1.02–1.79	Serum total peroxides (μmol /l)	0.85$\pm$0.73	0.14–2.27
			LDL peroxides (μmol/l)	0.28$\pm$0.23	0.10–0.80

Values are mean$\pm$s.d. of 14 women.
*Determined at 37°C.

Table 2 Energy and nutrient intake per person and day

	Mean ±*s.d.*	Minimum	*Maximum*	*% women below 2/3 Spanish RDI*
Energy (kJ)	7335±774	5774	8933	0
Carbohydrates (g)	197±29	123	242	
Carbohydrates (%en)	42.0±3.0	33.4	46.4	
Protein (g)	51.4±5.8	41.6	63.3	0
Protein (%en)	11.7±0.4	10.4	12.2	
Fat (g)	90±8.2	80.3	109	
Fat (%en)	46.4±3.0	41.6	54.8	
Cholesterol (mg)	401±33.9	355	480	
SFA (g)	23.1±3.6	15	29.4	
SFA (%en)	11.9±1.7	8.5	14.0	
MUFA (g)	39.9±3.4	35.1	47.5	
MUFA (%en)	20.6±1.2	17.3	22.6	
PUFA (g)	20.7±1.93	17.9	24.2	
PUFA (%en)	10.7±0.7	8.1	12.5	
PUFA+MUFA/SFA	2.68±0.50	2.43	4.15	
Fibre (g)	16.5±2.1	12.7	20.7	
Calcium (mg)	786±134	395	1009	7.1
Iron (mg)	8.7±1.0	6.3	10.4	7.1
Magnesium (mg)	186±28	147	259	78.6
Zinc (mg)	6.6±0.95	4.1	8.1	100
Sodium (mg) (from foods)	1.06±0.22	0.4	1.3	
Potassium (mg)	2.81±0.28	2.4	3.4	
Retinol equivalents (μg)	735±86	569	911	7.1
Riboflavin (mg)	1.46±0.2	0.9	1.8	0
Thiamine (mg)	0.88±0.11	0.7	1.1	0
Vitamin B_6 (mg)	1.21±0.13	1.1	1.5	0
Vitamin B_{12} (μg)	3.8±0.4	3.0	4.6	0
Niacin equivalents (mg)	16.3±1.7	13.9	19.7	0
Folate (μg)	173±16	148	207	100*
Vitamin C (mg)	114±10	98	136	0
Vitamin D (μg)	1.66±0.15	1.5	2.0	100
Vitamin E (mg)	19.5±1.8	16.8	22.7	0

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
*RDI (recommended dietary intakes) of folate in Spain has been recently increased to 400 μg/day.

and diastolic pressure was measured at the end of the study period.

Laboratory analyses

After overnight fasting (12 h), blood samples were collected by venepuncture. Routine determinations for erythrocyte and leucocyte counts, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using standard methods (Izasa, Barcelona, Spain) in a Hot-line coulter model MDII (Coulter-Corporation, Miami, FL, USA). Serum glucose, urea, uric acid, total cholesterol (TC), triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and total bilirubin were determined in a Technicon RA-500 autoanalyzer (Tarrytown, NY, USA) using routine standard methods (Bayer, Barcelona, Spain). Apolipoprotein (Apo) A-I and Apo B were determined by immunoturbidimetry, following the standard Behring Institute method (Barcelona, Spain). Lipoproteins were isolated by 22 h of density-gradient ultracentrifugation (Terpstra *et al*, 1981) at 8°C instead of 20°C, to ensure less lipoprotein thermal damage. Low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol and the TC/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios were also determined. Serum peroxide and LDL-peroxide concentrations were determined as thiobarbituric acid reactive substances (TBARS) following the method of Yagi (1984).

Results

Anthropometric and dietary assessment

Anthropometric characteristics are shown in Table 1. The BMI suggests that any woman presented obesity. Body weight did not show any significant changes throughout the study period. Physical activity was categorised as light.

The diet was characterised by the absence of meat, meat products and alcoholic beverages. Milk was the food consumed in greatest quantity (500 g/day), followed by vegetables (419 g/day), fruits (288 g/day), cereals (141 g/day), eggs (65 g/day, equivalent to nine eggs per week), oils (59 g/day), fish (48 g/day), sugars and chocolates (23 g/day) and legumes (15 g/day). Mean daily intake of nutrients and the dietary energy contribution of macronutrients are presented in Table 2.

Food intake conformed to energy expenditure (Tables 1 and 2). Carbohydrates contributed 42.0%en, lipids 46.4%en and protein 11.7%en. Nutrient intake and some nutritional quality indexes are shown in Table 2. The absence of meat-group items did not prevent covering protein requirements and assuring high-quality protein (32% came from milk and dairy products, 21% from cereals, 14% from eggs and 13% from fish and shellfish). All members of the community displayed low zinc, folic acid and vitamin D intakes.

Health markers

In general terms, the study population displayed biochemistry and haematology routine marker values within the normal ranges. Only one individual presented moderate hyperglycaemia, another had a low erythrocyte count, haematocrit and MCHC and two women displayed a low haemoglobin concentration.

Lipids and lipoproteins

Lipid and lipoprotein contents are shown in Table 1. Among the participants, 42.8% were hypercholesterolaemics (TC >6.21 mmol/l) and 35.7% had LDL-cholesterol levels

>3.88 mmol/l. However, none of them had triglyceride levels >1.21 mmol/l. According to HDL-cholesterol levels, only two women were at moderate CHD risk (values 1.16–1.42 mmol/l) while 12 were at low risk. The prevalence of women with an LDL-cholesterol/HDL-cholesterol ratio >3 was null. Only one individual had Apo B levels above 1.5 g/l, while 12 of the 14 women had Apo B values <1.2 g/l. Two women displayed an Apo A-1/Apo B ratio <1.1. The LDL fraction carried 33% of total peroxides.

Discussion

Diet assessment

The diet consumed by the community was relatively monotonous and deficient in some essential nutrients. Although a nutrient intake that is less than the RDI does not necessarily indicate that a given individual has not met the criterion of adequacy, a usual intake that is well below the RDI may be an indication of the need for further assessment of nutritional status by biochemical test or clinical examination (IOM, 2000). We have selected the two-third of the RDI as cut-point for this assessment. Thus, variable degrees of deficiency of these nutrients were a real possibility in the women studied. The most important dietary limitation was in relation to vitamin D. These results coincide with those of others (Moreiras *et al*, 1992). Ageing is accompanied by reduced absorption of this vitamin from the diet, reduced conversion to its active metabolites and resistance to its action in the intestine and in bone (Simon *et al*, 2002). Moreover, the women studied have a sedentary life and limited exposure to sunlight, both of which aggravate the suboptimal intake of this vitamin. The relative dietary limitation of iron and folic acid did not have clear negative repercussions on those women as, according to their MCH and MCHC, none displayed macrocytic anaemia. According to the new Spanish RDI for folic acid, diet of all women contained a low amount of this vitamin that in turn would increase the homocysteine CHD-related risk (Krauss *et al*, 2000). However, diet contained reasonable amounts of green vegetables; thus, a further increase would be difficult to be obtained only from diet.

According to the energy contribution, diet was normoproteic, hypocarbohydrated and hyperlipidic. These figures did not come close to reflecting current nutritional guidelines (Krauss *et al*, 2000; Mataix *et al*, 2001). However, controversy now exists between the advisability of following a diet containing >55% carbohydrates and 30% lipids (eg NCEP step 1) with respect to a Mediterranean diet poorer in carbohydrates but richer in lipids and monounsaturated fatty acids (MUFA). Thus, several studies do not confirm the benefits of the former diet over that of the Mediterranean diet with regard to serum triglycerides, HDL-cholesterol and fibrinolysis (Grundy, 1997; López Segura *et al*, 1996).

The low prevalence of elevated serum lipid, LDL-cholesterol and Apo B values and the high HDL-cholesterol and Apo A-I levels in this population suggest that, except for their relatively high cholesterol levels, these women may be considered to be reasonably protected with regard to CHD. Their values are compatible with the characteristics of the diet, adjusted to energy expenditure and with an adequate fatty acid profile (Krauss *et al*, 2000; Mataix *et al*, 2001). Taking into account the considerations of Dietschy (1998), the relatively high amount of dietary cholesterol seems not to be important because MUFA and, to a lesser degree, polyunsaturated fatty acids (PUFA) are good substrates for cholesterol esterification, maintaining the free cholesterol pool level low and thus the gene expression of the LDL receptor high. This process contributes to maintaining a low LDL-cholesterol level. A large proportion of the 400 mg cholesterol consumed per day came from eggs but, as hen-egg cholesterol is poorly absorbed (Jiang *et al*, 2001), it contributed less to serum cholesterol levels than cholesterol from other foods. Moreover, high intake of oleic acid has been related to high levels of HDL-cholesterol (Mata *et al*, 1992; Grundy, 1997). The n-3/n-6 ratio was lower than that recommended by the BNF (1992). Although fish and oily fish consumptions were about 48 and 15 g/day, respectively, the high contribution of sunflower oil (as part of the SO mix) decreased the n-3/n-6 ratio. As this population has eaten the present diet for decades, inclusion of n-3 PUFA-enriched foods (eg n-3 PUFA eggs, n-3 PUFA milk) was recommended in order to avoid stressful dietary changes.

About one-third of the serum peroxide values were found in the LDL fraction. Szczeklik and Gryglewski (1980) and Hagihara *et al* (1984) report that LDL transports 40% of the peroxide, suggesting that other lipoproteins are also important peroxide carriers. HDL may exert an antiatherosclerotic role by maintaining low LDL peroxidation and carrying oxidised cholesterol and acyl groups to the liver (Witztum, 1994). The high consumption of fruits, vegetables and vegetable oils implied a high intake of tocopherol, carotenes and minor compounds with antioxidant properties (Boskou, 1999), conferring a low peroxide status and thus decreasing the CHD risk in this population.

Conclusions

The present study shows that postmenopausal women who consume a variant of the Mediterranean diet, very rich in lipids but with an SFA/MUFA/PUFA (SFA = saturated fatty acids) ratio of 1/2/1, present a reasonably good lipoprotein profile and a low prevalence of CHD risk factors. However, nutritional advice was offered in order to improve the dietary content of some nutrients and thereby the health determinants of these women.

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